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Risk estimates of arsenic related skin lesions in two large villages in Rajshahi division, Bangladesh

by

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DEDICATION

To
***Prof. John Corbett McDonald**, my teacher of distinction*
for his unending commitment to the causes of Bangladesh

Mahamuda Khatun** and **Md. Mizanur Rahman
(my mother and father in law)
for their continuing inspiration for my education

***Labin Rahman, Siam and Saima** (my wife, son and daughter)*
who accepted happily my prolong absence from them for this work

ABSTRACT

In rural Bangladesh people drink water from an estimated 10-11 million tube wells, many with arsenic concentrations well above the national standard of 50µg/L. Characteristic skin lesions and more serious health effects are probable. The research reported here was designed to determine the relation between arsenic concentration and skin lesions on hands and feet controlling for education, use of tube well water, protein consumption and body mass index (BMI). The investigation included a well survey, a prevalence survey and a nested case-referent analysis. Studies were conducted in two villages (population 11670) in northern Bangladesh. In the well survey 1509 wells in use were identified and measurements of arsenic concentration made in 1422 (94%). Three estimates were made for each well, which yielded correlation among repeats = 0.93. The overall median was 47µg/L and the highest recorded 1760 µg/L.

Paramedics examined soles and palms of 11087 individuals for skin lesions and identified 168 cases (1.5%). In the third phase, cases (over 16 years) were matched by age, gender and village to referents (target of 3) without skin lesions. Cases and referents were interviewed about protein in diet, use of well water, education and residential history. Conditional logistic regression was used to assess the effects of arsenic

concentration, controlling for confounders. Subjects with lesions had a higher median concentration (250μg/L) than those without (47μg/L). Prevalence increased with both age and arsenic concentration, reaching 26.7% in those over 50 years of age and >500 μg/L. Of the 160 cases (≥16 years) 137 were interviewed, 127 with arsenic concentration measured in well water, together with their 504 referents. Cases were somewhat more likely to have lived in the villages throughout their lives and less likely to report using tube well water for cooking. The final model confirmed the high risk of lesions associated with arsenic concentrations. Using ≤50μg/L for comparison, an odds ratio of 15.2 (95%CI 7.2-32.2) was observed for those using tube wells with concentrations >500 μg/L, adjusting for use of tube well water in cooking and lifetime residence in the villages.

The results from this research provide continuing support for the use of 50μg/L as a useful national standard. While the enforcement of this standard has immediate value, it cannot be considered final without comparable information on more serious health risks.

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Symbols:

µg/L	Microgram/liter
As	Arsenic
Fe	Iron
NH_4	Ammonium
SO_4	Sulphate

Abbreviations:

AD	*anno domini* (in the year of the Lord)
ADB	Asian Development Bank
ATSDR	Agency for Toxic Substances and Disease Registry
AAS	Atomic Absorption Spectrophotometer
BC	Before Christ
BDHS	Bangladesh Demographic and Health Survey
BGS	British Geological Survey
BMI	Body Mass Index
BRAC	Bangladesh Rural Advancement Committee
CAE	Cumulative Arsenic Exposure
CAI	Cumulative Arsenic Index
CCA	Chromated Copper Arsenate
CDC	Centers for Disease Control
CI	Confidence Interval

DCH	Dhaka Community Hospital
DPHE	Department of Public Health Engineering
DMA	Dimethyl arsenic
DANIDA	Danish International Development Agency
DFID	Department for International Development
FDA	Federal Drug Administration
FI-HG-ASS	Flow Injection Hydride Generation Atomic Absorption Spectrophotometry
FEV_1	Forced Expiratory Volume
FVC	Forced Vital Capacity
GBM	Ganga Brhammaputra Meghna
GIT	Gastrointestinal Tract
GK	Gonosasthaya Kendra
GPS	Global Positioning System
HEALS	Health Effect of Arsenic Longitudinal Study
HG-AAS	Hydride generation Atomic Absorption Spectrometry
IARC	International Agency for Research on Cancer
ICCDRB	International Center for Diarrheal Disease Research Bangladesh
ICMH	Institute of Child and Mother Health
Kg	Kilogram
mg	Milligram
MMA	Monomethyl arsenic

MOHFW	Ministry of Health and Family Welfare
MSMA	Monosodium methyl arsenate
NGO	Non Governmental Organization
NH	Nazmul Huda
NHS	National Hydro-chemical Survey
NIPSOM	National Institute of Preventive and Social Medicine
LGRD	(Ministry of) Local Government and Rural Development.
LGED	Local Government Engineering Department
OR	Odds Ratio
PET	Positron Emission Tomography
POR	Prevalence Odds Ratio
ppb	Parts per billion
PPM	Parts per million
RERI	Relative excess risk for interaction
SAM	S-adenosylmethionine
SD	Standard Deviation
SMR	Standardized morbidity ratio
SPSS	Statistical Package for Social Scientists
TLIA	Total lifetime ingestion of arsenic
TW	Tube well
TWA	Time weighted arsenic
UK	United Kingdom
UN	United Nations

Unicef	United Nations Children Fund
USA	United States of America
USGS	United States Geological Survey
WHO	World Health Organization

Chapter 1: INTRODUCTION AND CONTEXT

1.1 Preamble:

This thesis is based on an epidemiological study of arsenic-related skin lesions in two large villages in the Kashinathpur area (Pabna district) in North Western Bangladesh. The study was conducted with helpful participation from a large non governmental organization in Bangladesh named Gonosasthaya Kendra (GK: the People's Health Center). This Introduction will review the more relevant aspects of arsenic and its geographical distribution in drinking water, toxicology, and response of the health care system, before proceeding to a review of what has been published about skin lesions due to exposure from arsenic in drinking water. Much of this introduction is based on two important assessments of the toxicology of arsenic, that published by the International Agency for Research on Cancer (IARC, 2004) and that of the US Agency for Toxic Substances and Disease Registry (ATSDR, 2007). These sources are implied for statements where no other reference is given.

1.2 Introduction

1. 2.1 Chemistry and history of arsenic:

Arsenic is a naturally occurring metalloid, a tasteless and colorless substance. It is the 20th most abundant natural element in the earth crust and because of its abundance in nature, potential for human exposure and toxicity, it is classified as the most hazardous substance on earth (ATSDR, 2003). In nature arsenic appears in solid phase, metalloid states, grey or black color, but yellow in color in non metallic form. In the general environment it is released through weathering of arsenic containing minerals and ores and by volcanic action. It may also be released into the environment from commercial and industrial processes as in the contemporary world arsenic is widely used in various industries. According to the United States Geological Survey (USGS), in 2005 China was the world's largest producer of white arsenic oxide, claiming 50% of the world share (USGS, 2008).

Arsenic is a chemical element with the symbol As and atomic number 33. It is found in two forms: inorganic and organic. In inorganic compounds arsenic combines with elements such as oxygen, chlorine and sulfur. In the organic form, it combines with carbon and hydrogen. In food, for example sea fish, sea weeds, vegetables and rice, arsenic concentration may be mainly organic. In drinking water and industrial contamination, inorganic arsenic is the predominant species. Upon entering the food chain, inorganic arsenic and its compounds are progressively metabolized by methylation and become less toxic. Organic arsenic in the form of arsenobetaine and arsenocholine has low toxicity in man and is rapidly excreted through urine.

Common valence states of arsenic are As^0 (metalloid arsenic), As^{3+}(arsenite), As^{3-}(arsine gas) and As^{5+}(arsenate). The toxicity of arsenic is related to its valence and oxidative status. Arsenic forms colorless, odorless, crystalline oxides As_2O_3 and As_2O_5.which are hygroscopic and dissolve in water to form weak acids. Some important arsenic compounds are, arsenic oxide or "white arsenic" (As_2O_3), yellow sulfide orpiment (As_2S_3), red reaglar (As_4S_4), Paris Green, calcium arsenate and lead hydrogen arsenate. Arsenic is chemically very similar to phosphorus. The similarity is such that arsenic partly substitutes phosphorus in biochemical reactions and thus express its toxicity more widely.

Arsenic in history dates back to the work of Aristotle in 400 BC. The name "arsenic" was derived from a Persian word "zarnikh" meaning "yellow orpiment". Greeks borrowed this word as "arsenikon" meaning masculine or potent. The Arabian alchemist Gerber is claimed to have isolated arsenic first in the 8th century. In Europe, scientist Alberta Magnus first isolated the element in 1250 AD. In history, arsenic is known as an agent for healing, homicide and better living. As it is tasteless, odorless and colorless and produces no defined symptoms, it was frequently used for political homicide. Because of its use in ruling class, potency, and poisoning it has been called the *"poison of kings and the king of poisons"*. It has been suggested that the Emperor Napoleon Bonaparte was killed by arsenic poisoning. In contrast, arsenic preparations have been used over the years for healing and better living. Arsenic preparations were eaten by men for potency and used as cosmetics by women for improving their complexion. Arsenic is still widely used in traditional and modern medicine. Asphenamine was used for treating syphilis and trypanosomiasis during the 18th-20th centuries. In sub toxic dose, arsenic compounds were also used as stimulants by healers in the 18th century. Arsenic trioxide has been used for treating cancer over the past 200 years and in 2000 the United States Federal Drug Administration (FDA) approved this agent for treating certain types of acute leukemia (Antman KH 2001). Arsenic-74, a positron emitter was used recently introduced in Positron Emission Tomography (PET) as an alternative to iodine 124 for producing a better quality image of cell functions (Jennewein et al 2008). Arsenical preparations have been used for various industrial and domestic purposes throughout the ages. In the 19th century, copper arsenate was used for coloring sweets and lead arsenate for spraying fruit trees well into the 20th century (Peryea 1998). More recently, a less toxic organic form (monosodium methyl arsenate-MSMA) had replaced lead arsenate in agriculture. For a long time in the wood industry, chromated copper arsenate (CCA) or Tanalith has been used as a fungicide for treating woods. Because of the risk of arsenic leaching into surrounding soil from the treated timber and therefore the possibility of human exposure and the risk related to burning older timber, its industrial use is now banned in many countries. In other industries, arsenic is widely used in insecticides, pesticides and herbicides. In the USA, arsenic is used in animal feed as a method of disease prevention and growth stimulation. In the computer industry, gallium arsenide is an important semiconductor material superior to silicon in integrated circuits.

1. 2.2: Geographical distribution:

Arsenic contaminated ground water is present in many areas of human habitation in all continents except Australia but differs greatly in level and size of population exposed. The countries most affected are in Asia but significant arsenic concentrations in water are also found in North America (USA, Canada) South America, Argentina, Bolivia, Brazil, Chile, Mexico and Peru, Africa (Ghana) and Europe (Greece, Hungary, Spain and UK). In Europe some 500000 persons are exposed to arsenic in drinking water at a concentration of 50µg/L or more. In Asia though Bangladesh and West Bengal (India) are more seriously affected, high levels are also found in China, Iran, Mongolia, Myanmar, Nepal, Pakistan, Taiwan, Thailand and Vietnam (World Bank, 2005). This well nigh ubiquitous distribution has, since the 1980s, gradually emerged as a major public health concern of greatest magnitude in Asia, most specifically in Bangladesh (East Bengal) and India (West Bengal) where hand pumped tube wells (TW) are the main source of drinking water in most rural areas (World Bank 2005). The fact that the water thus obtained was free from microbial pathogens led to the rapid development of these wells in the past 30-40 years, prompted and heavily supported by both governmental and non governmental agencies. Today it is estimated that up to 11 million TWs are in operation in Bangladesh alone, in a high proportion of which the level of inorganic arsenic exceeds the national standard of 50μg/L and the WHO recommendation of 10μg/L.

The extent of the problem was demonstrated in Bangladesh by an extensive systematic survey undertaken by the British Geological Survey (BGS) in collaboration with the Government of the Peoples Republic of Bangladesh, details of which were published in 2001 (BGS 2001). The survey was based on water analysis from 3534 tube wells systematically selected from a stratified random sample of one well per 37 Km^2. In addition 243 samples were tested from three selected study areas. Although the global and regional pattern of arsenic concentration is relatively clear cut (Appendix 1, 2) there is great local variation. Results from the special study areas showed a high degree of spatial variability with concentrations varying over four orders of magnitude (<3 to 986 μg/L, <3 to 1460μg/L, <3 to 2342 μg/L in the 3 areas). Similar variability was evident within most geographical areas in the general survey, with resulting difficulty in prediction of whether a further well close to a tested well would provide the same result (BGS 2001). However a clear regional pattern of arsenic concentration was evident with the south and the south east part of the country mostly affected and the north west and uplifted central part least so (BGS 2001).

Investigation of the geochemical and geographical distribution pattern of arsenic in ground water showed that most shallow alluvial aquifers in the holocene plain lands of the Ganges delta were vulnerable to arsenic concentration (Ahmed et al, 2004). The Ganges, Brahmaputra and Meghna river systems transport a huge amount of sediment, forming one of the largest sedimentary basins in the world. This sediment, in places more than 16km thick, is derived from the Himalayan and Indo-Burma range (Nordstrom 2002). Bangladesh itself is formed from three major geomorphological units-tertiary hills, pleistocene uplands and holocene plains (Nordstrom 2002). The shallow aquifers of the holocene plains, except in lake areas (haors), are all moderate to seriously contaminated by arsenic. In these regions, geologically young sediments forming low lying flat, closed basins with sluggish ground water, are the factors associated with high arsenic concentration.

There is as yet no consensus, about the precise cause of the high ground water concentration of arsenic in Bangladesh but several plausible mechanisms have been described. The geochemical pathway for concentration differs with geothermal conditions. In non mining areas, with low temperature ground water, a strong reducing environment leads to arsenic desorption from ferrous oxyhydroxide and water concentration. In confined aquifers, iron compounds may be oxidized to release arsenic in a pH (>8) condition (Smedley and Kinniburgh 2002). Akai et al (2004) has described the positive role of microbes in releasing arsenic in a reducing environment. Young sediments, characterized by organic matter and bacteria, ultimately influence the release of arsenic from ferrous compounds. Iron compounds are thus commonly claimed as the source of arsenic in ground water (Smedley and Kinniburgh 2002).

Globally arsenic concentration of ground water would thus seem essentially accidental, anthropogenic or geogenic. In the Bengal basin, it was thought to be anthropogenic, with mention of fertilizers, chromated copper arsenate (CCA) treated timber and pesticides as the source of arsenic concentration. Gradually however suspicion has shifted to the geogenic origin of arsenic. Recently, it was suggested that the concentration could be triggered by two specific factors in agriculture: the over exploitation of water from upper aquifers for irrigation and the use of phosphate fertilizer.

There has been little discussion concerning the demographic distribution of arsenic in drinking water. While the Ganga, Brahmaputra-Meghna (GBM) basin may not be larger than many other geographical areas with high arsenic concentration of ground water else where in the world, some

60 million people in this region are exposed out of approximately 75million people globally. In addition, in other regions populations may not rely totally on contaminated well water for drinking, whereas that is the fact for those on the banks of the GBM. There arsenic concentration of ground water has probably created the largest mass poisoning in history.

1.2.3: Toxicology:

Human exposure to arsenic varies with route, media and type of compound. It may be brief or continuous, accidental, occupational, or environmental and may be by inhalation, ingestion or dermal contact. Having regard for the objective of the present research undertaken, the toxicology of chronic arsenic will be limited to exposure by ingestion only. From a single dose of dissolved arsenic in the form of arsenite (As^{III}) or arsenate (As^{IV}), 70%-90% is absorbed. The rate of absorption is rapid as it is followed quickly by a high concentration of arsenic in urine. After absorption, arsenate (As^{IV}) is rapidly reduced to arsenite and as such the subsequent distribution of any other arsenic species follows the course of arsenite. Arsenite is then bound with SH groups in protein and low molecular weight compounds such as glutathione, and cystine in different organs of the body. Most of the arsenic in blood is rapidly cleared following an exponential clearance curve. Half life in the first phase is 2-3 hours, but in the second and third phase it is some 200 hours. In experimental animals, including mammals, arsenic is retained, depending on species, in the squamous epithelium of the upper gastrointestinal tract (GIT), skin, hair, liver, kidney, blood, epididymis, thyroid, skeleton and lens.

In man, the relative amount of different species of arsenic in the urine are 10-30% inorganic arsenic, 10-20% mono methyl arsenic $(MMA)_{(total)}$ and 60-80% dimethyl arsenic $DMA_{(total)}$. The major methylated metabolites in human urine are MMA^{V} and DMA^{V} with arsenic in pentavalent state. The mechanism of methylation is not clearly understood, although S-adenosylmethionine (SAM) seems to be the main methyl donor. Considerable intra individual variation in arsenic metabolism is reported though an individual's efficiency remains stable over time. The major route for excretion of most arsenic compounds is via the urine. The biological half life of inorganic arsenic is about 4 days, but shorter following exposure to arsenate than to arsenite.

In human beings, both inorganic arsenic and its methylated metabolites cross the placenta to the fetus. For pregnant women exposed to arsenic in drinking water at 200 ppb, the umbilical cord quickly reaches almost the same level (in late gestation). More than 90% of the arsenic in the urine and blood in the mother and fetus are in DMA compared to 70% in non pregnant women indicating an increase in the arsenic methylation capacity during pregnancy.

Acute toxicity after ingestion of high doses of inorganic arsenic, mainly As^{III}, is well documented. Cholera-like symptoms marked by diarrhea, blood tinged stool and vomiting are related to profound gastrointestinal lesions. Other symptoms and signs of acute toxicity are muscular cramps, facial odema and cardiac abnormalities.

Skin lesions are reported from many parts of the world as a manifestation of chronic arsenic toxicity but numerous studies have documented that chronic exposure adversely affects multiorgan systems. The non cancerous health effects of arsenic intoxication are insidious in onset and dependant on the magnitude of the dose and time course of exposure.

The skin lesions caused by chronic arsenic ingestion are characterized by pigmentation and keratosis. Arsenic induced hyperkeratosis appears mainly on the palms of the hand and the plantar aspect of the foot, although involvement of the dorsum of the extremities and trunk is also described. The lesions may be nodular or horny in appearance. The hyperpigmentation of chronic arsenic poisoning commonly appears in a finely freckled, raindrop pattern that is particularly pronounced on the trunk and extremities and is distributed bilaterally and symmetrically. It may also involve mucous membranes such as the under surface of the tongue and the buccal mucosa. Other pattern of skin pigmentation include diffuse hyperpigmentation or melanosis, localized or patchy pigmentation involving skin folds, and leukomelanosis in which the hypopigmented macules takes a spotty white appearance. The magnitude of dose, and the duration of exposure needed to induce skin lesions have been investigated only to a very limited scale. Studies relating to these effects and other manifestations of human exposure are reviewed in the following chapter of this thesis. Effects relevant to the situation in Bangladesh include, in addition to the skin lesions described above, skin cancers, other malignancies (particularly lung and bladder), non-malignant respiratory disease, reproductive outcomes and intellectual development.

1.2.4: Impact and reactions to the arsenic problem in Bangladesh:

Bangladesh is a low income country, long known for socio economic and political uncertainties (Unicef 2009). About 41 percent of the population lives on less than a dollar a day. Natural calamities, especially floods and cyclones, often dominate the headlines of the world press. Political and governance problems are generally considered the major concerns, and climate change, especially rising sea levels, is forecast to affect much of Bangladesh over the next few decades.

The nutrition and health of the population are far below any acceptable level. The most recent Bangladesh Demographic and Health Survey (BDHS 2004) gives annual birth and death rates of 26/1000 and 8/1000 respectively. Infant mortality, though much improved since the nineties, remains at 52/1000 live births (Unicef 2009) and the maternal mortality ratio at 3.2/1000 live births. About 48% of children under five suffer from chronic malnutrition. Though Bangladesh has a fairly comprehensive governmental primary health care infrastructure, it functions poorly because of technical, management and financial deficiencies. In 2002, there was one physician per approximately 4500 population, one nurse per 8500 population and one hospital bed (in the public sector) per 6500 population (Statistical Year Book of Bangladesh, 2002). The country's vital statistics are far from complete or reliable, and there is no registry for cancer or any other disease.

Non governmental organizations (NGOs) play some role in delivering primary care, especially for mothers and children. The Bangladesh Rural Advancement Committee (BRAC) operates a chain of over a hundred primary care centers in various peripheral towns, and is also involved in tuberculosis treatment and prevention, and in child nutrition programs, and so on. The Grameen Kalayan Foundation, a sister concern of the famous micro-credit known Grameen Bank, also provides rudimentary health care services through rural clinics. The International Center for Diarrheal Diseases (ICDDR,B) is the country's leading health, population and nutrition research center, and provides technical assistance and policy support to the Ministry of Health and Family Welfare (MOHFW). ICDDR,B also operates the largest diarrheal diseases hospitals, in Dhaka and in Matlab. Apart from these large NGOs, small NGOs provide services in many rural areas through small outpatient services. For example, Gonosasthaya Kendra (GK) works in over 600 villages, with over a million population. In these villages, trained paramedics provide comprehensive health care and have been gathering mortality and birth data for many years. Using the data from the BGS, it is possible to examine the exposure outcome of arsenic on different health aspects in GK villages, and a number of studies on skin lesion prevalence in relation to exposure have been completed (McDonald et al 2006, 2007).

Bangladesh first became aware of the arsenic problem from the news media in West Bengal in the mid nineties. In fact, the first case with skin lesions from arsenic in Bangladesh was diagnosed in Kolkata in 1984. Soon, arsenic in drinking water was recognized as a new problem but – being a country already overwhelmed by many other issues – unsurprisingly, Bangladesh could do little immediately to analyze the situation, due to a lack of financial and technical resources. At that time, an extensive mass campaign for tube well water promotion was in process. Countless tube wells were being installed, supported by government and international donors. However, the government acted quickly in collaboration with international agencies; particularly the UK's Department for International Development (DFID) and the DANIDA-

Danish International Development Agency. In 1996, the Bangladesh government initiated the most comprehensive national well survey ever undertaken, in collaboration with DFID, and the British Geological Survey (BGS) and almost 4000 wells were systematically assessed for arsenic and for 20 other metal contaminants (BGS, 2001).

Understanding the magnitude of the problem, Bangladesh set a water standard of 50 µg/L and began to achieve it (World Bank 2005). The first major arsenic mitigation effort undertaken by the Department of Public Health Engineering (DPHE) was to screen and mark every well at the opening with a green or red colour, according to the 50 µg/L standard, with those exceeding the standard being marked in red. An NGO forum was formed for water and sanitation to engage numerous small NGOs active at the local level. DPHE in collaboration with international agencies, and NGOs screened and marked a high proportion of tube wells in most rural areas, though the exact figure is not known. Various qualitative field test kits were used, and many field workers and volunteers were given training for this enormous task.

Further arsenic-related work in Bangladesh falls under two main headings, not readily summarized. First is the effort to find alternative sources of drinking water which are arsenic-free. Possibilities include the collection of rain water, deep wells into uncontaminated aquifers and various methods of filtration and treatment. None of these seem broadly effective at a manageable cost. The second approach, also unlikely to be entirely successful, involves the testing or retesting of all wells, and closing those with an arsenic concentration above 50μg/L. Justification for this approach clearly requires considerably more epidemiological information than is currently available, not only regarding skin lesions but also lung and other cancers.

DPHE also undertook research, in collaboration with local and foreign institutions, to explore the arsenic concentration behavior throughout the country. The Word Health Organization researched the effect of vitamins and micronutrients on arsenic-induced skin lesions. More recently, ICDDR,B, Columbia University, and BRAC have also begun arsenic research projects.

The research activities on arsenic have thus, so far, focused on the well survey and on the development and testing of alternative water options. There has now been some research on arsenic-induced skin lesions, although the cancer concerns have so far been little studied.

Translating research into action has become a priority for the country. The safety of the Bangladesh standard of arsenic in drinking water has yet to be established by epidemiological research. The possible health effects of long term exposure to arsenic in drinking water need to be defined, and the effectiveness of the alternative water options will require further study.

Chapter 2. LITERATURE REVIEW

2.1: Literature search strategy:

A comprehensive literature search was made to ensure a complete list of publications relating to the epidemiology of arsenic-induced skin lesions in Bangladesh and West Bengal. The search engines used were Medline, Academic OneFile, Global Health, and Google, focusing on “arsenic, drinking water and skin lesions in Bangladesh and West Bengal, India”. A number of additional papers were also identified from the reference list of the already accessed papers. All papers published in English in peer reviewed journals before October 2008, available with full texts, were reviewed except for those from Taiwan, where the arsenic related “black foot” disease, with a high rate of gangrene and amputation (Tseng, 2002) bore little clinical resemblance to the skin lesions in Bangladesh.

2.2: Reports from Bangladesh and West Bengal, India:

The first case of arsenic-induced skin lesions from Bangladesh was said to have been diagnosed in a Khulna district resident by Dr. KC Saha in Kolkata in December 1984 *(*Saha 2002*).* Epidemiological studies initially from four districts in Bangladesh (Tondel et al., 1999) but mainly from the Matlab study area of ICDDR,B , were published in 2006 (Rahman M et al.2006a, 2006b), 2007 (Hore et al.), and in 2008 (Lindberg et al.).From West Bengal there were early studies published 1998 (Guha Mazumder) in 2003 (Haque et al.) and, looking at diet, in 2004 (Mitra et al.).From Araihazar, Bangladesh there was a series of three reports based on a large cohort study published in 2006 (Ahsan et al.), 2007 (Argos et al.) and, on respiratory effects, in 2008 (Parvez et al.). Three papers based on villages under the care of GK included two on skin lesions (McDonald et al., 2006, 2007) and one on stillbirth (Cherry et al. 2008), This group also published on arsenic and lung cancer (Mostafa et al, 2008) Ahmad et al. published on both skin lesions (1999) and pregnancy outcomes (2001). Milton et al. published on respiratory effects in 2002 and on skin lesions, nutritional status and arsenic in 2004. Other reports on skin lesions and arsenic in Bangladesh were published from Pabna, looking at diet, by McCarty et al. in 2006; and a clinical study from Jessore by Kurokawa et al. in 2001. Chowdhury, in a paper published in 2000, reviews the work of his group in studies in both West Bengal and Bangladesh. Finally recent papers from 6 other countries are relevant though less so; Nepal in 2005 and 2006(Maharjan et al.), Chile (Smith et al, 2000), Turkey (Dogan et al 2005) the USA in 2005 (Tollestrup et al.) Inner Mongolia, China in 2001 (Guo et al.) and in 2006 (Guo et al.), in Iran in 2007 (Mosaferi et al.),

These papers will now be described individually, though retaining the identified groups of investigators.

Tondel et al. (1999) carried out a skin lesion prevalence survey in four villages from four districts in Bangladesh. The villages were selected with arsenic concentrations ranging from 10 to 2040μg/L. 1794 individuals – aged ≥30 years, who had lived in their villages all their life and who had used the same well as long as it had existed – were identified for the study by door-to-door visits. The well test results were obtained from an arsenic survey by the National Institute of Preventive and Social Medicine. After exclusion of 199 persons not available for interview and 141 not exposed at a high level, 1481 individuals (903 male and 578 female) were interviewed and examined by two experienced physicians, who identified 430 cases. Arsenical skin lesions were defined as pigmentation on an unexposed body surface and/or keratosis on the palm or sole. The dose index (μg/L) used was the arsenic level in the well divided by body weight of the subject in kg. It was categorized in three groups: <5 μg/L/Kg, 5-10 μg/L/Kg, and >μg/L/Kg. The estimated overall crude prevalence rate of skin lesion was 29% and log linear regression showed a significant trend for both sex with relative risk of 1.55 per 1000 μg/L for male and 1.42 per 1000 μg for female. The age-adjusted prevalence rate for female was 19.7% in the lowest exposure category and 30.8% in the highest category. For males, it was 19.6% and 34.8% respectively.

Rahman et al. (2006a) conducted the largest population-based survey for arsenical skin lesions in Bangladesh in the ongoing comprehensive health and demographic surveillance system of ICDDR,B in Matlab. This area is one of the highest contaminated areas within the country. In 142 villages, from a total population of 220,000, the study included 180,811 subjects, who stated that they drank water from local wells at least once a week. 166,934 individuals were available for initial screening and interview at their home by the field workers. Cases of hyperpigmentation and of bilateral keratosis cases initially identified by the field workers were referred to a research physician at a static clinic. The research physician made the final diagnosis, and cases were photographed. These photographs were independently reviewed by two dermatologists. There was agreement among these experts and physicians that 504 cases had skin lesions caused by arsenic.

Water samples from 13,286 wells out of 16,430 wells were tested later using HG-AAS technology. For any tube well now destroyed, the village mean was used as a proxy measure. For any tube wells out of the Matlab area, the BGS data were used as a proxy indicator. Cumulative exposure was estimated from the residential history and data on water source levels.

A crude prevalence of skin lesions of 3/1000 was found. Pigmentation was the most common skin lesion, present in 39% of all cases; 5.1% had keratosis only; and 56% had both hyperpigmentation and keratosis. Men were more at risk (standard morbidity ratio of men: women, 1.56:1). The highest prevalence of skin lesions were noted in the 35-44 age group in both sex. A positive association for the cases was found with higher socioeconomic status and educational attainment.

Rahman et al. (2006b) conducted a case-referent study nested in the cross-sectional survey of Tondel et al. (1999). This population-based case-referent study included the 504 cases identified from Matlab. Targeting a case-control ratio of 1:2, 1830 unmatched referents from the study population were interviewed. The mean level of arsenic exposure was 200µg/L and 211µg/L for male and female cases respectively, and 143µg/L and 155µg/L for male and female controls respectively. A significant dose-response relationship was found in lesions for each sex. Males were at a higher risk of skin lesions than women; most prominent (OR 10.9 for male and 5.8 for female) in the highest exposure quintile.

Hore et al. (2007) evaluated the case selection process in the studies of Rahman et al. (2006a) and Rahman et al. (2006b). Of the 1682 cases initially selected by the field workers, the study physicians diagnosed 579 (30%) as probable cases. These 579 were photographed and independently assessed by two expert dermatologists; final agreement was reached in 504 cases. A good diagnostic agreement between study physicians and the experts was found, with an overall probability of correct diagnosis of 87% (94% in females and 82% in males).

Lindberg et al. (2008) studied the methylation pattern of arsenic compounds in urine among arsenicosis cases in the Matlab area (Rahman et al. 2006b). A higher arsenic methylation capacity in women than in men was suggested as a possible explanation for the higher rates in men of arsenic-induced skin lesions.

Guha Mazumder et al. (1998) conducted the first large scale population-based, arsenic-induced skin lesion survey in West Bengal, India. Prevalence was assessed in a “convenience” sample of 7683 subjects - 4093 female and 3590 male - in 25 high exposure villages and 32 low exposure villages. Arsenic levels ranged from zero to 3400µg/L;80% of the population were exposed to >500µg/L. Study participants were interviewed by the field workers about source of drinking water, smoking, diet, water intake, medical symptoms, weight and height. One of two study physicians examined the subjects, who had initially been screened by field workers for evidence of bilateral keratoses and pigmented skin lesions. Samples of drinking and cooking water were collected and tested using atomic absorption spectrophotometer-hydride generation (ASS-HG) technology. A daily dose/kg body weight was calculated on the basis of water consumption.

Of 644 wells tested for arsenic in the study area, 43% exceeded 50µg/L. The average prevalence of keratotic skin lesions was 1.2% in women and 3% in men, but increased with dose per kg body weight (divided in three tertiles: <3.2µg/kg lowest tertile to 14.9µg/kg highest tertile). The prevalence of keratosis was 0.8% for both men and women, but in the highest tertile went up to 3.5% and 11% for men and women respectively. The prevalence of hyperpigmentation was 3.1% and 6.4% for men and women, again with a dose-response relationship. The mean drinking water concentration for subjects with skin lesions was 640µg/L, and 210µg/L for the controls

Large sample size and population-based design were important strengths of this study. As it was conducted in 1995-96, just when the problem first emerged in Bengal, it was not likely to have been affected by well-switching. All the subjects were examined by two physicians with long experience in arsenic-induced skin lesions. As the well water samples were tested after the interview and examination, there was one possible bias.

The result of the study was adjusted for age, sex, smoking habit and socioeconomic status (though how socioeconomic status and smoking habit were classified was not elaborated).

Haque et al. (2003) conducted a case-control study, nested in the skin lesion prevalence survey of Mazumder et al. (1998), limited to subjects exposed to <500 µg/L of arsenic. 265 cases and a similar number of age- (± 5years) and sex matched controls were identified, of which 192 cases and 213 controls were finally interviewed. Of the 192 cases, 72 did not have skin lesions at the time of the data collection, whereas 25 of the 213 controls did. A physician collected information from the study subjects at home concerning lifetime residential history, present and past water sources at home and work, fluid consumption pattern, smoking habits and socioeconomic characteristics. Skin lesions were photographed for some cases for expert review, with 87% agreement between the field diagnosis and the review by the dermatologist.

Estimates of latency for 42 cases with skin lesions and for whom water samples from all known sources could be collected yielded an average of 19 years, with a range of 3-42 years. The peak arsenic concentration for cases was 325 µg/L and for controls 183 µg/L. Of 69 cases with a complete water history, all had peak arsenic concentrations of 100 µg/L or higher. Conditional logistic analysis of age- and sex matched pairs, and the unconditional logistic analysis of all 405 study participants, demonstrated a clear trend of increasing risk by peak and average arsenic concentrations. In the unconditional multivariate analysis, age, sex, smoking habit and socioeconomic status were not associated with skin lesions. In the adjusted matched analysis, an OR of 16.3 in the highest exposure category with reference to <50μg/L exposure was reported.

Mitra et al. (2004) published a further analysis of the case-control study of Haque et al. (2003), assessing the role of certain nutrients in relation to the risk of skin lesions. A significant risk was obtained with low intake of animal protein (OR=1.94, 95% CI 1.05-3.59), low calcium (OR=1.89, 95% CI 1.04-3.43) and low fiber (OR=2.20, 95% CI 1.15-4.21).

Ahsan et al. (2006) reported the baseline prevalence of skin lesions in the HEALS cohort (Health Effects of Arsenic Longitudinal Study) project in Bangladesh. The analysis was based on married persons between 18 and 75 years of age, resident in the project area for at least five years and who used one of 5966 designated wells. Of 14,828 eligible subjects, 11,746 were examined; 810 with premalignant skin lesions were initially identified; 96 were excluded after further clinical review as cases of solar or occupational keratosis. Of 714 cases of skin lesions, 421 (337 men and 84 women) had only melanosis, and 293 had both melanosis and keratosis. Compared to <8.1 μg/L of arsenic, adjusted prevalence odds ratios were 1.91 (95% CI 1.26-2.89) for 8.1-40.0 μg/L (3.69-7.86). Males were at higher risk (OR 4.15, 95% CI 3.27-5.26).

Argos et al. (2007) also analyzed the baseline survey data from the HEALS project to assess the relationship of socioeconomic variables with arsenic toxicity. From the source population (n=65,876), 11,438 persons (714 with skin lesions) – who had lived in the study area for at least 3 years, were married and aged 18-75 years – were included. Cumulative arsenic exposure was used in the analysis, which included several socioeconomic variables such as education, television ownership, land ownership, use of cooking oil and comprehensive food indices. Borderline significant association with arsenical lesions was reported only with land ownership status. It concluded that landless people were at particular risk.

Parvez et al. (2008) conducted a study of 128 cases and 113 referents from the HEALS project cohort, examining the effect of chronic arsenic poisoning through drinking water with lung injury. Subjects were non smokers, with blood and urine samples available. The study found an inverse relationship between primary methylation capacity, risk of skin lesions and adverse respiratory effects. In a small subset (n=31), cases were found with possible evidence of a reduced FEV_1 and FVC.

McDonald et al. (2006) carried out a representative assessment of prevalence and risk of arsenic-related skin lesions in rural Bangladesh. The survey was conducted in a stratified random sample of 53 villages, from some 600 villages served by GK in four of the six divisions of the country. 13,705 women aged 18 years or more were examined by village paramedics at their home. The study included only women because of their relatively more straightforward well use. Also, as the paramedics were all women, enquiries and examination about skin lesions – and examinations for keratotic skin lesions in palms and soles – were more convenient. For exposure assessment, the initial study used the mean arsenic concentration of the geographical units of upazilla published by the National Hydrochemical Survey (NHS). Average arsenic levels ranged from 5 μg/L in 13 villages (24.5%) to >50 μg/L in 7 villages. 98% of women had drunk water for about 20 years at their present address. The overall prevalence of skin lesions was 1.3%: 4/1000 at the lowest exposure level (<5 μg/L) 4/1000 at the 6-10 μg/L level; 7/1000 at 11-50 μg/L; and 69/1000 at >50 μg/L. The skin lesions recorded were more commonly nodular than thickening and more prevalent on palms than on the soles.

Using the same GK villages and study population, McDonald et al. (2007) further refined the ecological study using upazillas as the unit of analysis and – then using a case-referent design – analyzed data of 176 cases identified in the prevalence survey with age-, sex and village-matched controls.

53 study villages were drawn from 12 upazilla in four divisions. Average arsenic concentration of five upazilla was 1 μg/L, where the keratotic and

pigmented skin lesion prevalence was 3.7/1000. In four upazilla, the average arsenic level was 21 μg/L and skin lesion prevalence was 6.2/1000, and two upazilla were at 43 μg/L, with skin lesion prevalence 6.4/1000. In one upazilla at the level of 81 μg/L, the skin lesion prevalence was 68.4/1000. In the case control study, details of water history were collected from the subjects, and arsenic concentration was measured using a digital arsenator. One of the investigating physicians examined all the cases and controls for skin lesions. The analysis showed significant risk of skin lesions (OR 2.96, 95% CI 1.02-8.59) for exposures above 50 μg/L.

Cherry et al. (2008) analyzed a large dataset of pregnancy outcome data (n=30,984) in some 600 villages of the GK network. Arsenic concentration per upazilla, obtained from the National Hydrochemical Survey (NHS), was used as the exposure indicator. In the NHS, seven to 14 wells from each sub-district were tested to estimate the sub-district mean arsenic concentration. The overall stillbirth rate of the cohort was 3.4%: 2.96% at <10 μg/L, 3.79% at 10 - <50 μg/L, and 4.43% at ≥50 μg/L. The odds ratios calculated for increased risk of stillbirth in reference to <10 μg/L arsenic were 1.23 (95% CI 0.87-1.74) and 1.80 (95% CI 1.14-2.86) for 10-50 μg/L and >50 μg/L respectively. The risk estimates were adjusted for a number of potential confounders including maternal age, education, gestational age, birth weight, place of delivery etc. The study included pregnancies from 16 sub districts (upazilla). Mean concentration in nine sub-districts was <10 µg/L, five sub-districts <50 µg/L and two sub-districts ≥50 µg/L. The range of concentration was 1µg/L-81 µg/L.

Mostafa et al. (2008) compared the relative risk of primary lung cancer among 3223 cases and 1588 referents with non malignant lesions. Subjects were living in villages for more than 10 years and used tube well water. The district mean arsenic concentrations as published by the NHS were used to assess exposure. At higher concentration (>100 µg/L) the study reported significant risk of lung cancer among the smokers. An odds ratio of 1.65 (95% CI 1.16-1.8) was calculated for males at >100 µg/L In the analysis, 13.8% subjects were from the districts with <10 µg/L arsenic, 47.8% were from ≤50 µg/L and 38.4% were from >50 µg/L. In the study the highest district mean arsenic concentration was 366 µg/L.

In 1999, Ahmad et al. published an arsenicosis prevalence survey from a village in Jessore, South Western Bangladesh. Data was collected from 200 subjects. The dose of arsenic, estimated from level of well concentration and duration of use, was observed to be lower for female cases than in men with skin lesions.

A further study published by Ahmad et al. (2001) concerned pregnancy outcome. This small cross-sectional study compared the pregnancy results of 192 women, half from an exposed area (≥10 μg/L), and the other half from a non-exposed area (≤10 μg/L). Significantly high abortion, preterm and stillbirth rates were found among the exposed group.

Milton et al. (2002) undertook a prevalence comparison of respiratory effects in chronic arsenic poisoning in Bangladesh. 169 subjects participated, 44 with skin lesions. Among the 169, 20 reported symptoms of chronic bronchitis, with an increasing, but non-significant, increase in odds ratio with increasing arsenic concentration (to 1000 μg/L).

Milton AH et al. (2004) matched 138 cases with skin lesions from 3 high arsenic villages with 144 subjects from arsenic free villages. The mean arsenic concentrations were 641 and 13 μg/L. Cases were more likely than controls to have a low body mass index (<18.5) with an odds ratio of 1.92 (95% CI: 1.33-2.78)

McCarty et al. (2006) also examined the modifying role of dietary elements on arsenical skin lesion risk. The case-referent analysis included 600 cases and 600 age and sex matched referents from the Pabna district in Bangladesh. Subjects were at least 16 years of age and permanent residents of Pabna. 80% of the subjects were from low- and 20% from high-concentration areas. A physician who was blinded to the well water concentration made the diagnosis. Monthly frequency for fruits, beef and milk consumption, and weekly frequency for other dietary elements except rice, were collected. The analysis did not find any significant relationship for beef, fowl, fish, milk, egg, vegetables or rice with risk of lesions. Increased consumption of fruits (OR=0.68, 95% CI: 0.51-0.89) was found to have a protective effect, and bean consumption (OR=1.89, 95% CI: 1.11-3.22) was associated with increased risk.

Kurokawa et al. (2001) described skin lesions in a group of cases in a highly contaminated village in Jessore, Bangladesh. In a population of 3555, 135 suspected cases, identified by local health workers, were examined by a group of Japanese dermatologists. Among the 135 there were 132 with hyperpigmentation and 128 had hyperkeratosis on the feet. 23 were thought, on clinic examination to have malignant lesions, of which 21 were early stage. One subject had multiple basal carcinomas and a second a squamous cell carcinoma.

Chowdhury et al. (2000) published commentaries on their work on health effects of arsenic in drinking water in West Bengal (for ten years from 1989) and in Bangladesh (for four years from 1995) Analysis of 12,135 water samples from tube wells from Bangladesh and 58,166 water samples from West Bengal showed that 34% wells in West Bengal and 59% in Bangladesh were contaminated at >50 μg/L. They identified contaminated wells in 42 districts in Bangladesh and in nine districts in

West Bengal. In 27 out of 42 arsenic-affected districts in Bangladesh and in seven of nine such districts in West Bengal, they examined 11,180 and 29,035 persons respectively, and found 24% and 15% respectively had skin lesions. Details of sample selection and examination procedure were not available in the paper. Keratosis and/or pigmentation change anywhere in the body was accepted as a skin lesion.

2.3: Reports from other countries:

Nepal

Maharajan et al. (2005) reported results of a prevalence survey in a highly contaminated area in the Terai region of Nepal. 1343 persons (80% of the population), aged 15 years or more, volunteered to be examined for any arsenical lesion by a physician. Detailed examination and sampling procedures were not described. 146 wells used by the subjects were tested for arsenic, and urine samples were also tested. The arsenic levels in wells ranged from 3 μg/L to 1072 μg/L; 87.6% of wells were >50 μg/L. The prevalence of skin lesions was 6.9% overall, though much higher in males (9.3%) than in females (4.4%). Although specific concentrations were not mentioned, a dose-response relationship was observed.

A further study (Maharjan et al, 2006) reported results from 6 villages in the Terai. The prevalence of arsenicosis was highest for both men and women in those >50 years with the prevalence again being higher in men.

Chile

Smith et al. (2000) published prevalence of skin lesions related to arsenic exposure in Chile. 44 members of 11 indigenous families exposed to a high level of arsenic in drinking water, and 31 members from 8 families who were not thus exposed, were studied for skin lesions. All the study subjects were examined by physicians who were blinded to the level of water concentration. Four of the six men, who had been exposed for more than 20 years – but none of the women – presented with arsenic-induced skin lesions. The study suggested that arsenic-induced skin lesions may occur despite thousands of years of potential exposure and despite good nutritional status.

Turkey

Dogan et al. (2005) reported the incidence of skin lesions from a small area in Turkey, contaminated by naturally occurring arsenic in water. A cross-sectional survey was conducted among 153 individuals in two villages, each contaminated at a low and a high level respectively. Concentration was 0.3-0.5 mg/L (300-500 μg/L) and 8.9-9.3 mg/L (8900-9300 μg/L) respectively in the two villages. Subjects were 25 years or older; 48.6% were male and all of them were smokers. Details on the sample selection procedure, and on who undertook the clinical examinations, were not available. It was found that 30.6% individuals in the highly contaminated village, and 5.41% individuals in the other, had arsenical skin lesions. They described a wide range of skin lesions, but the most common was palmoplanter keratosis.

USA

Tollestrup et al. (2005) assessed arsenical skin lesions in the USA. Populations living in the south west may be exposed to high levels of arsenic in drinking water. Tollestrup et al. sent simple postcard questionnaires to 240 dermatologists in the region to determine the frequency of arsenic-induced hyperkeratosis and pigmentation cases seen in the previous year and in the past ten years. 37 dermatologists responded that they had seen 237 cases in the past ten years and 35 cases in the previous year.

Inner Mongolia

Guo et al. (2001) reported that 26% of all the wells of the Hetao plain area of Inner Mongolia are contaminated at ≥50 μg/L of arsenic. The authors conducted a well survey and skin lesion prevalence survey in two areas. In one area, 96% of wells exceeded 50 μg/L and in the other area 69%. The highest arsenic levels were 1354 μg/L and 1088 μg/L respectively. The study reported a 45% prevalence of dermatosis in one area and 37% in the other. In a subsequent paper, Guo et al. reported that an increasing concentration of arsenic in water was significantly related to an increased risk of pigmented lesions, but the association with hyperkeratosis was not significant.

Iran

Mosaferi et al. (2008) conducted a study in an arsenic-contaminated area in Bijar County, Kurdistan to determine the correlation between skin lesions and total lifetime ingestion of arsenic (TLIA). In eight villages, they selected 752 subjects aged ≥ 10 years, who had lived in the village for 10 years or more; 44% male and 56% female. The villages were classified into four categories of exposure: 1) no exposure <0.05 ppm, 2) low exposure: 0.05-0.15 ppm, 3) medium exposure 0.15-0.3 ppm, 4) high exposure >0.3 ppm. All the subjects were interviewed and examined at their home. For skin lesion diagnosis, the study referred to UN guidelines. Water concentration in the villages was monitored for one year, taking one sample each season to estimate an annual mean concentration for each village. Total lifetime ingestion of arsenic was estimated in gms from arsenic level in the source well, amount of water consumed per day and length of residence in the village. Hyperkeratosis in the medium and high exposure groups was found to be 4.9% and 15.4% respectively. The study concluded that TLIA seemed to be a reliable indicator of risk in an exposed population.

2.4: Malignant skin lesions:

The arsenic induced skin cancer burden is uncertain in Bangladesh and West Bengal, although it is accepted by IARC (2004) as causally related to arsenic in drinking water. In a dermatological survey of a high arsenic village in Bangladesh, Kurokawa (2001) reported 23 cases (21 early stage) of malignant skin lesions, in a village of 3555. In West Bengal, in a study population of 4865 from 1206 villages, followed for 16 years, 212 (4.3%) developed skin malignancies, 161 squamous cell and 51 cases reported as 'Bowen's disease or intraepidermal carcinoma'. (Saha 2001).

2.5: Summary of Chapter 2:

The literature reviewed suggests that arsenic in drinking water is associated with characteristic skin lesions, malignancies including lung and skin cancer, and stillbirth. Because of differences in research design there remains substantial uncertainty about the concentration of arsenic in drinking water that may be associated with skin lesions in Bangladesh and their relation to duration of exposure and age (or latency). The research reported here was designed to estimate the relationship between level of exposure and the occurrence of lesions and to identify possible modifying factors, to allow evidence-based policies aimed at reducing the adverse outcomes of contaminated tube well water.

Chapter 3: OBJECTIVES

The study was designed to address the following four specific objectives:

1) To determine the prevalence of skin lesions in the two study villages in relation to gender, age and concentration of arsenic in drinking water.

2) To estimate the relative risk of skin lesions in relation to levels of arsenic in drinking water when cases are matched to referents on gender, age and village.

3) To determine whether the relation between arsenic concentration and skin lesions was modified by factors such as completed years of education, BMI, dietary protein consumption, the amount of well water drunk and use of well water for cooking.

4) To investigate whether duration of exposure to arsenic through drinking water provides a better predictor of case status than current arsenic level alone.

Chapter 4. METHODS

4.1: Background of present study and study setting:

The study was conducted in a field area of Gonoshasthaya Kendra (GK), which had already participated as the field partner in our previous arsenic-research. GK is an NGO, well known nationally and internationally since 1970 for their contribution in developing and testing the concept of primary healthcare. GK currently provides comprehensive healthcare for the entire population of some 600 villages in four of the six divisions of Bangladesh, from static centers and by door to door visits by trained female paramedics (McDonald et al 2006).

In 2004, a skin lesion survey was conducted among all women aged more than 18 years in 53 GK villages, described above (McDonald et al 2006). GK had since become aware of an increased prevalence of skin lesions in the Kashinathpur area for which it was becoming responsible, and was anxious to have the situation investigated in detail. As a result of this interest, an initiative was taken in 2005 to investigate the situation systematically. GK and its staff were thus committed to assist in this research project, the methods of which are described below.

The proposed study setting was a cluster of two adjacent villages (namely Syedpur and Ahmedpur: (appendix 3) in Sujanagar sub-district, Pabna district, Rajshahi division in northern Bangladesh, 210km northwest of Dhaka. The area lies on the river Atrai, a branch of the river Jamuna, which lies about 2km from the villages. The geographical location of the area was selected in part because naturally occurring arsenic in Bangladesh originated in the Himalayas, and was dispersed through the main river systems (Rahman M et al 2006b). The study area lies on the Jamuna, a main river in Bangladesh. The population of the two villages is about 12000, for an area of approximately 10km^2.

Though high levels of arsenic were reported from most of the 60 districts in Bangladesh covered by the National Hydrochemical Survey, the southern and southwestern regions were the most affected (BGS 2001). However, there were reports of considerable variation in small local geographical areas, sometimes referred to as “hot spots”, with exceptionally high arsenic groundwater levels. The study area was suspected of including one such area, though very few of the wells had actually been tested there.

The following three studies were conducted in the selected study area:

A. A survey of all wells in the area to measure the levels and distribution of inorganic arsenic in drinking water used by the inhabitants.

B. A comprehensive skin lesion prevalence survey.

C. A case referent study to examine the relative risk of arsenic-related skin lesions in relation to arsenic in drinking water and in context of protein consumption, education and other factors

4.2: Well survey:

Several types of wells were used for drinking water in the study area, including hand pumped tube wells, dug wells and wells with pumped water storage provision: this last we termed" supply water". Altogether 1508 functioning wells in the two villages were systematically tested for arsenic during May 2006 to August 2006 using the following procedure.

4.2.1: Listing and marking:

First, all the wells were listed according to their GPS location irrespective of type and functional status. All the tube wells with head present and dug wells with surrounding walls intact were given a unique identification number, handwritten by paint on a prominent point on the well. The identification numbers were prefixed with the initial of the village name (S for Syedpur and A for Ahmedpur). After completing the first round of well marking and numbering, a second search was done for any missed wells. Some were found, marked and numbered in the same manner.

4.2.2: Well data recording instrument:

An instrument was developed in English, pre-tested and finalized for well data recording (appendix 4). The data recorded included well location, household name, two GPS coordinates, and the results of three water sample tests (direct samples and diluted samples) and any additional relevant information.

4.2.3: GPS reading of all the wells:

The well number and village initial painted on each well was matched with that on the well list, GA (family cluster), and PA (family) number. First, GPS coordinates at the point of the handle of the well were recorded. Then moving thirty steps away from that point, the GPS coordinates were again recorded for comparison. If the well was in a covered place or the satellite signal was low, the coordinates were taken standing at the doorway of the well or from the nearest point with a good satellite signal. GPS readings were recorded immediately on a card labeled with the well number.

GPS readings for dug wells were recorded as close as possible to the well. For supply water, GPS coordinates were recorded in a similar manner.

4.2.4: Well water testing:

Arsenic concentration in wells used for drinking water was measured by a field test instrument (the Arsenator, developed by Wagtech International. Berkshire, UK) which provided a digital read-out of arsenic concentration. Arsenetor has been used for arsenic testing in Bangladesh and Myanmar (WHO 2003). As a field based instrument, it was evaluated in Myanmar and gave an above average consistency (Swash 2003). The Pearson correlation between the silver diethyldithiocarbamate method and the Wagtech digital arsenator was found to be 0.87 for arsenic concentrations in the range 0-100 μg/L (Sankaramakrishnan 2008). The overall performance of the Wagtech arsenator was rated as excellent by the Shri Ram Institute for Industrial Research (Shriram Institute 2006). The method had been used by the British Geological Survey (BGS 2001) alongside samples later analyzed by AAS, with a good concordance recorded.

Wagtech field kit model SE 10500s with sufficient refills and other supplies were taken to the field every day on a rickshaw van. This van served as a table for water testing, and placed at a convenient location close to the wells. Provision was made to protect the testing table in the event of adverse weather.

For tube well water sample collection, water was at first expelled by 20 full pumps. The sample collection vessel - an empty 600ml mineral water bottle (MUM brand) marked with the well number in water-insoluble ink - was first washed with water from the same well. A full bottle of water was then collected and closed, and quickly transported to the test table. For dug well sample collection, a glass bottle tied to the end of a rope was thrown into the well and allowed to go to one foot below the surface. A full bottle was then pulled out. For collecting supply water samples, we allowed the water to drain for 10 seconds through the tap and then collected the sample.

Three different samples were collected from each well and tested without dilution using the Wagtech field kit strictly following the manufacturer's testing methods. The investigator (NH) tested all the well samples personally, with a few exceptions. If any visual or digital test result was found equal to or more than 100μg/L, three additional samples were tested at five times dilution using measuring cylinder. For dilution purpose, MUM bottled water (sold as arsenic free) was used, which was consistently arsenic-free in our tests. The dilution process was repeated until all three test results (visual and digital) were below 100μg/L. The results were immediately recorded on the well card. If any problem in the testing process was noted, that test was repeated.

4.2.5: Quality monitoring of well survey:

All the wells were tested systematically following an explicit guideline, including those of the manufacturer. The investigator (NH), himself trained by the kit manufacturer and the department of public health engineering (DPHE) did all but a few of the tests. Tests were done in daylight to avoid any error in reading. Any problem in testing was immediately discussed with the study supervisor. The well cards were all kept in a safe place for later cross-checking if necessary. The test results were checked by the investigator one by one for completeness, clarity and consistency. A code book with instructions was then prepared for well data entry (appendix 5).

4.2.6: Well data entry:

The well test results were entered in a spreadsheet (SPSS version 12) by experienced data enterers. The complete spreadsheet was printed and finally checked against each well data card one by one.

4.2.7: Well data analysis:

Descriptive analysis of the arsenic concentration of well water was done using SPSS. Three final concentrations for each well were calculated, multiplying by the dilution factor where applicable. Mean, median, highest and lowest concentrations were calculated from the 3 values for each well. Correlations of three individual tests results of water samples were computed. The distribution of the different summary measures of arsenic concentration was also examined. Well test results were compared by villages and types of wells. Histogram, box plots, scatter plots and probability plots were prepared to help visualize the data.

Test results (both visual and digital) from the well data file were later merged with the prevalence survey and case-referent data files using the well identification number as the key variable.

4.2.8: Ethics in well survey:

Before well testing, the permission of the well owner was obtained. After completion of testing of all the wells in the village, the results were reported to the well owner in writing, as required by GK and the government of Bangladesh.

4.3: Skin lesion prevalence survey:

A comprehensive skin lesion prevalence survey was conducted in 2005-6 in the study area.

4.3.1: Population listing:

After defining the geographical boundary of the study setting, each person in these two villages was listed. All families in the study area were identified by a unique family number (PA) and family group number (GA) according to GK policy. At the outset, three GK paramedics collected detailed population data through home visits. They gathered name, age, gender and serial number of all family members by talking to the head of the family or any informed family member present at the time of a door to door visit. The data were then entered into a Word file to make a complete list of the population in the study area.

4.3.2 Data collection instrument:

A data collection instrument (appendix 6) was then developed to gather information on thickened and nodular lesions on the palms and soles as a prevalence measure of skin lesions for the persons listed. It included recorded information on how long the family head had drunk water from a tube well, and the tube well mostly used currently. The data collection instrument was pre-tested and finalized. It was then printed on a card with family number, group number, family members' name, gender and age inserted from the Word file using mail merge. The data collection instrument was initially developed in English and then translated to Bangla and again translated back to English by a second person to check consistency.

4.3.3: Interviewer recruitment and training:

Three interviewers were recruited, all GK trained paramedics, who were given hands-on practical experience in field data collection with demonstration of cases of thickening and nodular lesions on the palm and/or sole. The experience included training in interview and physical examination of five subjects. The investigator (NH) provided the training and supervised the data collection throughout.

4.3.4: Skin lesion prevalence survey:

Initially it was planned that three paramedics would work in three different areas of the villages starting from Syedpur village. But in the field, the three paramedics worked together because of security and other conveniences (transport, food etc). They went together to a point and from there worked in three directions.

The paramedic then talked to the head of the family or other responsible person present during the visit and collected information about the name of the family head, the name of the landlord (if the family was a tenant), how long the family head had drunk water from a tube well and the tube well currently used most often. The information was immediately recorded on the card.

4.3.5: Skin lesion examination:

After identifying individual family members, each subject was requested to wash their hands and feet (if soiled); these were then examined in daylight in a comfortable private environment. The presence, number and location (palm, sole) of any thickened or nodular lesion was immediately recorded on the card, the number of lesions on the most affected palm / sole being selected. For any child under five, the mother was asked about any skin lesion on the palm or sole. Paramedics were instructed to examine those children if their mother reported any lesion.

4.3.6: Exact location of individual households:

To complement the skin lesion prevalence study, at a later date, during January 2006 to March 2006, the global positioning of each household was recorded with a hand held GPS machine. This was used to determine each individual household's geographic coordinates. The interviewers went door to door with prevalence survey cards for this purpose. At first each household was identified by village and para (hamlet within the village), family number, group number, full name of family head, and other family members' names. The GPS readings were at the front door of the house. If that location was covered or the satellite signal was obstructed, the reading was taken from the nearest point at which a reading could be made. Two GPS readings were taken; after taking the first, the data collector moved thirty steps in any direction. He or she would then return to the same spot, and take the second reading. The GPS readings were delayed if the machine showed a deviation of more than 100 feet. All the prevalence survey cards were stamped with a record of the two GPS readings. These data were also entered in the prevalence survey family file.

4.3.7: Severity of skin lesions:

The severity of skin lesions were defined for analysis as follows:

No lesion

Thickening only:	Thickening (keratosis) only on palm and/or sole.
1-2 nodules:	Presence of 1-2 nodules on palm and/or sole with or without thickening
3+ nodules:	Presence of three or more nodules on palm and/or sole with or without thickening.

4.3.8: Missing cases:

If the family or any member of the family was not found on the first visit, information on their whereabouts was recorded from a member of the family or a neighbor. Families or family members who were expected to be back shortly were visited again at least three times at later dates. If someone was abroad or living elsewhere, this was recorded and no further attempt to meet them was made.

4.3.9: Monitoring and supervision:

The data collection process was systematically monitored, supervised and recorded as part of the research management and quality assurance plan. The investigator (NH) was personally responsible for this throughout. Progress and field problems were discussed from time to time with the thesis supervisor at the University of Alberta, who visited Bangladesh on several occasions during the fieldwork.

4.3.10: Data cleaning and entry:

All the data survey cards were checked by the investigator (NH) for completeness and consistency. A code book was then developed (appendix 7) for data entry and the data recorded on an Excel spreadsheet, in two files: individual-specific and family-specific. Computerized data were checked item by item on the spreadsheet, then checked on the computer for any inconsistent values. Data entry was by two experienced assistants.

4.3.11: Analysis of prevalence survey data:

Key variables:

Dependant variable:

Skin lesion status or case status (yes/no), categorical.

Independent variables:

Key exposure variable:

Total arsenic in the well water as measured by the digital arsenator: (Independent continuous variable) was used as a categorical variable in the analysis. To determine village wise and overall prevalence calculation, exposure variable was categorized as <10 μg/L (WHO standard value,); 11-50 μg/L (Bangladesh standard value); 51-100 μg/L, 101-500 μg/L and

>500 μg/L. In other analysis exposure variable was categorized as ≤50 μg/L, 51-100 μg/L, 101-500 μg/L and >500 μg/L. Three water test results for every well were available; in the analysis, the median of the three was used in categories.

Gender:

Gender was treated as a categorical independent variable.

Age:

Age was treated as an independent, as a categorical variable. To describe the village population and overall prevalence rate, in the descriptive analysis it was categorized as ≤5years, 6-18 years, 19-30 years, 31-50 years, 51-70 years, ≥71 years. In the logistic regression and chi square analysis it was categorized as 6-18 years, 19-30 years, 31-50 years, >50 years.

Village:

Used as a categorical variable.

Severity of lesions:

According to the presence of thickening and number of nodules, severity of skin lesions were categorized as no lesions, thickening only, 1-2 nodules and 3+ nodules.

4.3.12: Statistical analysis:

Prevalence survey data were finally transferred to SPSS version 16 files for analysis. Descriptive analysis of the study population was done. Chi square statistics were used to compare skin lesion prevalence and severity by gender, village, age group and arsenic concentration. Unconditional logistic regression analysis was done to calculate prevalence odds ratio against arsenic concentration, gender, village and age group.

4.3.13 Ethics: Prevalence survey:

The interviewing paramedics explained to the families the nature and purpose of the study and ensured a private environment for interview and examination, and confidentiality of the information assured. Verbal consent was obtained from all individual family members and for minors by their guardians.

4.4: Case referent study:

The case referent study nested in the skin lesion prevalence survey was conducted in 2007.

4.4.1: Definition of a case

Men and women aged at least 16 years at the time of the prevalence study for whom any skin lesion (keratosis, hyperkeratosis or keratotic nodules) on the palm and/or sole had been recorded were defined as a case. A total of 160 such cases were identified from the prevalence survey data.

4.4.2: Definition of a referent

Cases were individually matched for age (±3 years), gender and village to individuals (referents), without any skin lesion recorded at the prevalence survey.

4.4.3: Selection of cases and referents:

A case-referent ratio 1:3 was targeted, but, due to the possibility of losing a referent, five referents (where available) were randomly selected from the list of matched individuals for each case. A total of 800 potential referents were selected, and a list of cases and referents was prepared, showing the five referents against each case in order (1-5) of selection.

4.4.4: Data collection instrument:

To meet the objectives of the case-referent study, a semi-structured data collection instrument was developed in English. The instrument was translated in to Bangla and then back again into English by a third person for consistency checking. The instrument (appendix 8) was reviewed by the thesis supervisor, pre-tested and finalized.

4.4.5: Interviewer recruitment and training:

Two paramedics, trained by GK, were recruited for collecting information from the cases and referents. Two further field assistants were also recruited to assist them. The interviewers and the field assistants were provided training with hands-on practice before starting data collection. They were also provided with a written guideline and checklist for data collection (shown in English translation in appendix 9). It was planned that interviews should be conducted blind as to case and referent status.

4.4.6: Data collection:

Two separate teams were formed to work in the field simultaneously. In each team there was one paramedic, one field assistant and one set of data collection equipment. The fieldwork was first started in Syedpur, where initially visits to cases and the first three referents were attempted for data collection. In the second round, if any of the first three referents were missed, visits to the 4th and 5th referents were attempted. However, following the experience in Syedpur, in Ahmedpur village all five referents were attempted in the first round of fieldwork. The interviewers visited the household of the subject, and identified the subject by the GA/PA number, name, gender, and family serial number. Having then sought the subject's permission for their participation in the study, the interview and measurement of height and weight were conducted in a comfortable and private environment. If the subject was not available on the first visit, further attempts were made later to interview and examine him or her. The data collection procedure for the key variables is described below:

Education:

Completion of school year, certificate or degree was recorded as a measure of educational attainment of all subjects.

Residential history:

Address/es lived at for at least six months by the subject since birth were recorded. This included post office, upazilla (sub-district) and district information or street and town name. If the person lived in another location within the study area, GPS location or an area reference was recorded. The subjects were asked about the time period (age to age or date to date) they lived elsewhere. Their reason for moving was also recorded. The main drinking water source at each place inhabited for more than six months was identified also recorded. If the previous address was in either of the two study villages, the specific well number and type or location of the well/water source was recorded.

Food habit recall:

Particular food items (vegetable protein-dal, animal protein-egg, fish and meat) described according to local cooking practice with serving sizes were recorded. To estimate serving size, subjects were shown in photographs (appendix 10) of three sizes of meat and fish (of known weight) on a plate of constant size. An amount of dal was cooked in different styles to estimate the amount of lentil in a serving. Most commonly used local kitchen vessels/serving size photos were shown to the subjects to estimate the amount and type of food consumed in the immediate past week.

Water drinking history:

The subject reported information on various sources of water at home and workplace including the amount and source of water used in cooking food and drinking water and tea.

Body Mass Index (BMI):

The subject was weighed in his or her usual clothing, without shoes, using a digital bathroom scale (graduated in 100 gm). Height without shoes was measured in cm using a height measuring stand.

4.4.7: Quality monitoring of data collection:

An explicit technical and management plan was used for all aspects of the work. A set of field data collection guidelines in Bangla was provided to the interviewers, who were given one full day of training, with hands-on experience, each examining five subjects and observing the interviews and examinations done by her colleagues. Operational definitions of technical and management terms were written down explicitly in the data collection guideline. This plan was verified by the thesis supervisor and discussed with other research team members. The interviewers were required to keep a field diary, and at the end of each day, the field team along with the investigator checked the completion of work undertaken, and preparation made for the next day's work.

A summary of progress was routinely sent to the thesis supervisor. In the event of any technical difficulties, the thesis supervisor was informed at once.

4.4.8: Data entry and cleaning:

Every day, after data collection, the completed questionnaires were checked by the investigator (NH) for clarity, completeness and consistency. The serving sizes of different food items were converted to gm (appendix 11). The data were coded, using a coding manual included as appendix 12 and entered by an experienced data entry clerk in a spreadsheet (SPSS version 12). After data entry, the data were checked again for any extreme or missing values and other inconsistencies.

4.4.9: Analysis of case referent data:

Key variables:

Dependant variable:

Skin lesion (case) status: categorical.

Independent variables:

Key exposure variable:

Total arsenic in the well water as measured by the digital arsenator: (Independent continuous variable) was used as a categorical variable in the analysis, and classified as <10µg/L (WHO standard value); 11-50µg/L (Bangladesh standard value); 51-100µg/L, 101-500µg/L and >500µg/L. Three water test results for every well were available; in the analysis, the median of the three was used.

Education:

Completed years of education was treated as an independent, continuous variable, and also used as a categorical variable, recoded as No education (no schooling), Primary (≤5 years schooling) and Post-primary (>5 years schooling).

Body Mass Index: (BMI):

BMI was calculated as weight in kilograms/(height in meters)^2 and categorized for analysis; as follows: <18.5, 18.6-24.9, 25-29.9, 29.9+

Food data:

Amounts of vegetable protein (lentil) and animal protein (fish and meat) were calculated on the basis of serving size and number of meals, in grams from recorded consumption in the past week. The food item consumed in grams was weighted by a protein factor (appendix 13) to calculate the protein content of the particular food item consumed. A total protein intake was then calculated as the protein content of all food items and categorized for analysis as $\leq$6gm/day, 6.1-$\leq$9 gm/day and >9gm/day.

Water drunk at home:

Subjects reported the number of glasses of well water (in a 250ml glass) drunk at home. In the analysis, it was categorized in three categories ($\leq$8, 9-12, >12 glass/day).

Number of addresses at which the subject had lived:

Addresses were collected about the number of places lived by the subjects for more than six months. This information was used in two categories, persons who had lived in the villages throughout and those who had lived elsewhere.

Use of well water for cooking:

The subject reported whether or not well water was commonly used for cooking at home.

4.4.10: Statistical analysis:

Descriptive analysis was done, using SPSS 16, to explore the data examining the relation of exposure (median arsenic concentration) to the outcome variable (skin lesions) and the relation between case status and confounding variables (life time residence in the village, well water for home cooking, drinking tea of well water, BMI, education and protein consumption). Conditional logistic regression was carried out using stata 10, initially considering the relation of case status to each of the independent variables one by one. The potential explanatory variables, in which a value of <0.10 was seen in the univariate model, were then put together in a multivariable analysis model. The final model contained only those variables that added significantly.

4.4.11: Ethics: Case-Referent study:

The study protocol was approved by the GK ethics committee and the University of Alberta Health Ethics Board. The interviewers obtained permission of all subjects for their participation in the study. The subjects were assured of the confidentiality of the information they provided and this was observed in all stages of the data entry and analysis. They were not paid or given any incentive for their participation in the project. A field ethics note (in English at Appendix 9) was provided to all field workers to ensure their compliance with these principles.

Chapter 5: RESULTS

5.1: Well Survey Results:

There were 1509 wells in the study area during the well survey period. Villagers were dependant on three types of well water: a) tube well, b) dug well and c) supply water system. There were 1399 tube wells (92.7%), 97 dug wells (6.4%) and 13 (0.9%) wells supporting a supply water system. Table 5.1.1 shows the type of well by village in the study area. Out of 1509 wells, 1422 were tested during the well survey. Of 55 tube wells not tested, 49 were out of order at the time of the well survey. The 32 dug wells not tested in Ahmedpur were out of order and it was not possible to collect water samples from them. By the time of well testing (and since the prevalence study) a supply water system had been installed for some households and the hand pumps of many dug wells were removed.

Table 5.1.1: Type of well by village (n=1509)

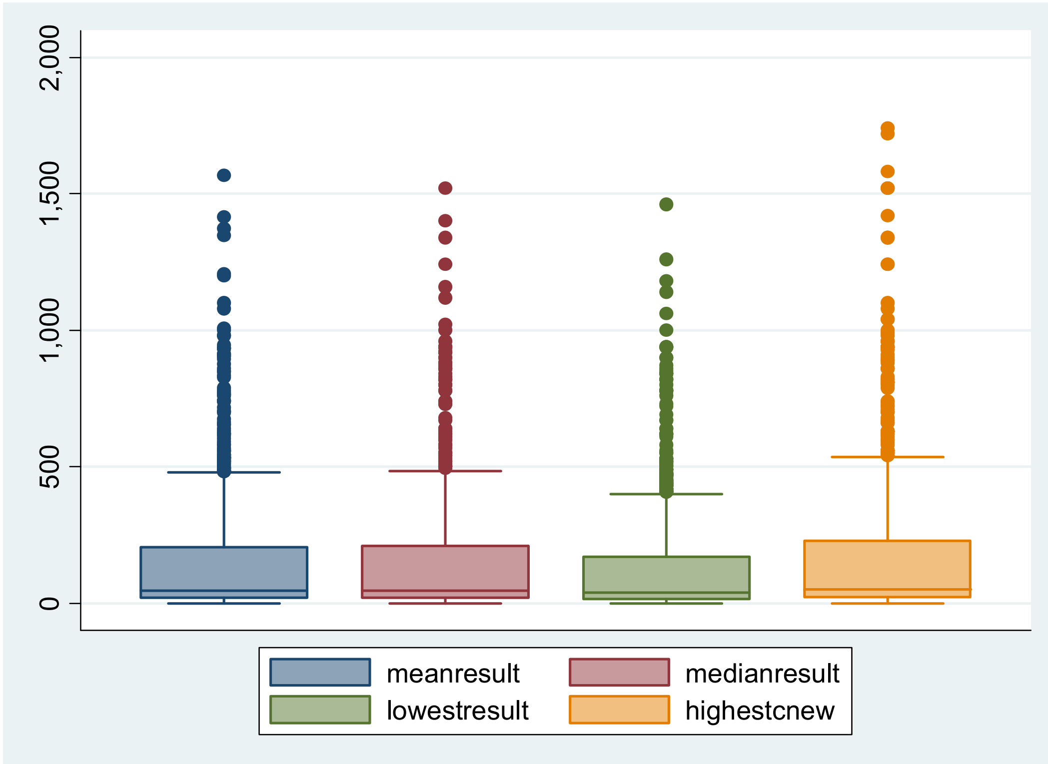
	Syedpur			**Ahmedpur**			**Total**		
	N	**Tested**	**%**	**N**	**Tested**	**%**	**N**	**Tested**	**%**
Tube wells	659	643	98	740	701	95	1399	1344	96
Dug wells	45	45	100	52	20	38	97	65	67
Supply water	7	7	100	6	6	100	13	13	100
Total	**711**	**695**	**98**	**798**	**727**	**91**	**1509**	**1422**	**94**

Results from three tests of one sample for each well were used in the analysis From these test results four summary statistics were computed for each well, the mean, the median, the lowest and the highest. The distribution of each of these statistics is shown in Figure 5.1.1 and in Table 5.1.2.

Table 5.1.2: distribution of summary statistics of arsenic concentration from three results/ well

Arsenic	**Mean**		**Median**		**Highest**		**Lowest**	
µg/L	N	%	N	%	N	%	N	%
0	129	9.1	156	11.0	129	9.1	221	15.5
<10	55	3.9	32	2.3	12	0.9	49	3.4
11-50	557	39.2	559	39.3	569	40.0	532	37.4
51-100	133	9.4	120	8.4	135	9.5	107	7.5
101-500	469	33.0	481	33.8	486	34.2	462	32.5
>500	79	5.6	74	5.2	91	6.4	51	3.6
Total	1422	100	1422	100	1422	100	1422	100

Figure: 5.1.1: Mean, median lowest and highest results of three tests

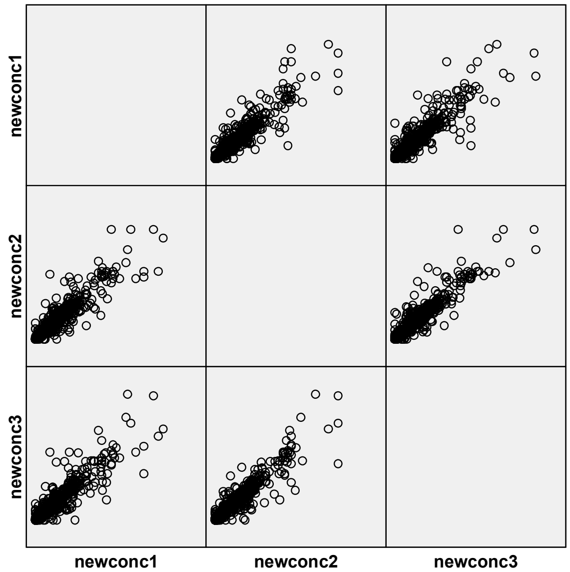


The results of three tests for each well were highly correlated. Table 5.1.3 and Figure 5.1.2 show the Pearson correlations of three tests results.

Table 5.1.3: Pearson Correlation of three results (N=1422)

	Result 1	Result 2	Result 3
Result 1	1	.946 P<.001	.936 P<.001
Result 2	.946 P<.001	1	.952 P<.001
Result 3	.936 P<.001	.952 P<.001	1

Figure 5.1.2: Correlation between three tests



The intention a priori had been to use the median of the three values and the examination of the results did not suggest any reason to change this decision. The distribution of the medians was then examined (Figure 3), showing a right skew. As concentration was not used as a continuous variable in these analyses (except descriptively as a mean), but as a categorical variable grouped as in table 5.1.2, it was not necessary to transform the data. The detection limit of the arsenator was 2-100 μg/L. A histogram of arsenic concentration of all the wells (in which less than 2 values replaced by half the limit of detection) of both the villages and by village are shown in Figure 5.1.3 and Figure 5.1. 4

Figure 5.1.3: Histogram of median values of three results

(showing fitted normal curve)

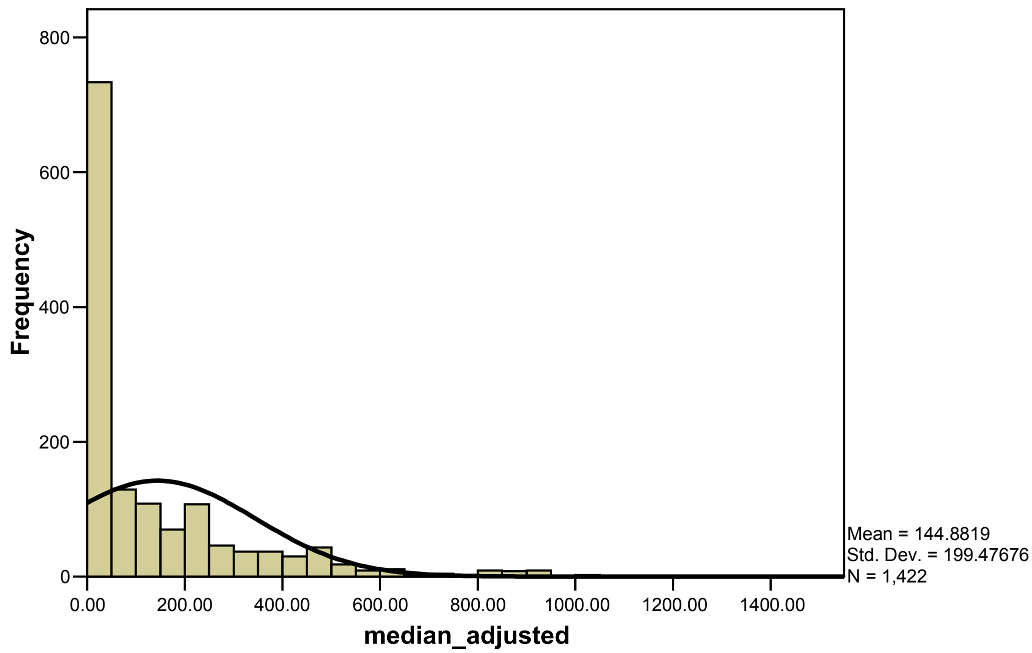


Figure 5.1.4

Figure 4: Arsenic concentration (Median) by village

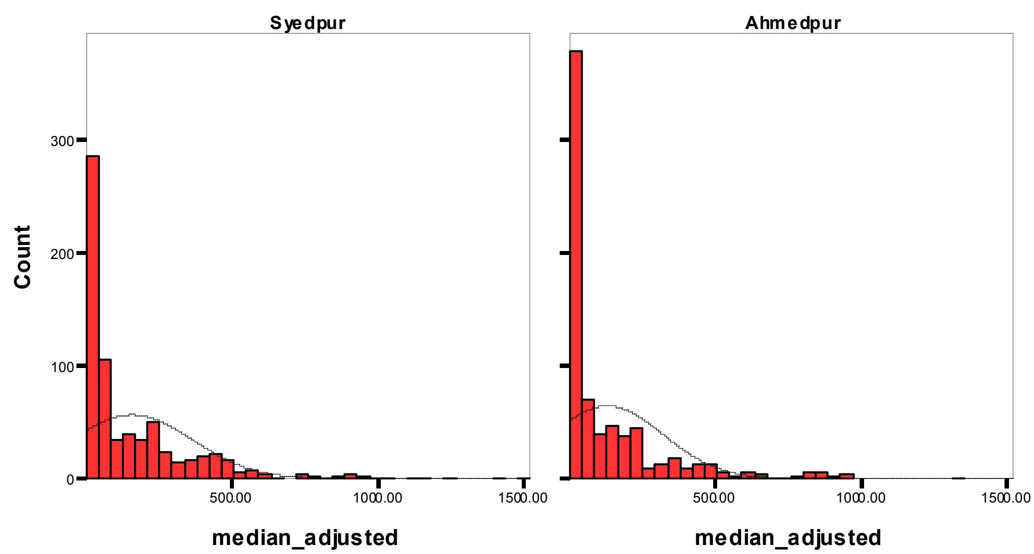


Table 5.1.4 shows summary statistics for arsenic concentration, indicated by the median of the 3 measures from each well by village and type of well. Syedpur village was more contaminated then Ahmedpur and tube wells had a higher concentration than the other two types of well.

Table 5.1.4: Arsenic concentration by village and type of well (n=1422)

	N	Mean (µg/L)	SD	Median (µg/L)
Syedpur	695			
Tube well	643	168.4	212.2	67
Dug well	45	31.6	42.3	20
Supply water	7	44.4	67.2	7
Ahmedpur	727			
Tube well	701	133.5	192.5	41
Dug well	20	82.4	132.0	17
Supply water	6	115.8	133.9	47
All	1422	144.8	199.5	47
Tube well	1344	150.2	202.9	49
Dug well	65	47.2	83.4	20
Supply water	13	77.4	105.3	27

5.1.1: **Summary of well results:**

The three test results from each well were highly correlated. The median of the three values for each well was retained as the index of arsenic concentration for further analysis. Using this measure 86.7% of wells were found to have a concentration of arsenic >10 µg/L (the WHO guideline) and 47.4% >50 µg/L (the Bangladesh guideline). Overall arsenic concentrations in Syedpur were higher than Ahmedpur and tube wells than other water sources.

5.2: Prevalence Survey Results:

The two villages in the study area, Syedpur and Ahmedpur were of almost equal geographical area and population size. There were 11670 people ascertained to be living in the two villages: in addition there were a few houses in which no contact could be made and which may have been uninhabited at the time of the survey. Table 5.2.1 shows the distribution of population in the villages by gender. In each village there were more men than women, more markedly so in Syedpur

Table 5.2.1: Population in household survey by village and gender (N=11670)

Gender	Syedpur		Ahmedpur		All	
	N	%	N	%	N	%
Male	3100	53.0	2952	50.7	6052	51.9
Female	2750	47.0	2868	49.3	5618	48.1
Total	5850	100	5820	100	11670	100

Out of 11670 residents in the households studied, 11078 (94.9%) were examined and interviewed during the survey period (including children aged less than 6 years who were only examined if lesions were reported by their mother). Table 5.2.2 shows the distribution of missed cases by village and gender. A larger number of men than women were not examined, particularly in Ahmedpur. In both villages the main reason for cases not being examined was that the subject was living out of the village during the survey. Thirteen subjects had died in the gap between the population listing and the prevalence survey.

Table 5.2.2: Household members not examined during survey by village

	Syedpur				Ahmedpur				All			
	Men		Women		Men		Women		Men		Women	
	N	%	N	%	N	%	N	%	N	%	N	%
Out of village	186	93.5	170	94.4	132	95.0	71	95.9	318	94.1	241	94.9
Died	04	2.0	01	0.6	06	4.3	02	2.7	10	3.0	03	1.2
Unknown	09	4.5	09	5.0	01	0.7	01	1.4	10	3.0	10	3.9
Total missed	199	100	180	100	139	100	74	100	338	100	254	100

Information on age was available for all subjects contacted except for one female infant whose family structure suggested an age of 3 years (used in the following analyses). The age distribution of the population was pyramidal with a relatively large younger population (Table 5.2.3).

Table 5.2.3: Age distribution by village and gender (N=11078)

Age	Syedpur				Ahmedpur				Total			
	Male		Female		Male		Female		Male		Female	
	N	%	N	%	N	%	N	%	N	%	N	%
≤5	343	11.8	308	12.0	362	12.9	371	13.3	705	12.3	679	12.7
6-18	861	29.7	702	27.3	831	29.5	754	27.0	1692	29.6	1456	27.1
19-30	760	26.2	757	29.5	673	24	782	28.0	1435	25.1	1539	28.7
31-50	591	20.4	549	21.4	663	23.6	625	22.4	1254	21.9	1174	21.9
51-70	273	9.4	224	8.7	250	8.9	207	7.7	523	9.2	431	8.0
≥71	73	2.5	29	1.1	32	1.1	55	2.0	105	1.8	84	1.6
Total	2901	100	2569	100	2813	100	2794	100	5714	100	5363	100

Villagers were asked about their main source of drinking water. This data was available for 10581(95.5%) subjects examined. Hand pump tube wells were the most common source of drinking water in both villages (Table 5.2.4). Only 5 subjects were using supply water at the time of the prevalence study.

Table 5.2.4: Source of drinking water of subject by village (N=11078)

Water source	Syedpur		Ahmedpur		All	
	N	%	N	%	N	%
Tube well	5167	94.4	5275	94.1	10442	94.3
Dug well	61	1.1	73	1.3	134	1.2
Supply water	5	0.1	0	0.0	5	0.1
Unknown	238	4.4	259	4.6	497	4.5
Total	5471	100	5607	100	11078	100

Out of 11078 persons examined, well identification number was available for 10863 subjects. At the time of well testing, wells of 356 persons were not functioning. Table 5.2.5 shows the distribution of well testing status and the reasons for not testing by village. The main reason for a well used by a subject not to be tested was that the well had been destroyed or was not functioning at the time of the well survey. A group of families in one village refused to allow their wells to be tested.

Table 5.2.5: Reasons for not testing subject source of water by village (n=10863)

Test	Syedpur		Ahmedpur		All	
	N	%	N	%	N	%
Done	5197	97.7	5247	94.7	10444	96.1
Subject refused	00	00.0	53	1.0	53	0.05
Well not functioning	119	2.2	237	4.3	356	3.3
Reason not recorded	5	0.1	5	0.1	10	0.1
Total	5321	100	5542	100	10863	100

Skin lesion survey:

Among the 11078 persons available for skin examination I68 cases with skin lesions on the palms and/or soles were found. The prevalence by age, gender and village is shown in table 5.2.6. No case occurred among children less than six years of age. Overall, the highest prevalence was observed among the 51-70 years age group. The prevalence rate appeared to be slightly higher in men in both villages, with the overall prevalence higher in Syedpur than Ahmedpur.

Table 5.2.6: Skin lesion prevalence (%) by age, gender and village (n=11078)

Age	Syedpur						Ahmedpur						All					
	Men			Women			Men			Women			Men			Women		
	N	Lesions	%	N	Lesions	%	N	Lesions	%	N	Lesions	%	N	Lesions	%	N	Lesions	%
≤5	343	00	0.0	308	0	0.0	362	0	0.0	371	0	0.0	705	0	0.0	679	0	00
6-17	861	2	0.2	702	5	0.7	831	5	0.6	754	1	0.1	1699	7	0.4	1462	6	0.4
18-30	760	17	2.2	757	6	0.8	675	8	1.2	782	5	0.6	1460	25	1.7	1550	11	0.7
31-50	594	26	4.4	549	29	5.3	1663	12	1.8	625	9	1.4	1292	38	2.9	1212	38	3.1
51-70	273	20	7.3	224	12	5.4	250	5	2	207	3	1.4	548	25	4.6	446	15	3.4
>71	73	1	1.4	30	1	3.3	32	1	3.1	55	0	00	107	2	1	86	1	1.2
Total	2901	66	2.3	2570	53	2.1	2813	31	1.1	2794	18	0.6	5714	97	1.7	5364	71	1.3

Table 5.2.7 shows arsenic concentration (as the median of the three tests for each well) in drinking water of subjects in the prevalence survey by village. In both the villages, more than half of the people were exposed to higher than the Bangladesh guideline for acceptable levels of arsenic in wells (50µg/l) and more than 90% to more than the WHO guideline of 10µg/l. People in Syedpur village were exposed to a higher concentration of arsenic in drinking water than those in Ahmedpur, with 54.5% in Syedpur exposed to >50µg/l compared with 43.1% in Ahmedpur.

Table 5.2.7: Arsenic concentration by village (n=10444)

Concentration µg/L	Village*					
	Syedpur		Ahmedpur		All	
	N	%	N	%	N	%
≤10	377	7.3	853	16.3	1230	11.8
11-50	1983	38.2	2132	40.6	4115	39.4
51-100	676	13.0	331	6.3	1007	9.6
101-500	1894	36.4	1774	33.8	3668	35.1
≥500	267	5.1	157	3.0	424	4.1
Total	5197	100	5247	100	10444	100

* X^2 = 340.03 p=<0.001

Figure 5.2.1 shows the exposure distribution by skin lesions. In both the villages, cases were exposed to higher mean and median arsenic concentrations than the non cases (Table 5.2.8). The mean and median exposures of cases in Ahmedpur were lower than cases in Syedpur (but higher than those for non-cases in that village).

Figure 5.2.1

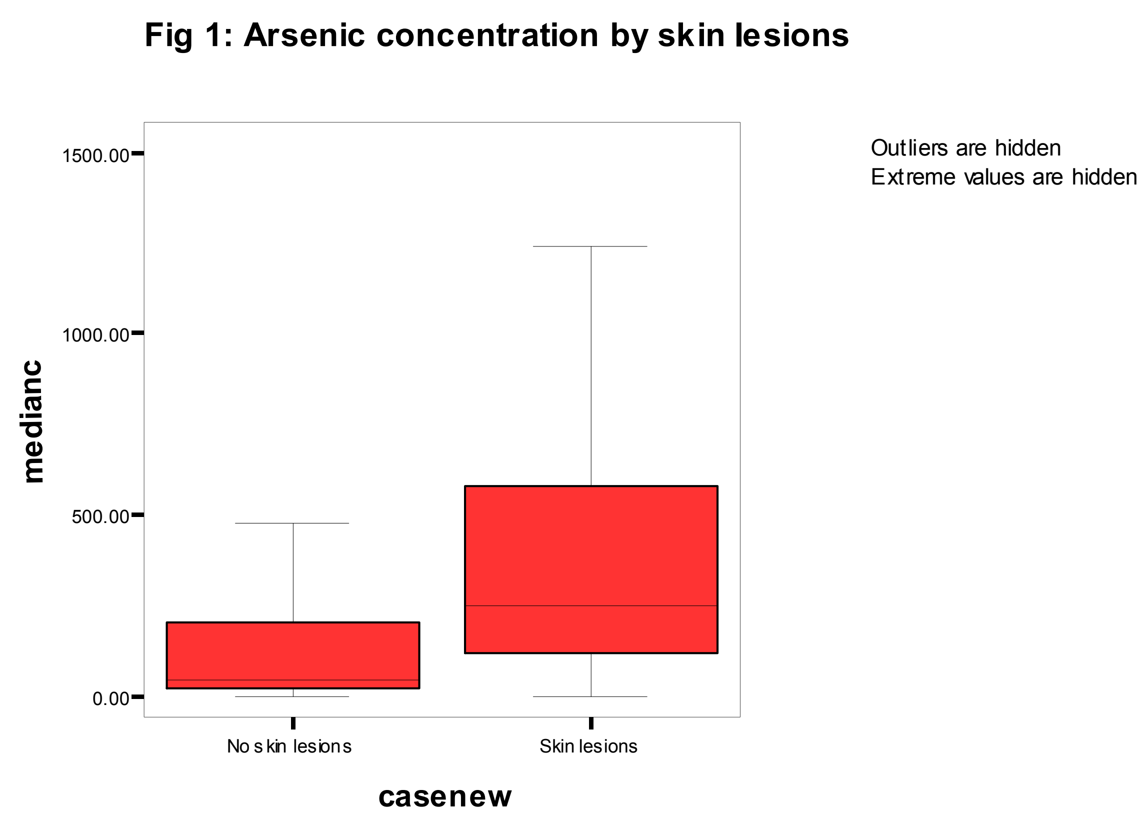


Table 5.2.8: Concentration of arsenic (median of three results) by skin lesions and village (n=10198)

	Village					
	Syedpur		Ahmedpur		All	
	Lesions	No lesions*	Lesions	No lesions	Lesions	No lesions
Mean	533.6	162.9	195.4	116.4	432.0	139.6
SD	437.5	218.8	235.9	157.6	417.4	192.1
Median	412.5	63.0	120.0	38.0	250.0	47.0
N	110	5021	47	5020	157	10041

• *Difference between the means Syedpur F=289.6, p<0.001, Ahmedpur F=11.6, p<0.001, All F=338.8 p< 0.001*

Overall there was a clear trend of increasing rate of skin lesion with increasing exposure in the study population, which was more marked at higher (>500 μg/L) concentrations, particularly in Syedpur (Table 5.2.9). The smaller number of skin lesions seen in Ahmedpur were not clearly related to increasing exposures above 50μg/l.

Table 5.2.9: Arsenic concentration by skin lesions and village (n=10444)

Concentration µg/L	Syedpur			Ahmedpur			All		
	N	Lesions	%	N	Lesions	%	N	Lesions	%
≤10	377	3	0.8	853	0	00	1230	3	0.2
11-50	1983	8	0.4	2132	10	0.5	4115	18	0.4
51-100	676	4	0.6	331	13	3.9	1007	17	1.7
101-500	1894	60	3.2	1774	17	1.0	3668	77	2.1
≥500	267	35	13.1	157	7	4.5	424	42	9.9
Total	5197	110	2.1	5247	47	0.9	10444	157	1.5

Table 5.2.10 shows the rate of skin lesion by age and exposure categories. Although a highest prevalence was seen in those over 30 years who were using drinking water with arsenic concentrations more than 500 ug/l, some trend of increasing rate of lesions with increasing arsenic concentrations was apparent in all the age groups.

Table 5.2.10: skin lesions by arsenic in wells and age (n=10444)

Arsenic in wells µg/L	Age groups											
	≤18 yrs			19-30 yrs			31-50 yrs			> 50 yrs		
	N	Total	%	N	Total	%	N	Total*	%	N	Total	%
≤50	2	2321	0.1	7	1285	0.5	8	1186	0.7	4	553	0.7
51-100	1	452	0.2	5	239	2.1	8	214	3.7	3	102	2.9
101-500	7	1586	0.4	13	909	1.4	36	791	4.6	13	382	3.4
≥501	4	187	2.1	7	95	7.4	19	97	19.6	12	45	26.7
Total	14	4546	0.3	32	2528	1.3	71	2288	3.1	40	1082	3.7

Tables 5.2.11-5.2.14 similarly shows the proportion of the population with skin lesions by arsenic concentration stratified by gender within each village. For both men and women in Syedpur there was a clear increase in lesions with age for those exposed to more than 100ug/L. In Ahmedpur the pattern was much less evident.

Table 5.2.11: skin lesions by arsenic (median of 3 results) in wells and age in the males in Syedpur (n=2763)

Arsenic in wells µg/L	Age groups											
	≤18 yrs			19-30 yrs			31-50 yrs			> 50 yrs		
	N	Total	%	N	Total	%	N	Total	%	N	Total	%
≤50	0	546	0.0	4	292	1.4	4	255	1.6	1	157	0.6
51-100	0	175	0.0	0	78	0.0	0	68	0.0	2	44	4.5
101-500	0	430	0.0	7	233	3.0	16	233	6.7	11	118	9.3
≥501	3	72	4.2	3	34	8.8	5	34	14.7	7	19	36.8
Total	3	1223	0.2	14	637	2.2	25	565	4.4	21	338	6.2

Table 5.2.12: skin lesions by arsenic (median of 3 results) in wells and age in female in Syedpur (n=2434)

Arsenic in wells µg/L	Age groups											
	≤18 yrs			19-30 yrs			31-50 yrs			> 50 yrs		
	N	Total	%	N	Total	%	N	Total	%	N	Total*	%
≤50	0	463	0.0	0	292	0.0	1	240	0.4	1	115	0.9
51-100	0	135	0.0	0	85	0.0	1	65	1.5	1	26	3.8
101-500	4	400	1	3	222	1.4	14	187	7.5	5	88	5.7
≥501	0	46	0.0	3	30	10.0	9	27	33.3	5	13	38.5
Total	**4**	**1044**	**0.4**	**6**	**629**	**1.0**	**25**	**519**	**4.8**	**12**	**242**	**5.0**

Table 5.2.13: skin lesions by arsenic (median of 3 results) in wells and age in males in Ahmedpur (n= 2659)

Arsenic in wells µg/L	Age groups											
	≤18 yrs			19-30 yrs			31-50 yrs			> 50 yrs		
	N	Total	%	N	Total	%	N	Total	%	N	Total	%
≤50	1	665	0.2	2	340	0.6	1	361	0.3	2	150	1.3
51-100	1	68	1.5	3	31	9.7	3	45	6.7	0	15	0.0
101-500	2	394	0.5	3	215	1.4	5	203	2.5	3	94	3.2
≥501	1	35	2.9	0	14	0.0	3	23	13.0	0	6	0.0
Total	**5**	**1162**	**0.4**	**8**	**600**	**1.3**	**12**	**632**	**1.9**	**5**	**265**	**1.9**

Table 5.2.14: skin lesions by arsenic in wells and age in females in Ahmedpur, (n=2588)

Arsenic in wells µg/L	Age groups											
	≤18 yrs			19-30 yrs			31-50 yrs			> 50 yrs		
	N	Total	%	N	Total	%	N	Total	%	N	Total	%
≤50	1	647	0.2	1	361	0.3	2	330	0.6	0	131	0.0
51-100	0	74	0.0	2	45	4.4	4	36	11.1	0	17	0.0
101-500	1	362	0.3	0	239	0.0	1	185	0.5	2	82	2.4
≥501	0	34	0.0	1	17	5.9	2	21	9.5	0	7	0.0
Total	**2**	**1117**	**0.2**	**4**	**662**	**0.6**	**9**	**572**	**1.6**	**2**	**237**	**0.8**

Table 5.2.15 shows the distribution of skin lesions by type of severity by village. In appears that severe cases were found in equal proportions in the two villages but that milder cases were less likely to be detected in Ahmedpur.

Table 5.2.15: Distribution of skin lesions by type of severity and village
$X^2 = 11.4$ p=0.003

Severity	Syedpur		Ahmedpur		Both	
	N	**%**	**N**	**%**	**N**	**%**
No lesions	5352	97.8	5558	99.1	10910	98.5
Thickening only	49	0.9	8	0.1	57	0.5
1 – 2 nodules	54	1.0	27	0.5	81	0.7
3+ nodules	16	0.3	14	0.2	30	0.3
Total	5471	100	5607	100	11078	100

A multiple logistic regression analysis was carried out to examine the effect of arsenic on skin lesions having allowed for gender, age group (4 categories), and village. As no lesions were reported for children aged less than 6 years, this analysis was limited to those age 6 years or older (N=8940). Table 16 shows the OR and 95% CI of the predictor variables. As indicated by the odds ratios, the risk of lesions sharply increased with increasing exposure intensity and age. Gender was not a significant risk factor, though males had a somewhat greater odds ratio than female. The risk of lesions was significantly higher in Syedpur village even with adjustment for arsenic concentration.

Table 5.2.16: Prevalence of skin lesions by arsenic concentration in drinking water gender, age and village: logistic regression (N=8940).

Factor	Odds Ratio	95%CI
Arsenic in wells		
≤50 µg/L	1	-
51-100 µg/L	3.9	2.1 – 7.6
101-500 µg/L	5.3	3.3 – 8.6
≥501 µg/L	26.9	15.6– 46.4
Gender		
Female	1	---
Male	1.3	1(0.95)– 1.3
Village		
Ahmedpur	1	--
Syedpur	2.0	1.4-2.8
Age		
6-18 yrs	1	---
19-30yrs	3.2	1.7-6.0
31-50 yrs	8.3	4.6-14.9
>50 yrs	9.4	5.0-17.4

In order to examine any interaction between age and concentration (which might throw light on the importance of duration) the logistic regression analysis was repeated with stratification for age. Table 5.2.17 summarizes the results of these analyses. An increase in risk with exposures above 500ug/l is seen at all ages, but with the highest odds ratios in the older subjects. The divergence of risk between the two villages also appears to increase with age.

Table 5.2.17: Prevalence of skin lesions by arsenic concentration within age groups: logistic regression

Arsenic in wells (µg/L)	Age							
	6-18 yrs		19-30 yrs		31-50 yrs		> 50 yrs	
	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI
≤50	1		1		1		1	
51-100	2.8	0.2- 30.9	3.9	.7-15.2	4.9	1.8-13.2	3.3	0.7-15.2
101-500	5.2	1.1-24.9	2.6	2.6-22.9	6.6	3.0-14.2	7.8	2.6-22.9
≥501	25.9	4.6-144.7	13.4	3.47, 29.8	33.1	14.0-78.5	42.0	12.7-139.2
Gender								
Female	1		1		1		1	
Male	1.1	0.4-3.2	2.3	1.1-5.0	1.03	0.6-1.7	1.46	0.7-2.9
Village								
Ahmed	**1**		**1**		**1**		**1**	
Syedpur	.8	0.3-2.2	1.2	0.6-2.6	2.3	1.3-3.9	3.53	1.5-8.2
N	**3198**		**2449**		**2224**		**1069**	
Cases	**14**		**32**		**71**		**40**	
%	**0.4**		**1.3**		**3.2**		**3.7**	

5.2.1: Summary of results from the prevalence survey.

Objective 1 of this thesis was to determine the relation between skin lesions and arsenic concentration by gender and age for all inhabitants of the two study villages. In this chapter it has been shown that men and women were equally likely to develop lesions but that there were systematic differences in the development of lesions by age (with older people having more lesions, at least to the age of 70 years). There was also a substantial increase in the risk of lesions with increasing arsenic concentration, those with concentrations greater than 500 µg/l having a risk more than 20 times that of people with exposures below the Bangladesh guideline of 50µg/l. An important difference in risk was seen between the two villages which could not be explained fully by differences in arsenic exposure: mild lesions may have been under-detected in Ahmedpur. The case-referent study that follows was designed to investigate the effects of potential confounders that might differ between villages, on the observed relation between arsenic and skin lesions.

5.3: Case Referent Study Results

Out of 160 cases aged 16 years or greater at the time of the prevalence study, 137 (85.6%) were available for interview during the field work for the case referent study. Table 5.3.1 shows the missed cases by village. About half of the missed cases had migrated to other places during the time between the prevalence survey and the case referent study. Seven cases had died before the case referent study begun. The reasons for missing cases between the villages were similar.

Table 5.3.1: Interview status of cases by village (n=160)

Interview status	Syedpur		Ahmedpur		Total	
	N	%	N	%	N	%
Interviewed	99	86.1	38	84.4	137	85.6
Not interviewed						
Dead	6	5.2	1	2.2	7	4.4
Migrated	8	7.0	4	8.9	12	7.5
Not at home	0	0.0	1	2.2	1	0.6
Married and migrated	2	1.7	1	2.2	3	1.9
	115	100	45	100	160	100

Table 5.3.2 shows the interview status of the referents by village. This was more difficult to tabulate because at the start of the study in Syedpur, once 3 referents had been interviewed for a case, no attempt was made to contact the remaining referents. Of the 784 referents where an attempt at contact was made 675 (86.1%) were interviewed. About 10% of the referents were living elsewhere because of work, study, business and marriage related relocation. 11 referents died in Syedpur and one in Ahmedpur during the time between the prevalence survey and the case referent study. 19 referents appeared twice by mistake in Syedpur village. The referents were used only with the cases appeared first in the list.

Table 5.3.2: Interview status of referents by village (n=784)

Interview status	Syedpur		Ahmedpur		Total	
	N	%	N	%	N	%
Interviewed	481	86.1	194	86.2	675	86.1
Not interviewed						
Dead	11	2	1	0.4	12	1.5
Living elsewhere	54	9.7	25	11.1	79	10.1
Not at home	8	1.4	4	1.8	12	1.5
Others	5	0.9	1	0.4	6	0.8
	559	100	225	100	784	100

Of the 812 cases and referents interviewed, data on arsenic concentration in the well used at the time of the prevalence study was available for 127 cases and 641 referents (Table 5.3.3). However only referents (N=504) who were matched to interviewed cases with data on arsenic concentration contributed to the matched case-referent analyses: the remainder of the analysis in this chapter is limited to these 127 cases and their 504 matched referents with interview and well data. The number of referents per case used in the analyses in this chapter is shown by village in table 5.34. For 11 (8.7%) cases, there were only two referents.

Table 5.3.3: Well result by village and case status (n=812).

	Case			**Referent**			**All**		
	N	**Missed**	**%**	**N**	**Missed**	**%**	**N**	**Missed**	**%**
Syedpur	99	8	8.1	481	22	4.6	580	30	5.2
Ahmedpur	38	2	5.3	194	12	6.2	232	14	6.0
All	137	10	7.3	675	34	5.0	812	44	5.4

Table 5.3.4: Number of referents interviewed per case by village (n=127 cases)

Number of Referent s	Syedpur		Ahmedpur		All	
	N cases	%	N cases	%	N cases	%
2	9	9.9	2	5.6	11	8.7
3	18	19.8	7	19.4	25	19.7
4	33	36.3	15	41.7	48	37.8
5	31	34.1	12	31.6	43	33.9
Total	91	100	36	100	127	100

Referents were matched with the cases on age and gender (as recorded in the prevalence survey), and village. Table 5.3.5 shows that matching on age was very close; in a conditional logistic regression analysis the difference in age between cases and referents did not approach significance (p=0.78)

Table 5.3.5: Age (mean and median) of cases and matched referents

Matching variables	Cases	Referents
Age (years)		
Mean	41.7	42.6
Standard Deviation	13.4	13.1
Median	41	42
N	127	504

Table 5.3.6 gives the mean and median values of arsenic concentration in the wells used for drinking water by cases and referents in the two villages. The mean arsenic level was higher than the median in the study population and in both the villages separately indicating, as before, a skewed distribution. However, the medians do confirm the earlier observation that cases were exposed to higher level of arsenic than the referents, and that Syedpur had higher arsenic concentrations than Ahmedpur.

Table 5.3.6: Arsenic concentration (median) by case status and by village (n=631)

	N	Arsenic in Wells ((µg/L)		
		Mean	**SD**	**Median**
Syedpur				
Cases	91	552.1	451.6	425
Referents	359	167.5	210.4	68
All	450	245.3	316.3	132
Ahmedpur				
Cases	36	210.8	249.4	120
Referents	145	147.6	187.7	48
All	181	160.2	202.3	68
All				
Cases	127	455.3	432.2	325
Referents	504	161.8	204.1	68
All	631	220.9	290.7	105

The distribution of arsenic concentrations for cases and referents are given in Table 5.3.7. Referents are much more likely than cases to have exposures of <50µg/L in their current source of drinking water.

Table 5.3.7: Arsenic concentration (median) by case status (n=631)

Arsenic level μg/L	Cases		Referents		Total	
	N	%	N	%	N	%
≤50 μg/L	17	13.4	228	45.2	245	38.8
51-100 μg/L	13	10.2	50	9.9	63	10.0
101-500 μg/L	60	47.2	206	40.9	266	42.2
≥ 500 μg/L	37	29.1	20	4.0	57	9.0
Total	127	100	504	100	631	100

Cases were found to be more likely to have lived in the two study villages throughout their lives than the referents (Table 5.3.8). Among males, the majority of both cases and referents lived in the study villages throughout but in both men and women cases were more likely to do so than referents.

Table 5.3.8: Lived in the villages throughout their lives by gender and case status (n=631)

	Male				Female				All			
	Case		Referent		Case		Referent		Case		Referent	
	N	%	N	%	N	%	N	%	N	%	N	%
Always lived in the villages	63	82.9	221	76.7	17	33.3	44	20.4	80	63.0	265	52.6
Did not always live in the villages	13	17.1	67	23.3	34	66.7	172	79.6	47	37.0	239	47.4
Total	76	100	288	100	51	100	216	100	127	100	504	100

Subjects were asked if they use tube well water for cooking at the time of the prevalence survey two years earlier. We found a higher proportion of referents compared to cases used tube well water for cooking. Table 5.3.9 shows the distribution of tube well water use for cooking at different concentrations of arsenic for cases and referents: in both there was a marked trend towards not using tube well water where drinking water was taken from more contaminated wells.

Table 5.3.9: Tube well water for cooking at home by case status and arsenic concentration (n=631)

Arsenic in wells (µg/L)	Case			Referent		
	N	Used	%	N	Used	%
≤50	17	13	76.5	228	209	91.7
51-100	13	13	100	50	45	90.0
101-500	60	36	60.0	206	151	74.5
> 500	37	20	54.1	20	14	73.3
Total	127	82	64.6	504	419	83.1

Drinking tea at home was not very common in either village. Only 5% of subjects used to drink at least one cup of tea made with tube well water at home everyday. Table 5.3.10 shows the distribution of drinking tea at home by gender and case status. Females, either cases or referents were less likely than males to drink tea. More male referents than cases drank tea at home . Overall there was no important difference between cases and referents in the likelihood of drinking tea made with tube well water.

Table 5.3.10: Drink tea made with TW water by case status by gender (n=631)

	Male				Female				All			
	Case		Referent		Case		Referent		Case		Referent	
	N	%	N	%	N	%	N	%	N	%	N	%
No Tea with TW water at home	64	84.2	209	72.6	50	98.0	210	97.2	114	89.8	419	83.1
Tea with TW water at home	12	15.8	79	27.4	1	2.0	6	2.8	13	10.2	85	16.9
Total	76	100	288	100	51	100	216	100	127	100	504	100

There seemed to be a difference in the water consumption pattern between the cases and referents. Inspection of the amount of tube well water drunk at home showed that a larger proportion of cases than referents drank more than 12 glasses of tube well water/day. This difference was more marked among the females. Table 5.3.11 shows the distribution of tube well water consumption by gender and case status.

Table 5.3.11: Drink TW water at home by gender and case status (n=631)

	Male				Female				All			
	Case		Referent		Case		Referent		Case		Referent	
	N	%	N	%	N	%	N	%	N	%	N	%
≤8 glass/day	33	43.4	111	38.5	26	51.0	108	50.3	59	46.5	219	43.5
>8-12 glass/day	29	38.2	133	46.2	14	27.5	91	42.1	43	33.9	224	44.4
>12 glass/day	14	18.4	44	15.3	11	21.6	17	7.9	25	19.7	61	12.1
Total	76	100	288	100	51	100	216	100	127	100	504	100

Body mass index, in tertiles not accounting for gender, was lower in males than females. In males, cases had a somewhat lower BMI than referents, but this was reversed for females (Table 5.3.12). Overall the BMI of cases and referents was virtually identical.

Table 5.3.12: BMI by gender and by case status (n=626)

	Male				Female				All			
	Case		Referent		Case		Referent		Case		Referent	
	N	%	N	%	N	%	N	%	N	%	N	%
Low	37	48.7	104	36.5	8	15.7	65	30.4	45	35.4	169	33.9
Medium	23	30.3	92	32.3	15	29.4	63	29.4	38	29.9	155	31.1
High	16	21.1	89	31.2	28	54.9	86	40.2	44	34.6	175	35.1
Total	76	100	285	100	51	100	214	100	127	100	499	100

Note: 5 referents, 3 male and 2 female, had missing BMI.

Table 5.3.13 shows the distribution of schooling by gender and case status. About two thirds of the subjects did not have any schooling. In males, the distribution of schooling was very similar between the cases and referents. In the females, cases appeared more likely to have some primary education, while referents appeared more likely to have some post primary education. Overall the level of education did not differ between cases and referents.

Table 5.3.13: Education by gender by case status (n=631)

	Male				Female				All			
	Case		Referent		Case		Referent		Case		Referent	
	N	%	N	%	N	%	N	%	N	%	N	%
No education	44	57.9	168	58.3	35	68.6	161	74.5	79	62.2	329	65.3
Primary	11	14.5	41	14.2	11	21.6	20	9.3	22	17.3	61	12.1
Post Primary	21	27.6	79	27.4	5	9.8	35	16.2	26	20.5	114	22.6
Total	76	100	288	100	51	100	216	100	127	100	504	100

The consumption of protein in the last week was compared for cases and referents (Table 5.3.14). About 40% of subjects ate some amount of egg in the last week. The pattern of egg consumption between cases and referents was similar. Fish was the most common type of protein, consumed by more than 95% subjects. Cases consumed an average of 205.5gm fish and referents an average of 198.5gm in the last week. Less than 40% of subjects ate meat. Of those who ate, mean meat consumption by the cases and referents were 46.2 gm and 44.5 gm respectively. The difference between the means was not statistically significant. Dal (lentil) was eaten by more than half of the subjects in the past week. Mean dal consumption by the cases and referents was 17.6 gm and 18.7gm respectively.

Table 5.3.14: Protein consumption by case status (n=631)

	n	Case		Referent		All	
		N	%	N	%	N	%
Egg (#) eaten last week	**631**						
Not Eaten		75	59.1	288	57.1	363	57.5
≤1 egg		32	25.2	116	23.0	148	23.5
1.1-3 egg		12	9.4	48	9.5	60	9.5
>3 egg		8	6.3	52	10.3	60	9.5
Fish (gm) eaten last week	**631**						
≤125 gm		24	18.9	116	23.0	140	22.2
126-200 gm		19	15.0	114	22.6	133	21.1
201-250gm		50	39.4	158	31.3	208	33.0
251-300 gm		22	17.3	72	14.3	94	14.9
>300gm		12	9.4	44	8.7	56	8.9
Meat (gm) eaten last week	**631**						
No meat		81	63.8	306	60.7	387	61.3
Some meat		27	21.3	120	23.8	147	23.3
More meat		19	15.0	78	15.5	97	15.4
Dal (gm) eaten last week	**631**						
Not eaten		60	47.2	232	46.0	292	46.3
≤15gm		13	10.2	66	13.1	79	12.5
16-25 gm		16	12.6	79	15.7	95	15.1
26-40 gm		22	17.3	56	11.1	78	12.4
>40gm		16	12.6	71	14.1	87	13.8

Total dietary protein content was estimated from the elements of table 5.3.14. Table 5.3.15 shows the distribution of protein consumption by case status and gender. Women reported eating less protein than men, but within gender the distribution of protein consumption by cases and referents was similar.

Table 5.3.15: Protein consumption by gender by case status (n=631)

	Male				Female				All			
	Case		Referent		Case		Referent		Case		Referent	
	N	%	N	%	N	%	N	%	N	%	N	%
≤6gm/day	21	27.6	82	28.5	18	35.3	91	42.1	39	30.7	173	34.3
9gm/day	27	35.5	86	29.9	19	37.3	68	31.5	46	36.2	154	30.6
>9gm/day	28	36.8	120	41.7	14	27.5	57	26.4	42	33.1	177	35.1
Total	76	100	288	100	51	100	216	100	127	100	504	100

Regression analysis:

Because of the matched design, conditional logistic regression was used to test the significance of each of the factors shown in tables 5.3.7-5.3.15., Table 5.3.16 shows the relation of individual variables with case status in a univariate analysis. Arsenic concentration in drinking water showed by far the strongest relation to case status ($p<0.0001$). Life time residence in the village ($p=0.04$), using tube well water in cooking (<0.001), and amount of tube well water used for drinking ($p=0.032$), were also significantly related to case status.

Table 5.3.16: Univariate conditional logistic regression analysis of individual variables by case status

Variable	n	OR	95% CI	X^2	p
Arsenic in water	**631**			**77.40**	**<.001**
<u><</u>50 µg/L		1			
51-100 µg/L		3.1	1.4-6.8		
101-500 µg/L		3.5	1.9-6.2		
<u>></u> 500 µg/L		18.3	8.8-37.9		
Lived always in the village	**631**			**3.95**	**0.04**
Didn't live		1			
Lived		1.6	1.0-2.7		
Duration of tube well use	**631**			2.67	0.44
0-9 yrs		1			
10-19 yrs		1.0	0.5-1.9		
20-30 yrs		0.8	0.4-1.6		
30+yrs		0.6	0.3-1.3		
Tube well water for home cooking	**631**			**20.84**	**<.001**
Used		1			
Not used		2.9	1.9-4.6		
Tube well water drinking at home	**631**			**6.86**	**0.032**
<8 glass/day		1			
8-12 glass/day		0.7	0.5-1.1		
>12 glass/day		1.5	.9-2.7		
Body Mass Index	**626**			0.92	0.63
<18.48		1			
18.48--21.2		0.8	0.5-1.3		
>21.2		1.0	0.6-1.5		
Schooling	**631**			0.02	0.90
No Schooling		1			
Some Schooling		1.0	0.7-1.6		
Protein consumption	**631**			1.53	0.47
<u><</u> 42 gm		1			
42-64 gm		1.3	0.8-2.1		
>64 gm		1.0	0.6-1.7		

Each factor was then examined in a model that included arsenic concentration in drinking water. Table 5.3.17 shows the effect of individual variable in presence of main exposure variable in the model. In the bivariate analysis,only tube well water for home cooking was only found significantly related to the case status (p=0.015), cases being less likely to report this. Residence in the villages throughout approached significance in this bivariate analysis (p=0.052).

Table 5.3.17: Factors adjusted for arsenic concentration: conditional logistic regression (n=631)

Variable	OR	95% CI	X^2	p
Duration of tube well use			-.8	1
0-9 yrs	1			
10-19 yrs	0.95	0.4-2.0		
20-30 yrs	0.99	0.4-2.1		
30+yrs	0.77	0.4-1.7		
Body Mass Index			2.1	0.350
<18.48	1			
18.48--21.2	0.8	0.5-1.4		
>21.2	0.9	0.5-1.6		
Schooling			0.4	0.527
No Schooling	1			
Some Schooling	1.2	0.7-1.9		
Protein consumption			4.9	0.086
< 42 gm	1			
42-64 gm	1.3	0.8-2.2		
>64 gm	0.9	0.5-1.6		
Lived always in the village			**3.8**	**0.052**
Didn’t live	1			
Lived	1.7	0.99-2.89		

Tube well water for home cooking			**5.9**	**0.015**
Used	1			
Not used	1.9	1.1-3.2		
Tube well water drinking at home			4.0	0.135
<8 glass/day	1			
8-12 glass/day	0.7	0.4-1.1		
>12 glass/day	1.3	0.7-2.4		

The effect of duration of well use was further examined by village and gender using conditional logistic regression, but limiting the analysis to those who had lived at the same address throughout (where duration was recorded unambiguously). (Table 5.3.18) There was no trend to suggest that duration of well use was related to case status in this group.

Table 5.3.18: Case status by concentration and duration of well use: men living at one address throughout their lives (n=363)

Over all X^2 =31.21 p<0.0001

Variables	OR	95% CI of OR	X^2	P value
Arsenic contamination of the wells			**29.57**	**<.0001**
<50 µg/L	**1**			
51-100 µg/L	**2.0**	**0.7-5.6**		
101-500 µg/L	**2.3**	**1.1-4.8**		
>500 µg/L	**11.4**	**4.8-28.9**		
Duration of use of TW water			1.64	.065
Less than 9 years	**1**			
10-19 years	**1.0**	**0.4-2.3**		
20-29 years	**1.4**	**0.6-3.3**		
30 years or more	**1.1**	**0.4-2.8**		

This analysis was then repeated for each village separately and restricted to men as there was only one case-referent set for women in which both case and referent had never moved. Neither in Syedpur (Table 5.3.19) nor Ahmedpur (Table 5.3.20) was there any indication of a sustained trend with duration.

Table 5.3.19: Case status by concentration and duration of well use: men living at one address throughout their lives: Syedpur village (n=250)

Over all X^2 =28.83 p<0.0001

Variables	OR	95% CI of OR	X^2	P value
Arsenic contamination of the wells			**27.71**	**<.001**
<50 µg/L	**1**			
51-100 µg/L	**0.7**	**0.1-3.4**		
101-500 µg/L	**2.2**	**0.9-5.4**		
>500 µg/L	**12.8**	**4.1-39.1**		
Duration of tube well water use			1.12	0.77
Less than 9 years	**1**			
10-19 years	**1.2**	**0.4-3.8**		
20-29 years	**2.0**	**0.6-6.3**		
30 years or more	**1.1**	**0.3-3.9**		

Table 5.3.20: Case status by concentration and duration of well use: men living at one address throughout their lives: Ahmedpur village (n=113)

Over all X^2 =12.00 p=0.0174

Variables	OR	95% CI of OR	X^2	P value
Arsenic contamination of the wells			**11.61**	**<.01**
<50 µg/L	**1**			
51-100 µg/L	**22.0**	**3.2-149.4**		
101-500 µg/L	**2.4**	**0.6-9.2**		
>500 µg/L	**12.8**	**1.8-93.4**		
Duration of tube well water use			0.39	0.94
Less than 9 years	**1**			
10-19 years	**0.2**	**.1-1.2**		
20-29 years	**0.3**	**.01-1.5**		
30 years or more	**0.6**	**0.1-3.6**		

A model was then fitted including the main exposure variable (arsenic in wells) and potential confounders that, in the univariate analysis, had p<0.10. This included tube well water for cooking, number of glasses of tube well water drunk and life time residence in the village. Conditional logistic regression was carried out to determine the effect of arsenic concentration and potential confounders on case status. The model fitted is shown in Table 5.3.21. Arsenic concentration retained a very strong relation to case status: the risk was three fold for those exposed to between 51-100 µg/L compared to those exposed to ≤50 µg/L. and increased substantially to 14 fold in those exposed to >500 µg/L. Cooking with tube well water continued to be protective, those not doing so being more likely to have skin lesions.. The number of glasses of tube well water drunk at home did not appear as a significant factor for skin lesions in the model, but living in the village throughout life so far approached significance.

Table 5.3.21: Case status as determined by selected variables in the model by conditional logistic regression (case n=127)

X^2 = 91.04 (p<.0001)

Variables	OR	95% CI of OR	X^2	P value
Arsenic contamination of the wells			**78.08**	**<0.0001**
<50 µg/L	**1**			
51-100 µg/L	**3.3**	**1.5-7.2**		
101-500 µg/L	**3.4**	**1.8-6.2**		
>500 µg/L	**14.2**	**6.7-30.2**		
Used Tube well water for cooking at home			**5.17**	**.023**
Yes	**1**			
No	**1.9**	**1.1-3.2**		
Tube well water consumption at home			4.67	.097
=<8 glass/day	1			
8-12 glass/day	0.67	0.4-1.1		
>12 glass/day	1.3	0.7-2.5		
Lived always in the villages			3.12	.077
No	1			
Yes	1.7	0.96-2.9		

This model was then repeated without the variable 'water consumption' but the effect of living in the village throughout did not change (Table 5.3.22)

Table 5.3.22: Case status as determined by selected variables in the final model by conditional logistic regression (case n=127)

X^2 = 86.37 (p<.0001)

Variables	OR	95% CI of OR	X^2	P value
Arsenic contamination of the wells			**78.08**	**<0.0001**
<50 µg/L	**1**			
51-100 µg/L	**3.3**	**1.5-7.1**		
101-500 µg/L	**3.3**	**1.8-6.0**		
>500 µg/L	**15.2**	**7.2-32.2**		
Used Tube well water for cooking at home			**5.17**	**0.023**
Yes	**1**			
No	**1.8**	**1.1-3.1**		
Lived always in the villages			3.12	0.077
No	1			
Yes	1.6	0.9-2.8		

Finally the model in Table 5.3.22 was run again, first for each village and again separating men and women. In Syedpur (Table 5.3.23) the effect of living in the village throughout was stronger and did reach significance, but the protective effect of using tube well water for cooking was less pronounced.. An increase in risk with arsenic was, though, only seen above 100µg/L. In Ahmedpur (Table 5.3.24) only arsenic concentration was significant, and even then there was no clear dose-response.

Table 5.3.23: Case status as determined by selected variables in the final model in Syedpur village by conditional logistic regression (case n=91)

(X^2 = 81.6, p<0.0001)

Variables	OR	95% CI of OR	X^2	P value
Arsenic contamination of the wells			**73.70**	**<0.0001**
<50 µg/L	**1**			
51-100 µg/L	**0.8**	**0.2-3.0**		
101-500 µg/L	**3.4**	**1.6-7.2**		
>500 µg/L	**17.7**	**7.0-45.0**		
Used Tube well water for cooking at home			3.69	0.055
Yes	**1**			
No	**1.8**	**1.0-3.2**		
Lived always in the villages			**4.21**	**0.040**
No	1			
Yes	1.7	1.0-2.9		

Table 5.3.24: Case status as determined by selected variables in the final model in Ahmedpur village by conditional logistic regression (case n=36)

X^2 =28.55, p<0.0001

Variables	OR	95% CI of OR	X^2	P value
Arsenic contamination of the wells			**27.30**	**<0.001**
<50 µg/L	**1**			
51-100 µg/L	**19.8**	**5.1-76.4**		
101-500 µg/L	**1.8**	**0.6-5.3**		
>500 µg/L	**7.7**	**2.0-30.2**		
Used Tube well water for cooking at home			**0.94**	**0.332**
Yes	**1**			
No	**1.8**	**0.6-5.9**		
Lived always in the villages			0.31	**0.578**
No	1			
Yes	0.7	0.2-2.3		

For both men (Table 5.3.25) and women (Table 5.3.26) increasing risk with arsenic concentration was clearly demonstrated and there was a consistent (although not significant) tendency for those who had lived in the villages throughout to be at greater risk. In men, but not women, there was a strong tendency for cases to report that well water was not used for cooking in their household.

Table 5.3.25: Case status as determined by selected variables in the final model in males by conditional logistic regression (case n=76)

Over all X^2 =54.95, p<0.0001

Variables	OR	95% CI of OR	X^2	P value
Arsenic contamination of the wells			**29.57**	**<0.0001**
<50 µg/L	**1**			
51-100 µg/L	**2.1**	**0.8-5.7**		
101-500 µg/L	**2.3**	**1.1-4.8**		
>500 µg/L	**9.6**	**3.8-24.4**		
Used Tube well water for cooking at home			**7.75**	**0.005**
Yes	**1**			
No	**2.5**	**1.3-4.6**		
Lived always in the villages			1.74	**0.187**
No	1			
Yes	1.6	0.8-3.2		

Table 5.3.26: Case status as determined by selected variables in the final model in females by conditional logistic regression (case n=51)

Over all X^2 =54.95, p<0.0001

Variables	OR	95% CI of OR	X^2	P value
Arsenic contamination of the wells			**54.03**	**<0.0001**
<50 µg/L	**1**			
51-100 µg/L	**7.5**	**1.9-29.3**		
101-500 µg/L	**6.4**	**2.0-20.0**		
>500 µg/L	**47.9**	**11.0-199.9**		
Used Tube well water for cooking at home			0.1	0.752
Yes	**1**			
No	**1.1**	**0.4-2.8**		
Lived always in the villages			0.82	0.365
No	1			
Yes	1.5	0.6-3.7		

5.3.1: Summary of results from the case referent study:

The case referent study addressed objectives 2-4

Objective 2:

The results of the case referent study supported the observation of the prevalence study. Even after matching for age, gender and village the risk of skin lesions increased with arsenic concentration in the wells used for drinking water, particularly in Syedpur village.

Objective 3:

Few of the potential confounders considered were related to the presence of skin lesions. Protein consumption, BMI and education bore no relation to skin lesions and were not confounders. Use of tube well water for cooking was found to be less usual, for both cases and referents, where the well used for drinking water had higher arsenic concentrations. Cases, particularly men, were much less likely than referents to report that the household used such water for cooking. A small increase in risk with drinking large volume of well water was not significant in the final model. Importantly none of the factors considered as a potential confounder modified the observed relation between arsenic concentration and skin lesions.

Objective 4:

This objective was to determine whether duration of well water use added to the prediction of skin lesions once current arsenic exposure had been considered. The data for testing this was not ideal because arsenic concentration at previous addresses was not known. In those with only one address (and who had thus lived in the villages throughout their life) no increased risk with duration of use was seen. However age and duration of use would have been highly correlated and a case-referent study matched on age (which was strongly related to lesions in the prevalence study) is probably over-matched for the study of duration. The observation that living in the villages throughout – and so potentially having a long duration of drinking water from a ‘hot spot’ of high arsenic – may be indirect indication that duration (or early exposure) might be important.

Chapter 6.1 DISCUSSION

The primary objective of the studies described here was to obtain information on risk of skin lesions in relation to arsenic concentration in drinking water. The prevalence study considered age and gender and the nested case-referent study sought to assess the modifying effect of amount of water consumed or used in home cooking and of estimated protein consumption, body mass index (BMI) and educational level. Analyses were also made to determine whether cumulative exposure was a better predictor of risk than current level of arsenic in well water.

These intentions were reasonably well fulfilled in that 95% of the large study population (n = 11,670) were examined, and that 94% of the wells and other water sources were tested satisfactorily for arsenic. Of 160 eligible cases, 137 (85%) were interviewed, and complete data obtained for 127 (79%) cases and matched controls. Though all of these procedures were monitored meticulously, it was not feasible, nor were there resources, to undertake independent checking on a representative sample of subjects and water sources. The limitations of the data are discussed later in this chapter.

No case was identified in children aged under six years, and relatively few below age 30 or over 70. In adults aged 31-70 the crude prevalence rate was 3.3%, and was somewhat higher in males (3.3%) than females (2.7%). The risk of lesions was clearly related to exposure intensity. Subjects exposed to more than

500µg/L were, having allowed for age, gender and village, at 26 times greater risk than those below the Bangladeshi standard (50µg/L). These findings were closely corroborated by the results of the case-referent analysis. Cases overall were exposed to a higher median concentration of arsenic (250µg/L) than referents (47µg/L). The odds ratio of being a case at 500µg/L was more than 15 times greater than at 50µg/L, and there was little evidence that BMI, schooling or consumption of well water and protein had any important modifying effect: use of well water for cooking was, as is discussed below, reported as less likely in cases. Those who had, since birth, lived in these two villages, which had been chosen because of suspected high arsenic concentrations, were somewhat more likely to be cases than those who had moved into the area (OR=1.6) but among those with only one address within the villages, duration of use of tube well water was not related to lesions. It is likely that a high correlation between age (used as a matching factor) and duration mitigated against the isolation of a direct effect of duration in the case-referent study.

Before comparing these results with those of other similar studies, it is worth considering the previous findings in other GK villages, since the methodology had many similarities, in particular with those described by McDonald et al. (2007). This also was a prevalence survey with nested case-control analysis, but there were important differences in design and methodology. For example, the earlier studies were in a fairly representative sample of 53 villages, most of which were at low arsenic levels, with case ascertainment by almost as many different paramedics as villages. The present study, on the other hand, was in two much larger villages, in a relatively high arsenic area, with case ascertainment entirely by only three, rather more experienced paramedics under close supervision. Nevertheless, to the extent that the exposures overlapped, the levels of risk and relative risk were quite compatible, both tending to suggest that there was little evidence of skin lesions below the Bangladeshi standard of 50µg/L, a crude prevalence of only 0.4% (21 cases) in the present study. Above that level, relative risk rose quite sharply.

Some limitations of the data and some incongruity in results should be considered before comparing these results with those of other groups. First the assessment of arsenic concentration was made with a field device lacking the checks normal to good laboratory practice. There were no spiked samples or quality control repeats, for example, and confidence in the results is based on validation studies reported elsewhere. Samples were only taken and tested on a single occasion, some months after the prevalence study, and insofar as arsenic concentrations might change over time, the measured concentrations are an imprecise estimate of even current exposures. Although residential histories were taken with the aim of estimating life time exposures, these could not be included in the analyses reported here.

Assessment of skin lesions was carried out by trained paramedics who examined the palms and soles of all inhabitants (age 6years or greater) of both villages.

Work began in Syedpur and, with the near completion of that village, continued in Ahmedpur. The presence of skin lesions in Syedpur has been shown to be markedly more consistently related to arsenic concentration than in Ahmedpur. Examination of the severity of lesions in the two villages suggested that there were approximately the same number of subjects with multiple nodules but that the paramedics were much more likely to identify skin thickening or isolated nodules in Syedpur. Examination of patterns of skin lesions recorded by the 3 paramedics did not identify any systematic difference by time or individual (data not shown) and it is unclear whether this difference between villages reflects a change in sensitivity to recording a case, common to all three paramedics, or a real difference between exposures not reflected in the arsenic concentrations measured.

The data on diet and water use was self reported. Considerable efforts were made to ensure that each subject used the same food quantity definitions, yet there is little data from Bangladesh about the accuracy of such reporting. While is is unlikely that the extent of error in reporting would be determined by case status, it might be that the non-differential misclassification was so great as to hide a true effect. The apparent ‘protective’ effect of using tube well water for cooking also deserves some comment. This use declined sharply in both cases and referents with increased arsenic concentration, probably because of the unpleasant taste. The reported lower use of well water in cooking in male cases (only) might simply be chance or an assumption (perhaps in ignorance of the true source of water) that such use in the kitchen would be avoided once lesions had appeared.

These observations on study limitations are not unique to the present investigation and the findings may usefully be compared with the results of the other three cross-sectional studies in the region (see Table 6.1). They appear quite similar to those of Guha Mazumder et al. (1998) in West Bengal, but less so with those of Ahsan et al. (2006), and far lower than those of Tondel et al. (1999), both in Bangladesh. The latter included only subjects over 30 years of age who had been exposed to any contaminated well during its existence; perhaps this explains their much higher rates.

Table 6.1: Comparison of prevalence estimate (%) in relation to arsenic concentration

Arsenic conc.	Tondel et al. (1999)	Ahsan et al. (2006)	Guha Mazumder et al. (1998)	Huda (2008)
≤10µg/L		2.5 (0.1-8.0)		0.2
11-50µg/L		4.2 (8.1-40.0)	0.1 (≤50) Keratosis only	0.4
51-100µg/L	17.5 (≤150)	6.5 (40.1-91.0)	0.9 (51-99)	1.7
101-500µg/L	23.4 (151-350)	7.4 (91.1-175.0)	3.3 (100-499)	2.1
>500µg/L	33.4 (>350)	11.1 (175.1-864.0)	6.5 (≥500)	9.9

The cross-sectional analysis examined the role of age and sex on skin lesion prevalence. The rate of prevalence increased with increasing age in both men and women. This was consistent with the earlier findings of McDonald et al. (2006) in GK villages, which reported that the mean age of cases was significantly higher than the non cases ($p<0.001$). In the present study, the rate of lesions in the thirty years or less age group was 0.6%, and over thirty years it was 3.2%. Guha Mazumder et al. (1998) reported 1.2% in his younger population (<30 years) compared to 3.4% in the older population. The skin lesion prevalence rate reported by Ahsan et al. (2006) in those aged under 30 was 1.5% compared to 7.9% prevalence in the over-30 population. These rates were much lower than the 29% reported by Tondel et al. (1999). Rahman et al. (2006a) also reported his highest prevalence rate in the 35-44 age group in both males and females.

Prevalence in adult males aged 18-70 was higher than in females (2.7% vs. 2.0%) in the present study. Tondel et al. (1999) also reported a higher prevalence in males than females (30.9% vs 26.1%). Ahsan et al. (2006) reported a risk of lesions four times higher in males than in females. Guha Mazumder et al. (1998) also found a higher prevalence in males (3.0%) than in females (1.2%), and from the large skin lesion prevalence survey in Matlab, Rahman et al. (2006a) reported a 1.58 male excess. The reason for this male excess and its variability has not been explained but the small excess in the present study was consistent with chance (OR=1.3; 95% CI 0.95-1.3)

The case-referent analysis examined the role of certain confounders including schooling, BMI, protein consumption, life time residence in the same village and duration of well use, well water use for home cooking and number of glasses of well water drank at home. More water was drunk by women in the home than by men, but possibly more men were exposed to more contaminated water outside the home.

In this study, there was no significant difference in schooling between cases and referents. Rahman et al. (2006b) examined the role of schooling on the risk of lesions in Matlab and reported a positive association ($p<0.01$), those with longer schooling being more likely to have lesions. Argos et al. (2007) reported a lower prevalence of lesions among subjects with more than two years of schooling in the Araihazar cohort. The conditional logistic regression analysis of Mitra et al.

(2004) did not find any significant relation between the risk of lesions and education in West Bengal. On the other hand, McCarty et al. (2006) found schooling associated with risk in Pabna, those with lesions being more likely to be illiterate.

There was no significant association of BMI with case status in this study. This was consistent with that of Mitra et al. (2004) in West Bengal, and of McCarty et al.(2006) in Pabna. Contrary to these findings, Milton et al. (2004) reported a higher prevalence risk ratio for subjects with a low BMI (p=0.001). The present study also examined the effect of protein consumption (lentil, fish, egg and meat) on the risk of lesions, but no significant relationship was found. Low animal protein consumption was reported with increased risk by Mitra et al. (2004), but this was not confirmed by McCarty et al. (2006).

A comparison of results from different studies is complicated by a large number of factors (see appendix 14); the inclusion criteria in particular. In the present study, for example, the whole population at all ages in two adjacent villages was included. Ahmad et al. (1999) included the whole population of a similar village. Guha Mazumder et al. (1998) included people of all ages and both sexes, but a questionable sampling technique was used to recruit subjects from 25 and 32 villages in two areas with quite different arsenic contamination levels. McDonald et al. (2006) included only women of 18 years or over in their study, in a stratified random sample of 53 widely scattered villages from four divisions of Bangladesh.

Tondel et al. (1999) included only subjects aged over 30 years who were lifelong residents of four villages in which there was a contaminated well. The four villages selected were each from one of four districts in Bangladesh. Ahsan et al. (2006) included 11,746 married subjects who had lived in their village for three years or more from a much larger source population. Rahman et al. (2006a) studied the large population aged four years or above from the community in Matlab.

Exposure to arsenic in drinking water has been measured and classified for analysis in different ways (see appendix 15). In this study, the arsenic concentration in all wells around the time of the prevalence survey was measured. Exposure indices in the various studies differed considerably, and there was also much variation in exposure classification. Most of the published studies compared prevalence over a range of exposure categories, but Rahman et al. (2006a), nor Ahmad et al. (1999) did not. The indices of exposure used varied enormously, and included current well concentration, daily dose/kg body weight, mean time-weighted arsenic concentration, cumulative arsenic exposure and geographical mean (Appendix 15). Differences in exposure quantification and category seriously limit the range of comparisons which can be made with other studies.

There were also important differences in the definition of skin lesions, sometimes described as “arsenicosis”, and in methods of examination, diagnosis and/or quantification (see Appendix 16). In this study, only thickening and keratosis on the palm and sole were included, and subjects were examined by paramedics, who were instructed to describe what they observed, and not what they considered to be the cause. In other studies physicians were sometimes asked to make a diagnosis, and even to assess causation (for example, Hore et al, 2007).

In considering the role of confounding variables, the various studies again defined each factor in different ways. In examining the effect of education, for example, Argos et al. (2007) compared subjects who had two years of schooling with those who had more than two years. Tondel et al. (1999) examined the effect of no education vs. college education and no education vs. primary education. Rahman et al. (2006b) classified education level as none, primary, secondary and higher. With regard to protein consumption, McCarty et al. (2006) considered weekly frequencies of fish, fowl, egg, beef, beans and milk consumption. The present study included chicken, mutton, beef and any other meat together, but considered the effect of lentils, egg and fish separately.

In summary, the epidemiological evidence to date on the nature of the risk between arsenic concentration in tube well water and incidence of keratotic skin lesions is far from exact, and there is little consensus on the effect of the various social and nutritional variables that have been studied. Although the risk appears to be strongly related to the current arsenic concentration, there remains uncertainty about the contribution made by duration of exposure. This is inevitably correlated closely with age – and complicated in women, who frequently move to another village on marriage. Of considerable importance is the general agreement that there is little evidence of skin lesions in persons exposed at concentrations below the Bangladesh standard of 50μg/L. This finding seems to hold whether the assessment of skin lesions is made by a physician or trained paramedic.

Although the keratosis may become severe and debilitating, there are other potential effects of arsenic exposure which are more serious. These are cancers of the lung, liver and urinary tract, and adverse effects in pregnancy. So far these risks, at least in Bangladesh (and India), have received little study, mainly because of the difficulty of having access to the necessary mortality and morbidity data.

As for malignant disease, the IARC (2004) concluded that there was sufficient evidence in man that arsenic in drinking water caused cancers of the skin, lung and urinary bladder. This evidence was based on studies in the USA, South America, Australia, Finland and Japan; none were cited from Bangladesh or India. Recently, however, Mostafa et al. (2008) reported excess risk of lung cancer from arsenic in drinking water in Bangladesh, based on subjects who underwent lung biopsy at a diagnostic centre in Dhaka, using referrals from throughout the country. Of patients who reported using tube well water for 10 years, 3223 subjects with previous lung cancer were compared with 1588 with more non-malignant lesions. Odds ratios for lung cancer increased steadily with mean arsenic concentration in the district in which they lived. The trend was seen only in smokers, where the odds ratio for exposures ≥ 100μg/L was 1.65 (95% CI 1.25 - 2.18).

In pregnancy, arsenic and its metabolites have been shown to cross the placental barrier (Ahmad et al. 2001), and a few studies have investigated the effect of arsenic in drinking water on pregnancy outcome, two in Bangladesh. Ahmed et al. (2001) investigated abortion, stillbirth and low birth weight through a cross-sectional analysis (n=192). This study reported significantly high rates of spontaneous abortion, stillbirth and preterm birth among the mothers who drank arsenic-contaminated water for 15 years or more. Cherry et al. (2008) analyzed 30,984 births in 600 GK villages in relation to geographical mean levels. Compared to the stillbirth rate in districts with a mean arsenic concentration ≤10 μg/L, a significantly higher rate of stillbirth was found in the areas with arsenic level ≥50μg/L (OR 1.8, 95% CI 1.14-2.86).

It thus seems probable that it is only a matter of time before not only keratotic skin lesions but also adverse pregnancy outcome and excess lung and other internal cancers will be found to be attributable to arsenic in concentrations in excess of 50 μg/L in village tube wells. The probable extent of this problem can be estimated, though hardly yet its gravity. An authoritative assessment of the situation is outlined in the two final sections of *Vol 1: Summary of the BGS Technical Report* (2001). In Section 12, entitled "Other Health Related Water Quality Problems", attention is drawn to the fact that while arsenic is by far the greatest health risk, 25% of all tube wells exceeded the Bangladesh standard of 50 μg/L, 35% also exceeded the WHO guideline for manganese, some significantly so. Indeed 36% of wells with water below the 50 μg/L standard for arsenic exceeded the guideline level for manganese. Nor was this situation limited to manganese, but applied to several other of the 20 metals quantified in the National Survey.

It follows that any arsenic mitigation policy should take account of water quality in general, and not be confined to arsenic. In Section 13 of the BGS Summary, entitled "Implications for Arsenic Mitigation", the urgency of the arsenic problem demanded that the aim should be to tackle the worst affected areas first; namely those in the south and east of the country. In addition, however, there were local "hotspots" in the north and west, only identified by evidence of skin lesion prevalence, which required equally urgent consideration. One such example are the two villages in the present study.

Any detailed consideration of strategy and methods of mitigation are beyond the scope of this thesis, but can be considered briefly. First and foremost is the need to have all wells in current use tested, and to close so far as possible any which exceed the Bangladesh standard. Usually these will be shallow wells, defined as less than 150 meter in depth. Even in areas of higher arsenic concentration, existing or new replacement wells of greater depth are likely to be safe provided care is taken during borehole construction to prevent “leakage” of contaminated water from shallow into deeper aquifers. Since this approach would eventually provide villages with a virtually permanent source of safe drinking water, it seems unlikely that any other approach would be as good. However, it would some take time to implement, and as it is important that ingestion of arsenic should cease without delay, other methods of mitigation may help to bridge the gap. These include rainwater harvesting, sand filters and other commercially available filters, and small-scale local supply systems – but all entail costs, and Bangladesh is a very poor country.

6.2 Safe water: lessons from working in contaminated villages

It is now well established, from the present and related studies, that chronic exposure to excess arsenic through drinking water and food chain is a significant hazard to the health of Bangladeshis taking water from contaminated tube wells. Availability of safe water is a basic human right and a citizens' movement is absolutely necessary to avert arsenic related morbidity and mortality in Bangladesh and West Bengal, India. Safe water must be put on the public agenda if there is to be sufficient political commitment to ensure investment of resources to alleviate the effects of arsenic exposure.

Although villagers using contaminated water have some understanding of the health risk, their reliance on this water is unlikely to change unless alternative water options are provided. The options so far tried in Bangladesh are not adequately evaluated and as such it is very difficult to recommend any particular technology. However, from experience in working with villagers in areas with arsenic contaminated wells, I would like to make following suggestions:

Developing a village well policy: In the study villages there was, on average, one well for every 8 persons, with an average distance between them of about 70 meters. Even in these contaminated villages one third of wells met the Bangladesh standard. Opportunities should not be missed to use these safe water sources for the benefit of the whole community. There should be a village well policy developed in a participatory process, facilitated by the government, where the villagers decide how best to manage water resources in their village. Further understanding of the spatial variation of arsenic in drinking water at the local level is very important and will help to answer villagers' questions about how far they will need to travel for water collection if contaminated wells are closed down. Local level participatory action research should document the outcome of this approach.

At a national level, an arsenic communication policy should be developed that will take into account the socio-cultural realities in Bangladesh. To do so, much more needs to be known about water utilization behavior and its relationship with arsenic toxicity; for example, we need to understand more clearly how arsenic is changed in cooking, water collection and storage at the village level. Such studies may generate valuable information for risk reduction. The resulting arsenic mitigation strategies and policies must be formulated and reformulated in such a way that it is really owned by its target community. In our study villages, trained paramedics identified simple skin lesions and village level health providers with some training may go on to play a vital role in arsenic related health surveillance. Technologies and practices for arsenic mitigation must be sustainable, suitable and simple if they are to have a real and lasting impact in reducing the use of arsenic contaminated water.

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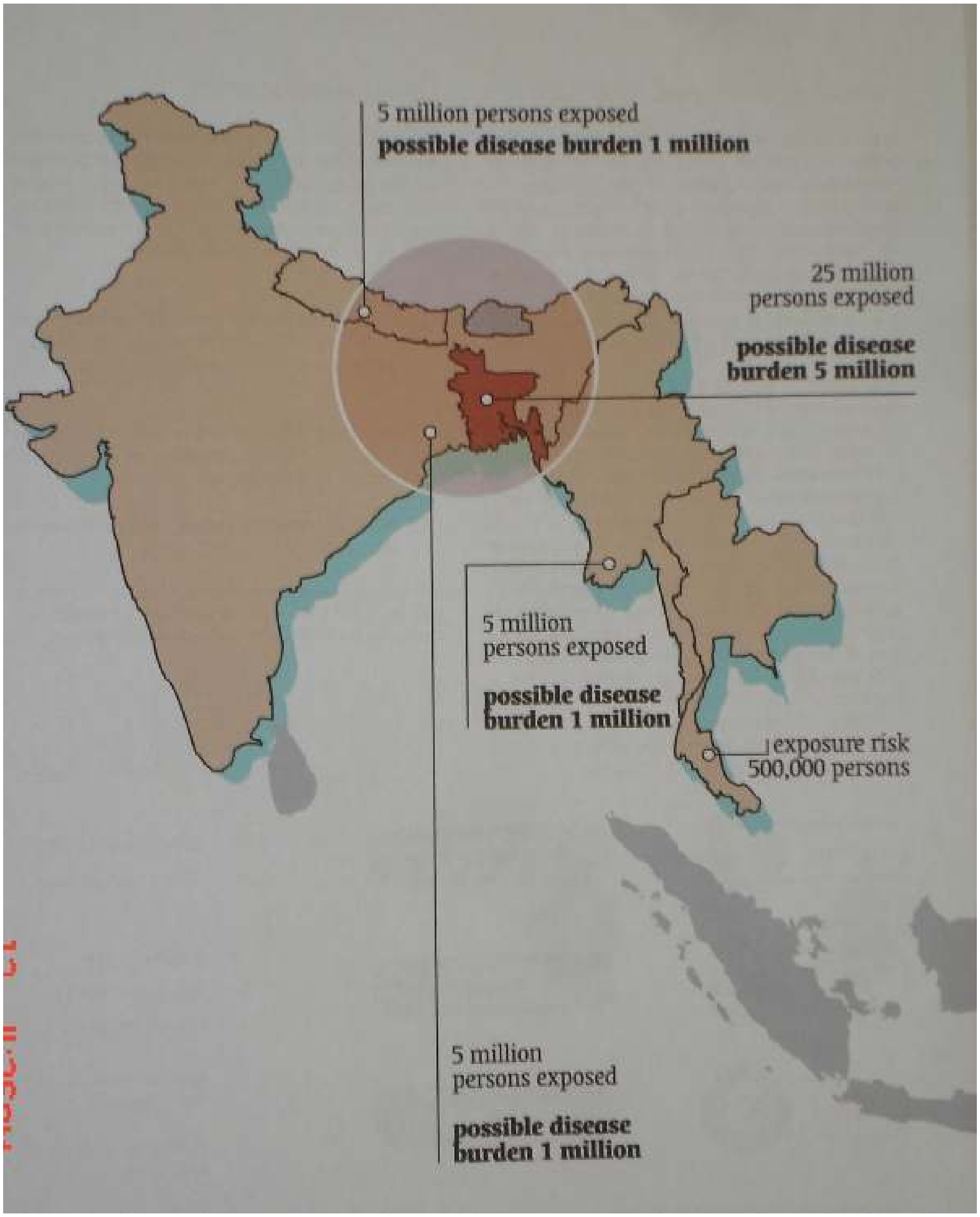
Appendix 1
Geographical distribution of naturally occurring arsenic in the world

Source: World Bank, Towards a more effective operational response: Arsenic concentration of ground water in South and East Asian Countries, Report No 31303, p 30


IBRD 33757
April 2005
Fairbanks, Alaska
Aleutian Islands
Coeur D'Alene
British Columbia
Lake Oahe
Yellowstone Park
Western, U.S.A.
Clark Fork River
Wisconsin
Halifax, Nova Scotia
Don Pedro, California
North-Central Mexico
Baja California
Comarca Lagunera, Mexico
Zimapan Valley
Dominica
El Salvador
Iceland
South-west England
South-west Poland
Great Hungarian Plain, Hungary, Romania
Massif Central, France
Styria, Austria
Lavrion Peninsula, Greece
Kamchatka
Xinjiang Province, China
Inner Mongolia, China
Gubong, Rep. of Korea
Kyushu, Japan
Punjab, Pakistan
Terai, Nepal
Taiwan Province, China
Bengal Delta, West Bengal, Bangladesh
Red River Delta
Irrawaddy Delta, Myanmar
Mekong River, Cambodia
Ron Phibun, Thailand
Sarawak
Ashanti Region, Ghana
Zimbabwe
Antofagasta, Chile
Minas Gerais, Brazil
Chaco-Pampean Plain, Argentina
Wairakei, New Zealand
LEGEND
Arsenic-affected aquifers
Arsenic related to mining and mineralization
Geothermal waters

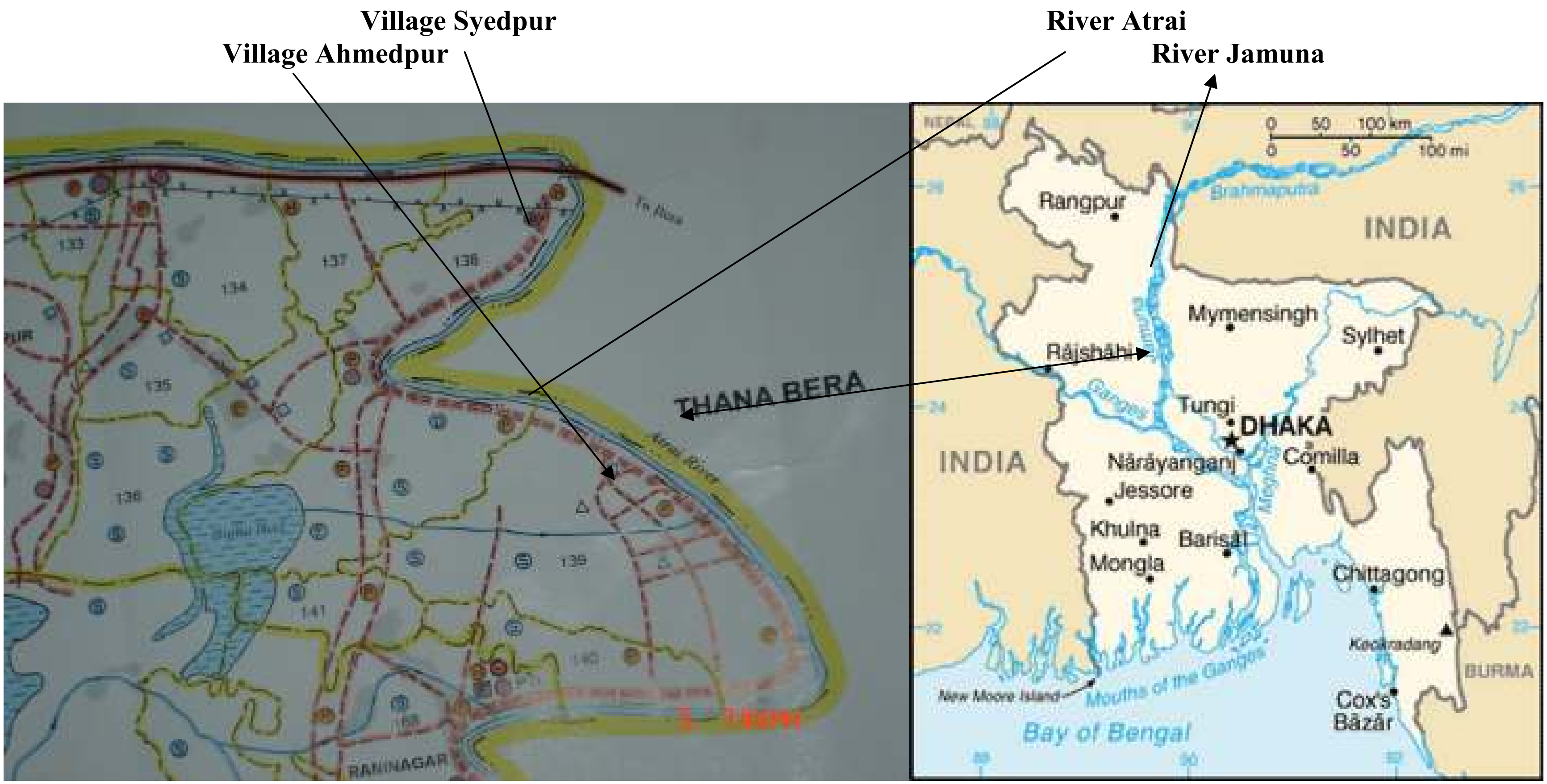
Appendix 2
Geographical distribution of naturally occurring arsenic in South Asia

Source: World Health Organization, Regional Office for South East Asia, A Field Guide for detection, management and surveillance of arsenicosis, (First working copy), WHO technical publication No SEA/EH/545


5 million persons exposed
possible disease burden 1 million
25 million
persons exposed
possible disease
burden 5 million
5 million
persons exposed
possible disease
burden 1 million
exposure risk
500,000 persons
5 million
persons exposed
possible disease
burden 1 million

Appendix 3

Geographical location of study villages
Source: Produced from the maps collected from Local Government Engineering Department (LGED) office, Dhaka


Village Syedpur
Village Ahmedpur
River Atrai
River Jamuna
THANA BERA
RANINAGAR
Atrai River
0 50 100 km
0 50 100 mi
Brahmaputra
Rangpur
INDIA
Mymensingh
Sylhet
Rājshāhi
Ganges
Tungi
DHAKA
Comilla
Nārāyanganj
INDIA
Jessore
Khulna
Barisāl
Mongla
Meghna
Chittagong
Keokradang
BURMA
New Moore Island
Mouths of the Ganges
Cox's Bāzār
Bay of Bengal

Appendix 4

Well Survey record card

Well Survey: Village Name

Well #	Village initial				Family ID				_					-		

GPS 1: N /_____/_____/_____/ E /_____/_____/_____/

GPS 2: N /_____/_____/_____/ E /_____/_____/_____/

Date:	Without Dilution			If diluted 1 2 3 4 5		
Color						
Digital						

Remarks: ____

____

Appendix 5

Code book for well data entry

Appendix 5

Codebook for well Survey date

Sl	Variables	Codes	Remarks
1	Village	1=Syedpur 2=Ahmedpur	
2	Well Type	1=Tube well 2= Deep Tube well 3= Dug well 4= Supply water 9=Not known	
3	Well ID	999= Not known	
4	Family ID	9999= Not known	
5	GPS N 1 degree	99 = Not recorded	
6	GPS N I minute	99 = Not recorded	
7	GPS N 1 second	99.9=Not recorded	
8	GPS E 1 degree	99 = Not recorded	
9	GPS E 1 minute	99 = Not recorded	
10	GPS E 1 second	99.9=Not recorded	
11	GPS N 2 degree	99 = Not recorded	
12	GPS N 2 minute	99 = Not recorded	
13	GPS N 2 second	99.9=Not recorded	
14	GPS E 2 degree	99 = Not recorded	
15	GPS E 2 minute	99 = Not recorded	
16	GPS E 2 second	99.9=Not recorded	
17	Test date		
18	Test Status	1=Yes 2=No 3=Out of order	

Sl	Variables	Codes	Remarks
19	Color 1	1=<10 µg/L 2= 20-40 µg/L 3=50 µg/L 4= 60-80 µg/L 5= 100 µg/L 6= 100-200 µg/L 7= 200-300 µg/L 8= 300-400 µg/L 9=400-500 µg/L 10= > 500µg/L 77= Faulty appearance 99= Not taken	
20	Digital 1	Actual reading 777= 100+ µg/L 888= Error 999= Not taken	
21	Color 2	As color 1	
22	Digital 2	As digital 1	
23	Color 3	As color 1	
24	Digital 3	As digital 1	
25	Dilution	Actual times 88=Not applicable	
26	Dilution color 1		
27	Dilution digital 1		
28	Dilution color 2		
29	Dilution digital 2		
30	Dilution color 3		
31	Dilution digital 3		
32	Comment	99=No comment	

Appendix 6

Skin lesions survey record card

Village GA PA	GK Skin Survey -Kashinathpur			Name of Paramedic:				
Name of Family Head:	Land lord:	How long family head drinking TW water:		Owner of the most commonly used TW:				
				TW#				

Skin examination (check with in appropriate boxes)														
				Palm of hands					Sole of feet					
Name of family members	Gender	Age (yrs)	Date of exam	Thickening		Nodules			Thickening		Nodules			
				No	Yes	No	1-9	≥10	No	Yes	No	1-9	≥10

Location of house hold:
GPS 1: N _____/______/______/ E: _____/______/______/ GPS 2: N _____/______/______/ E: _____/______/______/

Any Additional Information:

Appendix 7

Code book for skin lesions data entry

Appendix 7

GK Skin Lesions Survey, Kashinathpur

Code Book

sl	Variable	Code	
01	GA	Actual #	
02	PA	Actual #	
03	Family serial	Actual #	
04	Village	1=Syedpur 2=Ahmedpur	
05	Sex	1=Male 2=Female	
06	Family Head?	1=Yes 2=No 8=Not Recorded 9=Not applicable	
07	Why not	1=Seen 2=Dead 3= Living elsewhere 4=Not done 5=Not seen 9=No information	
09	Age	Actual age 99=No information	
10	Palmthick	1=Yes 2=No	
11	Solethick	1=Yes 2=No	
12	Palm nodules	1=No 2= 1-9 3= >9	
13	Sole Nodules	1=No 2= 1-9	

		3= >9	
14	GPS1 N Deg	Actual reading	
15	GPS1 N Minute	Actual reading	
16	GPS1 N Sec	Actual reading	
17	GPS1 E Deg	Actual reading	
18	GPS1 E Minute	Actual reading	
19	GPS1 E Sec	Actual reading	
20	GPS2 N Deg	Actual reading	
21	GPS2 N Minute	Actual reading	
22	GPS2 N Sec	Actual reading	
23	GPS2 E Deg	Actual reading	
24	GPS2 E Minute	Actual reading	
25	GPS2 E Sec	Actual reading	

Appendix 8

Questionnaire for case referent study

Appendix 8

Paramedic: __________________ Sl # ________

Study on Arsenic problem in Syedpur and Ahmedpur village

Administrative information:

1. GA_______/ PA _______ Serial # ________ Family No (New): _______
2. Name in Family Card: ____
3. □Male □Female
4. Description of home visit for interview:
   a) First time date: _________________ outcome: _____________
   b) Second time date:________________ outcome: _____________
   c) Third time date: _________________ outcome: _____________
5. Place of Interview: ____
6. Interview starts: __________________ ends: _____________________
7. Date of Interview: ____

General Information:

8. Your name please (including nick name): ____________________________
9. How old are you? _________ years Actual: _____ Tentative: ___________
10. What class or degree in school you have completed? __________________

11. Description of places lived continuously for more than 6 months since birth (present to past):
    a.1) Period: Since ____________ till date.
    a.2) Complete address: ____
    a.3) Do you drink Tube well water at this address: Yes No
    If yes, ID number of most commonly used tube well __________________
    If the well number is not known, or if it does exist any more, record the owner or the exact location of the well.
    a.4) How long you drank tube well water at this address? ________ years.

b.1) Period: From ___________________ to : _______________________.

b.2) Complete address: ____

b.3) Do you drink Tube well water at this address: Yes No

If yes, ID number of most commonly used tube well ___________________

If the well number is not known, or if it does exist any more, record the owner or the exact location of the well.

b.4) How long you drank tube well water at this address? ________ years.

b.5) Reason for leaving this address? ______________________________

b. 6) GPS location 1: N ____/_____/______ E ____/_____/______

GPS location 2: N ____/_____/______ E ____/_____/______

c.1) Period: From ___________________ to : _______________________.

c.2) Complete address: ____

c.3) Do you drink Tube well water at this address: Yes No

If yes, ID number of most commonly used tube well ___________________

If the well number is not known, or if it does exist any more, record the owner or the exact location of the well.

c.4) How long you drank tube well water at this address? ________ years.

c.5) Reason for leaving this address? ______________________________

c. 6) GPS location 1: N ____/_____/______ E ____/_____/______

GPS location 2: N ____/_____/______ E ____/_____/______

d.1) Period: From ___________________ to : _______________________.

d.2) Complete address: ____

d.3) Do you drink Tube well water at this address: Yes No

If yes, ID number of most commonly used tube well ___________________

If the well number is not known, or if it does exist any more, record the owner or the exact location of the well.

d.4) How long you drank tube well water at this address? ________ years.

d.5) Reason for leaving this address? ______________________________

d. 6) GPS location 1: N ____/_____/______ E ____/_____/______

GPS location 2: N ____/_____/______ E ____/_____/______

e.1) Period: From __________________ to : ______________________.

e.2) Complete address: ____

e.3) Do you drink Tube well water at this address: Yes No

If yes, ID number of most commonly used tube well __________________

If the well number is not known, or if it does exist any more, record the owner or the exact location of the well.

e.4) How long you drank tube well water at this address? ________ years.

e.5) Reason for leaving this address? _____________________________

e. 6) GPS location 1: N ____/_____/______ E ____/_____/______

GPS location 2: N ____/_____/______ E ____/_____/______

f.1) Period: From __________________ to : ______________________.

f.2) Complete address: ____

f.3) Do you drink Tube well water at this address: YesNo

If yes, ID number of most commonly used tube well __________________

If the well number is not known, or if it does exist any more, record the owner or the exact location of the well.

f.4) How long you drank tube well water at this address? ________ years.

f.5) Reason for leaving this address? _____________________________

f. 6) GPS location 1: N ____/_____/______ E ____/_____/______

GPS location 2: N ____/_____/______ E ____/_____/______

g.1) Period: From __________________ to : ______________________.

g.2) Complete address: ____

g.3) Do you drink Tube well water at this address: Yes No

If yes, ID number of most commonly used tube well __________________

If the well number is not known, or if it does exist any more, record the owner or the exact location of the well.

g.4) How long you drank tube well water at this address? ________ years.

g.5) Reason for leaving this address? _____________________________

g. 6) GPS location 1: N ____/_____/______ E ____/_____/______

GPS location 2: N ____/_____/______ E ____/_____/______

h.1) Period: From ___________________ to : _______________________.

h.2) Complete address: ____

h.3) Do you drink Tube well water at this address: Yes No

If yes, ID number of most commonly used tube well ___________________

If the well number is not known, or if it does exist any more, record the owner or the exact location of the well.

h.4) How long you drank tube well water at this address? ________ years.

h.5) Reason for leaving this address? ______________________________

h. 6) GPS location 1: N ____/_____/______ E ____/_____/______

GPS location 2: N ____/_____/______ E ____/_____/______

i.1) Period: From ___________________ to : _______________________.

i.2) Complete address: ____

i.3) Do you drink Tube well water at this address: Yes No

If yes, ID number of most commonly used tube well ___________________

If the well number is not known, or if it does exist any more, record the owner or the exact location of the well.

i.4) How long you drank tube well water at this address? ________ years.

i.5) Reason for leaving this address? ______________________________

i. 6) GPS location 1: N ____/_____/______ E ____/_____/______

GPS location 2: N ____/_____/______ E ____/_____/______

12. Description of food items consumed in past seven days:

12.a) How many eggs have you eaten in past seven days? If whole egg/ or omelet etc eaten what portion was eaten?

Times________ X portion _____________ Total: ____ egg

Times________ X portion _____________

12.b) In last seven days, how many times have you eaten dal? Describe in common serving cup: Full cup/ ½ cup or ¼ cup/ Thick (like yogurt) / Medium thick or thin (like milk) dal?

Times ________ X amount X Thickness Total: ____ cups

Times ________ X amount X Thickness

12.c) How many times in past seven days, have you eaten fish? Pieces of big fishes (big, medium or small pieces), or fish fries (½ cup. Or ¼ cup) describe?

Times ______ X Size ________ X Pieces Total: ____ gm

Times ______ X Size ________ X Pieces

12.d) How many times in past seven days have you eaten meat? Describe in serving size: big/ medium or small piece:

Times ______ X Size ________ X Pieces Total: ____ gm

Times ______ X Size ________ X Pieces

13. Where do you spend most of the time of a day: at own house or at work or friends or relations house?

1) Always at own home
2) Some time at other place (at work / in friends or relations house: ____)
   If yes, how long ______ hours /day
3) If some time spend days or weeks outside, please describe?

____

13.a) When you stay at home, how many glasses of TW water do you drink?
___ glass/day

13.b) (if do not stay at home always) While away from home, how many glasses of TW water you drink per day?

glass/day, Well # _________ Not applicable

13.c) When you stay at home, how many cups of tea made of TW you drink

#______ cup tea/day

13. d) (if do not stay at home always) While away from home, how many cups of tea made of TW water you drink per day?

_____ cup of tea   Not applicable

13.e) At your home, do you use well water for cooking (rice, curry or dal cooking)?

Yes/ No   Not applicable

13. f)) (if do not stay at home always) While away from home, if TW is used for cooking (rice, curry or dal) at that place?

Yes/ No   Not applicable

Now you please remember your things two years before

14. Two years ago, where do you spend most of the time of a day: at own house or at work or friends or relations house?

1) Always at own home
2) Some time at other place (at work / in friends or relations house: ____)
   If yes, how long   ______ hours /day
3) If some time spend days or weeks outside, please describe?

____

14.a) When you stay at home, how many glasses of TW water you used to drink two years ago?   ___ glass/day

14.b) (if do not stay at home always) While away from home, how many glasses of TW water you used to drink per day?

glass/day,   Well # _________   Not applicable

14.c) When you stay at home, how many cups of tea made of TW you used to drink ? #______ cup tea/day

14. d) (if do not stay at home always) While away from home, how many cups of tea made of TW water you used to drink per day?

_____ cup of tea Not applicable

14.e) At your home, did you use well water for cooking (rice, curry or dal cooking)?

Yes/ No Not applicable

14. f)) (if do not stay at home always) While away from home, if TW was used for cooking (rice, curry or dal) at that place?

Yes/ No Not applicable

15. Weight (in kg upto one decimal point): _____ 16. Height in cm: _________

Thank you for your participation in the study

Appendix 9

Field ethics note for case referent study

Appendix 9

We are conducting a research in Ahmedpur and Syedpur village on Arsenic related health problems. It will help us to understand some health problems caused by arsenic in water. You have been selected for interview in the study. If you agree, we would like to interview you. Your participation is optional. The information we gather from you will be kept confidential and will be used for research purposes only.

Appendix 10

Show cards indicating portion size

10.1 Meat
10.2 Large fish
10.3 Small fish
10.4 Thick dal (45 gm)
10.5 Medium dal (30 gm)
10.6 Thin dal (20 gm)


Sage
hyme

Appendix 10.2

Appendix 10.3

Appendix 10.4


Thyme
SALTER
296


Thyme
SALTER

Appendix 11

Serving size of protein food items

Serving size of different type of protein food

Meat (Beef, Mutton, Chicken etc):

Large piece	50 gm
Medium piece	25 gm
Small piece	15 gm

Fish (big and fish fries):

Large serving	50 gm
Medium serving	25 gm
Small serving	15 gm

Lentil (200ml cup):

Thick serving	45 gm
Medium thick	30gm
Thin	20 gm
Mesh/khichuri	45 gm

Appendix 12

Code book for case referent study

Study on Arsenic in two villages
Master Code Book

Ref	Variable ID	Code	
	Serial #	Serial #	|___|___|___|
	Village	Syedpur = 1 Ahmedpur = 2	|___|
1	GA	Actual number	|___|___|___|
1	PA	Actual number	|___|___|___|___|
1	Family Serial	Actual number	|___|___|
3	Sex	Male=1 Female=2	|___|
	Is it a case or control?	Case=1 Control =2	|___|
	If control, Control #	1st Control=1 2nd Control=2 3rd Control=3 4th control=4 5th Control=5 Not applicable=8	|___|
	If control, referent case sl #	Actual case sl # Not applicable=8888	|___|___|___|___|
4	Outcome of final attempt for interview	Interviewed=1 Dead=2 Migrated =3 Not at home=4	|___|
5	Place of interview	Home=1 Field=2	|___|

		Market=3 Not applicable=8 Not recorded=9	
6	Hour -interview started (on 24 hr clock)	Time interview started Not applicable=88 Not recorded=99	|___|___|
6	Mnts-interview started	Time interview started Not applicable=88 Not recorded=99	|___|___|
6	Hour-interview finished on 24 hr clock	Time interview finished Not applicable=88 Not recorded=99	|___|___|
6	Mnts-interview finished	Time interview finished Not applicable=88 Not recorded=99	|___|___|
	Is the interview complete	Yes=1 No=2	|___|___|
	If not complete, why	Very sick=1 Disable=2 Not applicable=8 Not recorded=9	|___|
7	Day of final attempt for interview	Day of the month Not applicable=88 Not recorded=99	|___|___|
7	Month of final attempt for interview	Month of the year Not applicable=88 Not recorded=99	|___|___|
7	Year of final attempt for interview (English calendar)	Year Not applicable=8888 Not recorded=9999	|___|___|___|___|
7	# attempts for interview	Not applicable=8 Not recorded=9	|___|
9	Age	Age in years Not applicable=888 Not recorded=999	|___|___|___|

9	Age estimation	Estimated=1 Actual age=2 Not applicable=8 Not recorded=9	|___|

10	Education	# completed years
		SSC=10
		HSC=12
		BA/BSc (pass course)=14
		BA/BSc (hons)=15
		MA/MSc/BSS (new sys)=16
		Can sign only=77
		Not applicable=88
		Not recorded=99
			|___|___|

	Number of addresses lived	Not applicable=88 Not recorded=99	|___|___|
11.A1	At Address1 (That is, current address) since	Year in English calendar	|___|___|___|___|
	Address1 in	Syedpur=1 Ahmedpur=2 Other=3 Not applicable=8 Not recorded=9	|___|
	Address during prevalence survey	First=1 Second =2 3rd=3 4th=4	|___|
11.A3	Used TW at Address1	Yes=1 No=2 Not applicable=8 Not recorded=9	|___|
11.A4	How long used TW at Address1	# years Not applicable=88 Not Recorded=99	|___|___|
	GPS Present address: North Degree1	Degree Not applicable=88 Not Recorded=99	

	North Minutes1	Minutes Not applicable=88 Not Recorded=99	
	North Seconds1	Seconds Not applicable=8888 Not Recorded=9999	
	GPS Present address: East Degree1	Degree Not applicable=88 Not Recorded=99	
	East Minutes1	Minutes Not applicable=88 Not Recorded=99	
	East Seconds1	Seconds Not applicable=8888 Not Recorded=9999	
11B1	At Address2 from IF no more addresses code 8888 here and leave rest of address 2 data blank.	# Year Not recorded=8888 Not applicable=9999	|___|___|___|___|
11B1	At Address2 to	# Year Not recorded=8888 Not applicable=9999	|___|___|___|___|
	Address2 in	Syedpur=1 Ahmedpur=2 Other=3 Not applicable=8	|___|
11B3	Used TW at Address2	Yes=1 No=2 Not Applicable=8 Not recorded=9	|___|
11B4	How long used TW at Address2	Not Applicable=88 Not recorded =99	|___|___|
	N degree of address 2	Actual reading Not applicable=88 Not recorded =99	|___|___|

	N mnts of address 2	Actual reading Not applicable=88 Not recorded =99	|___|___|
	N seconds of address 2	Actual reading Not applicable=88 Not recorded =99	|___|___|___|___|
	E degree of address 2	Actual reading Not applicable=88 Not recorded =99	|___|___|
	E mnts of address 2	Actual reading Not applicable=88 Not recorded =99	|___|___|
	E seconds of address 2	Actual reading Not applicable=88 Not recorded=99	|___|___|___|___|
11C1	At Address3 from IF no more addresses code 8888 here and leave rest of address 3 data blank.	#Year Not applicable=8888 Not recorded=9999	|___|___|___|___|
11C1	At Address3 to	# Year Not applicable=8888 Not recorded=9999	|___|___|___|___|
	Address3 in	Syedpur=1 Ahmedpur=2 Other=3 Not applicable=8	|___|
11C3	Used TW at Address3	Yes=1 No=2 Not Applicable=8 Not recorded=9	|___|
11C4	How long used TW at Address3	# years Not applicable=88 Not recorded=99	|___|___|
	N degree of address 3	Actual reading Not applicable=88 Not recorded =99	|___|___|
	N mnts of address 3	Actual reading	

		Not applicable=88 Not recorded =99	|___|___|
	N seconds of address 3	Actual reading Not applicable=88 Not recorded =99	|___|___|___|___|
	E degree of address 3	Actual reading Not applicable=88 Not recorded =99	|___|___|
	E mnts of address 3	Actual reading Not applicable=88 Not recorded =99	|___|___|
	E seconds of address 3	Actual reading Not applicable=88 Not recorded =99	|___|___|___|___|
11D1	At Address4 from IF no more addresses code 8888 here and leave rest of address 4 data blank.	#Year Not applicable=8888 Not recorded=9999	|___|___|___|___|
11D1	At Address4 to	#Year Not applicable=8888 Not recorded=9999	|___|___|___|___|
	Address4 in	Syedpur=1 Ahmedpur=2 Other=3 Not applicable=8	|___|
11D3	Used TW at Address4	Yes=1 No=2 Not Applicable=8 Not recorded=9	|___|
11D4	How long used TW at Address4	# years Not applicable=88 Not recorded=99	|___|___|
	N degree of address 4	Actual reading Not applicable=88 Not recorded =99	|___|___|

	N mnts of address 4	Actual reading Not applicable=88 Not recorded =99	|___|___|
	N seconds of address 4	Actual reading Not applicable=88 Not recorded =99	|___|___|___|___|
	E degree of address 4	Actual reading Not applicable=88 Not recorded =99	|___|___|
	E mnts of address 4	Actual reading Not applicable=88 Not recorded =99	|___|___|
	E seconds of address 4	Actual reading Not applicable=88 Not recorded =99	|___|___|___|___|
11E1	At Address5 from IF no more addresses code 8888 here and leave rest of address 5 data blank.	#Year Not applicable=8888 Not recorded=9999	|___|___|___|___|
11E1	At Address5 to	#Year Not applicable=8888 Not recorded=9999	|___|___|___|___|
	Address5 in	Syedpur=1 Ahmedpur=2 Other=3 Not applicable=8	|___|
11E3	Used TW at Address5	Yes=1 No=2 Not Applicable=8 Not recorded=9	|___|
11E4	How long used TW at Address5	# years Not applicable=88 Not recorded=99	|___|___|
	N degree of address 5	Actual reading Not applicable=88 Not recorded =99	|___|___|

	N mnts of address 5	Actual reading Not applicable=88 Not recorded =99	|___|___|
	N seconds of address 5	Actual reading Not applicable=88 Not recorded =99	|___|___|___|___|
	E degree of address 5	Actual reading Not applicable=88 Not recorded =99	|___|___|
	E mnts of address 5	Actual reading Not applicable=88 Not recorded =99	|___|___|
	E seconds of address 5	Actual reading Not applicable=88 Not recorded =99	|___|___|___|___|
11F1	At Address6 from IF no more addresses code 8888 here and leave rest of address 6 data blank.	#Years Not applicable=8888 Not recorded=9999	|___|___|___|___|
11F1	At Address6 to	#Year Not applicable=8888 Not recorded=9999	|___|___|___|___|
	Address6 in	Syedpur=1 Ahmedpur=2 Other=3 Not applicable=8	|___|
11F3	Used TW at Address6	Yes=1 No=2 Not Applicable=8 Not recorded=9	|___|
11F4	How long used TW at Address6	# years Not applicable=88 Not recorded=99	|___|___|
	N degree of address 6	Actual reading Not applicable=88	|___|___|

		Not recorded =99	
	N mnts of address 6	Actual reading Not applicable=88 Not recorded =99	|___|___|
	N seconds of address 6	Actual reading Not applicable=88 Not recorded =99	|___|___|___|___|
	E degree of address 6	Actual reading Not applicable=88 Not recorded =99	|___|___|
	E mnts of address 6	Actual reading Not applicable=88 Not recorded =99	|___|___|
	E seconds of address 6	Actual reading Not applicable=88 Not recorded =99	|___|___|___|___|
11G1	At Address7 from IF no more addresses code 8888 here and leave rest of address 7 data blank.	#Years Not applicable=8888 Not recorded=9999	|___|___|___|___|
11G1	At Address7 to	#Year Not applicable=8888 Not recorded=9999	|___|___|___|___|
	Address7 in	Syedpur=1 Ahmedpur=2 Other=3 Not applicable=8	|___|
11G3	Used TW at Address7	Yes=1 No=2 Not Applicable=8 Not recorded=9	|___|
11G4	How long used TW at Address7	# years Not applicable=88 Not recorded=99	|___|___|
	N degree of address 7	Actual reading	

		Not applicable=88 Not recorded =99	|___|___|
	N mnts of address 7	Actual reading Not applicable=88 Not recorded =99	|___|___|
	N seconds of address 7	Actual reading Not applicable=88 Not recorded =99	|___|___|___|___|
	E degree of address 7	Actual reading Not applicable=88 Not recorded =99	|___|___|
	E mnts of address 7	Actual reading Not applicable=88 Not recorded =99	|___|___|
	E seconds of address 7	Actual reading Not applicable=88 Not recorded =99	|___|___|___|___|
11H1	At Address8 from IF no more addresses code 8888 here and leave rest of address 8 data blank.	#Years Not applicable=8888 Not recorded=9999	|___|___|___|___|
11H1	At Address8 to	#Year Not applicable=8888 Not recorded=9999	|___|___|___|___|
	Address8 in	Syedpur=1 Ahmedpur=2 Other=3 Not applicable=8	|___|
11H3	Used TW at Address8	Yes=1 No=2 Not Applicable=8 Not recorded=9	|___|
11H4	How long used TW at Address8	# years Not applicable=88 Not recorded=99	|___|___|

11I1	At Address9 from IF no more addresses code 8888 here and leave rest of address 9 data blank.	#Years Not applicable=8888 Not recorded=9999	|___|___|___|___|
11I1	At Address9 to	#Year Not applicable=8888 Not recorded=9999	|___|___|___|___|
	Address9 in	Syedpur=1 Ahmedpur=2 Other=3 Not applicable=8	|___|
11I3	Used TW at Address9	Yes=1 No=2 Not Applicable=8 Not recorded=9	|___|
11I4	How long used TW at Address9	# years Not applicable=88 Not recorded=99	|___|___|
11J1	At Address10 from IF no more addresses code 8888 here and leave rest of address 10 data blank.	#Years Not applicable=8888 Not recorded=9999	|___|___|___|___|
11J1	At Address10 to	#Year Not applicable=8888 Not recorded=9999	|___|___|___|___|
	Address10 in	Syedpur=1 Ahmedpur=2 Other=3 Not applicable=8	|___|
11J3	Used TW at Address10	Yes=1 No=2 Not Applicable=8 Not recorded=9	|___|
11J4	How long used TW at Address10	# years Not applicable=88 Not recorded=99	|___|___|

11K1	At Address11 from IF no more addresses code 8888 here and leave rest of address 11 data blank.	#Years Not applicable=8888 Not recorded=9999	|___|___|___|___|
11K1	At Address11 to	#Year Not applicable=8888 Not recorded=9999	|___|___|___|___|
	Address11 in	Syedpur=1 Ahmedpur=2 Other=3 Not applicable=8	|___|
11K3	Used TW at Address11	Yes=1 No=2 Not Applicable=8 Not recorded=9	|___|
11K4	How long used TW at Address11	# years Not applicable=88 Not recorded=99	|___|___|
12.A	Egg eaten last week	Yes=1 No=2 Not applicable=8 Not recorded=9	|___|
	Number of Eggs eaten last week	Not applicable=88 Not recorded=99	
12.B	Eaten Dal last week	Yes=1 No=2 Not applicable=8 Not recorded=9	|___|
	Amount of Dal eaten last week	Not applicable=88 Not recorded=99	
12C	Eaten fish last week	Yes=1 No=2 Not applicable=88 Not recorded=99	|___|___|
	Amount of fish eaten last week	Not applicable=88 Not recorded=99	

12.D	Eaten meat last week	Yes=1 No=2 Not applicable=8 Not recorded=9	|___|
	Amount of meat eaten last week	Not applicable=88 Not recorded=99	
13	Regularly spend time outside	No=1 Outside for short time=2 Outside for days=3 Not applicable=8 Not recorded=9	|___|
13	Hours outside/day	# hours Not applicable=88 Not recorded=99	|___|___|
13.A	Glass of TW drank at home/day	# glasses Not applicable=88 Not recorded=99	|___|___|
13.B	Glass of TW outside home	# glasses Not applicable=88 Not recorded=99	|___|___|
13B	TW number	TW # Not applicable=888 Not recorded=999	|___|___|___|
13C	Cups of tea of TW at home/day	# cups of tea Doesn't drink tea=77 Not applicable=88 Not recorded=99	|___|___|
13D	Cups of tea of TW at outside/day	# cups of tea Doesn't drink tea=77 Not applicable=88 Not recorded=99	|___|___|
13E	TW for cooking at home	Yes=1 No=2 Others=7 Not applicable=8 Not recorded=9	|___|

13F	TW for cooking outside	Yes=1 No=2 Others=7 Not applicable=8 Not recorded=9	|___|
14	2 years ago, time spend out of home	No=1 Outside for short time=2 Outside for days=3 Not applicable=8 Not recorded=9	|___|
	Hours outside/day	# hours/day Not Known=88 Not Applicable=99	|___|
14A	Glass of TW drank at home/day	# glasses Not applicable=88 Not recorded=99	|___|___|
14B	Glass of TW outside home	# glasses Not applicable=88 Not recorded=99	|___|___|
	TW number	TW# Not applicable=888 Not Recorded=999	|___|___|___|
14C	Cups of tea of TW at home/day	#cups of tea Doesn't drink tea=77 Not applicable=88 Not recorded=99	|___|___|
14D	Cups of tea of TW outside/day	# cups of tea Doesn't drink tea=77 Not applicable=88 Not recorded=99	|___|___|
14E	TW for cooking at home	Yes=1 No=2 Others=7 Not applicable=8 Not recorded=9	|___|
14F	TW for cooking outside	Yes=1 No=2 Others=7	|___|

		Not applicable=8 Not recorded=9	
15	Weight	# kg Not applicable=8888 Not recorded=9999	|___|___|___|___|
16	Height	# cm Not applicable=888 Not recorded=999	|___|___|___|

Appendix 13

Reference table for protein portion estimation

There is difference in protein portions in food items as reported by different sources. Also the food items were not identical. We use following table to approximately estimate the protein content.

Food	**Protein portion**
Meat	25%
Fish	20.2%
Egg	8.6 gm/egg
Lentil	7.6%

Appendix 14

Major studies on arsenic related skin lesions in Bangladesh and West Bengal

Appendix 14

Major studies on arsenic related skin lesions in Bangladesh and West Bengal

Study	Study design	Study features	Key result
Mazumder DNG et al. (1998)	Cross sectional N=7683, West Bengal, India convenient sampling,	Arsenic up to 3400 µg/L, 80% < 500 µg/L Skin lesions keratosis and pigmentation change	Over all keratosis 2.0% Male 3.0%, Female 1.2% Male more susceptible Low BMI more susceptible Dose response relationship
Ahmad SA et al. (1999)	Cross sectional N=3606 Jessore, Bangladesh	About 60% well above 50 µg/L	Over all prevalence 10% Male more affected 52.6% Highest prevalence 20-29 yrs
Tondel M et al. (1999)	Cross sectional N=1481 Above 30 years	Ranged up to 2040 µg/L Only exposed and lifetime resident in the village were included	Over all prevalence 29.0% Males were more affected
Haque R et al. (2003)	Case referent Cases 192 Referents 213	Nested in Mazumder DNG study	Cases exposed to 325 µg/L Referents 180 µg/L
Milton AH et al. (2004)	Cross sectional 138 exposed cases 144 unexposed referents	Cases exposed to 641.15 µg/L Referents to13.5 µg/L	More risk at low BMI (<18.5) OR 1.92 (95% CI 1.3-2.8)
Mitra SR et al. (2004)	Case referent 192 cases 213 referents	Cases exposed <500 µg/L	Education and BMI no effect Animal protein consumption reduces risk
Rahman et al. (2006)	Case referent 504 cases 1830 referents Nested in their cross sectional survey	Arsenic up to 3644 µg/L 70% well above 10 µg/L	Cases were exposed to higher level of arsenic Cases had higher schooling, Male were more susceptible Cases had higher asset score
Rahman M et al. (2006)	Cross sectional N=166934 (above 4yrs) Entire population, Matlab, Bangladesh	Arsenic up to 3644 µg/L 70% well above 10 µg/L	Over all prevalence 3/1000 Male more susceptible Highest prevalence in 35-44 yr age group Higher schooling more susceptible

Study	Study design	Study features	Key result
McDonald et al. (2006)	Cross sectional survey of 53 villages Stratified random sample	Women aged 18 or more only	Overall prevalence 1.3% More than 20 times more risk at above 50 µg/L compared to ≤5µg/L
McCarty KM et al. (2006)	Case referent Pabna, Bangladesh 600 cases 600 referents	Examined the risk modifying effect of dietary variables	No significant difference in beef, beans, fowl, egg and fish consumption between the cases and referents.
Ahsan H et al. (2006)	Cohort/Cross sectional analysis N=11746		Risk of lesion positively modified by male gender, older age and low body mass index.
McDonald et al. (2007)	Cross sectional & case referent N=13705 Cases 155 Referents 155	All relevant wells tested with Arsenator	About 3 times risk of skin lesion at >50 µg/L Relative risk of lesion was consistent at all ages
Argos M et al. (2007)	Cohort N=11438 Married, resident for 3 years, 18-75 yrs of age	Cases 165.8 µg/L Referents 97.2 µg/L	Men more susceptible (POR 4.8, 95% CI 3.9-5.9) Lower schooling (≤2 yrs) increased risk (POR 1.6 95% CI 1.3-1.8) Older age group (36-75) at higher risk (POR 2.7 95% CI 2.2-3.3)

Appendix 15

Exposure measurement in different studies

Appendix 15

Exposure measurement in different studies

Study	Exposure measurement	Exposure classification
Mazumder DNG et al. (1998)	Water tested by HG-ASS	Daily dose /kg/body weight ≤ 50 µg/L, 50 - 99 µg/L 100-149 µg/L, 150-199 µg/L 200-349 µg/L, 350-499 µg/L 500-799 µg/L, >800 µg/L
Ahmad SA et al. (1999)	AAS	Mean time weighted arsenic exposure (mg) and Mean cumulative arsenic exposure (mg) Prevalence comparison not shown against concentration category
Tondel M et al. (1999)	Arsenic at present well Water data pooled from NIPSOM	≤ 150 µg/L, 151-350 µg/L 351-550 µg/L, 551-1000 µg/L >1000 µg/L
Rahman et al. (2006)	Well tested after prevalence survey Water tested by HG-AAS For non functioning well village average was used as proxy	Cumulative arsenic exposure (CAE) <100 µg/L, 100-499 µg/L 500-999 µg/L, 1000-4999 µg/L 5000-9999 µg/L, ≥10000 µg/L No comparison of prevalence in different exposure category
McDonald et al. (2006)	Geographical mean from BGS	≤5 µg/L, 6 -10 µg/L 11-50 µg/L, ≥50 µg/L
McDonald et al. (2007)	Average concentration of upazilla	1 µg/L 21 µg/L 43 µg/L 81 µg/L
Ahsan H et al. (2007)	HG-AAS, present well	<8.1 µg/L, 8.1-40.0 µg/L 40.1-91.1µg/L, 91.1-175.0µg/L 175.1-864.0 µg/L,

Appendix 16
Comparison of outcome measurement and method

Comparison of outcome measurement and method

Study	Skin lesion characterization	Examined by
Mazumder DNG et al. (1998)	Whole body Keratosis and pigmentation change	Physician
Ahmad SA et al. (1999)	Whole body Keratosis and pigmentation change	Physician
Tondel M et al. (1999)	Whole body Keratosis and pigmentation change	Physician
Rahman et al. (2006)	Whole body Keratosis and pigmentation change	Dermatologist
McDonald et al. (2006)	Keratosis on palm and sole	Trained Paramedics
Ahsan H et al. (2007)	Whole body Keratosis and pigmentation change	Physician

Appendix 17

Ethical approval letters

Health Research Ethics Board

213 Heritage Medical Research Centre
University of Alberta, Edmonton, Alberta T6G 2S2
p 780.492.9724 (Biomedical Panel)
p 780.492.[illegible] (Health Panel)
p 780.492.[illegible]
p 780.492.[illegible]
f 780.492.7808

ETHICS APPROVAL FORM

Date: **July 6, 2007**

Name(s) of Principal Investigator(s): **Prof. Nicola Cherry**

Department: **Medicine**

Title: **Risk estimates for arsenic related skin lesions in two large villages in Rajshahi Division, Bangladesh**

The Health Research Ethics Board (Biomedical Panel) has reviewed the protocol involved in this project which has been found to be acceptable within the limitations of human experimentation.

Specific Comments: This study will be conducted in Bangladesh, where verbal consent will be obtained because of the high illiteracy rate in the subject population.

J. S. Bamforth, M.D.
Associate Chairman, Health Research Ethics Board
Biomedical Panel

JUL - 6 2007
Date of Approval Release

This approval is valid for one year

Issue #6939

UNIVERSITY OF ALBERTA Capital Health Edmonton area CARITAS HEALTH GROUP

গণস্বাস্থ্য কেন্দ্রের ৩৫ বছর ২০০৭
years of Gonoshasthaya Kendra 1972-2007

GONOSHASTHAYA KENDRA
PEOPLES HEALTH CENTRE
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September 5, 2007

FAXED
492 9677

Professor Nicola Cherry
Community and Occupational Medicine Program
12-102 Clinical Sciences Building
University of Alberta, Edmonton
T6G 2G3, AB, Canada

Dear Professor Cherry,

Risk estimate of arsenic related skin lesions in two large villages in Rajshahi Division, Bangladesh

We have reviewed your research proposal "Risk estimate of arsenic related skin lesions in two large villages in Rajshahi Division" and recognize the importance of the work for understanding arsenic induced health issues in Bangladesh. The research proposal is found fully compliant with the ethical requirement of GK. As a partner in the research project, we would be pleased to provide facilities for the field study.

Yours sincerely,

Dr. Zafrullah Chowdhury
Projects Coordinator

Copy to:
Prof. Corbett McDonald, Imperial College School of Medicine, London
Dr. Sk. Nazmul Huda, School of Public Health, University of Alberta

Health Research Ethics Board

213 Heritage Medical Research Centre
University of Alberta, Edmonton, Alberta T6G 2S2
p.780.492.9724 (Biomedical Panel)
p.780.492.0302 (Health Panel)
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ETHICS APPROVAL FORM

Date: **July 2008**

Name of Principal Investigator(s): Dr. Nicola Cherry

Department: **Public Health Sciences**

Title: **Risk estimates for arsenic related skin lesions in two large villages in Rajshahi Division, Bangladesh**

The Health Research Ethics Board (Biomedical Panel) has reviewed the file on this project for which all documentation is currently up to date. The research has been found to be acceptable within the limitations of human experimentation.

Specific Comments:

This is the annual re-approval and is valid for one year. Next year, a few weeks prior to its expiration, a Progress Report will be sent to you for completion. If no major issues are identified, your approval will be renewed for another year.

For studies where investigators must obtain informed consent, signed copies of the consent form must be retained, as should all study related documents, so as to be available to the HREB on request. They should be kept for the duration of the project and for at least seven years following its completion. In the case of clinical trials approved under Division 5 of the Food and Drug regulations of Health Canada, study records must be retained for 25 years.

S.K.M. Kimber, MD, FRCPC
Chair, Health Research Ethics Board
Biomedical Panel

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