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Prenatal Arsenic Exposure and Consequences for Pregnancy Outcome and Infant Health

Epidemiological Studies in Bangladesh

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Abstract

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The aim of this thesis was to analyse possible effects of prenatal arsenic exposure on foetal and infant health. The setting is Bangladesh, where two cohorts were studied, both part of a health and demographic surveillance system in Matlab. A historical cohort 1991-2000 included 29,134 pregnant women with information on drinking water sources and arsenic testing of tube well water. A prospective cohort study included pregnant women 2002 - 2003 where urinary arsenic concentrations were assessed twice during pregnancy; 2,924 women and their pregnancy outcomes were evaluated for foetal loss, perinatal and infant mortality; 1,578 mother-infant pairs were analysed for size at birth; and 1,552 were analysed for morbidity during infancy.

Women exposed to arsenic levels ≥ 50 µg/L in water had an increased risk of foetal loss and infant death in comparison with women exposed to arsenic levels < 50 µg/L. These findings were confirmed in the prospective cohort study. Women with urine arsenic concentrations at the 5th quintile had 62% increased risk of spontaneous abortion (OR 1.62, 95% CI 1.04 - 2.55) in comparison with women who had arsenic concentrations at the 1st quintile level. Increased risks of perinatal morality (RR 3.01, 95% CI 1.07 - 8.45) and infant mortality (RR 5.01; 95% CI: 1.41 - 17.84) were also observed at the 5th quintile of exposure.

Significant negative dose-effect associations were found between arsenic exposure and birth weight, head and chest circumferences at a relatively low level of exposure (< 100 g/L in urine). In this range of exposure birth weight decreased by 1.68 g (SE 0.62) for each 1 µg/L increase of arsenic in urine.

In comparison with exposure at the 1st quintile level the risk of lower respiratory tract infection was significantly increased (RR 1.68, 95% CI 1.35-2.07) for women who had urinary arsenic concentrations at the 5th quintile level. The risk was also increased for diarrhoeal diseases.

The study findings highlight the negative effects of arsenic exposures on pregnancy outcomes and infant health. Mitigation programs need to be strengthened and women of reproductive ages should be prioritized in arsenic affected regions worldwide.

Keywords: arsenic, groundwater, foetal loss, birth weight, lower respiratory tract infection, diarrhoeal diseases, infant mortality, cohort, Bangladesh

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List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals I - IV:

I. Rahman A, Vahter M, Ekstrom EC, Rahman M, Mustafa AHMG, Wahed MA, Yunus M, Persson LÅ. Association of arsenic exposure during pregnancy with fetal loss and infant death: a cohort study in Bangladesh. Am J Epidemiol 2007; 165: 1389-96.

II. Rahman A, Persson LÅ, Arifeen SE, Nermell B, Ekström EC, Smith AH, Vahter M. Arsenic Exposure in Pregnancy Increases the Risk of Fetal Loss and Infant Death: A Prospective Cohort Study in Bangladesh. *Submitted for publication.*

III. Rahman A, Vahter M, Smith AH, Yunus M, Arifeen SE, Persson LÅ, Ekström EC. Arsenic exposure during pregnancy and size at birth: a prospective cohort study in Bangladesh. Am J Epidemiol 2009;169:304-12.

IV. Rahman A, Vahter M, Ekström EC, Persson LÅ. Arsenic exposure in pregnancy is associated with increased risk of lower respiratory tract infection and diarrhea: a prospective cohort study in Bangladesh. Manuscript.

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Abbreviations

As	Arsenic
AS(III)	Arsenite
As(IV)	Arsenate
AsMat	As and health consequences, Matlab
BGS	British Geological Survey
CHRWs	Community health research workers
CI	Confidence intervals
HDSS	Health and demographic surveillance system
iAs	Inorganic arsenic
ICDDR,B	International Centre for Diarrhoeal Disease Research, Bangladesh
LRTI	Lower respiratory tract infection
MINIMat	Maternal and Infant Nutrition Interventions, Matlab
OR	Odds ratio
RR	Relative risk
SES	Socio-economic status
SD	Standard deviation
WHO	World Health Organization

Glossary and definitions

Cohort study: The analytical method of epidemiological studies in which sub-sets of a defined population can be identified who are, have been, or in the future may be exposed or not exposed, or exposed in different degrees, to a factor, or factors hypothesized to influence the probability of occurrence of a given disease or other outcome.

Cross-sectional study: A study that examines the relationship between diseases and other variables of interest as they exist in a defined population at one particular time.

Dose-response relationship: The quantitative relationship between the amount of exposure to a toxicant and the incidence of adverse effect.

Developmental toxicity: The occurrence of adverse effects on the developing organism that may results from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Ecological study: An ecological study is an epidemiological study in which the unit of analysis is a population rather than an individual.

Morbidity: State of being diseased; morbidity rate is the incidence or prevalence of disease in a specific population.

Mortality: Death; mortality rate is a measure of the number of deaths in a population during a specified period of time.

Risk factor: An aspect of personal behaviour or lifestyle, an environmental exposure, or an inborn or inherited characteristic that is associated with an increase occurrence of disease or other health-related event condition.

Reproductive toxicity: The occurrence of adverse effects on the reproductive system that may result from exposure to chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behav-

iour, fertility, pregnancy outcome, or modification in other functions that are dependent on the integrity of this system.

Note: the definitions were derived from JM Last “A dictionary of Epidemiology” ,fourth ed. New York, Oxford University Press 2001, and from Agency for Toxic Substances and Disease Registry; Division of Toxicology and Environmental Medicine; 1600 Clifton Road NE, Mailstop F-32; Atlanta, GA 30333. “Toxicological profile of arsenic”, (2007).

Introduction

Contamination of ground water with high arsenic (As) content exists in many parts of the world. Millions of people worldwide, mainly in low income countries, are exposed to As concentrations in drinking water above the World Health Organization (WHO) guideline value of 10 µg/L [1-3]. Arsenic is a ubiquitous element and it exists in a variety of states in water, food, soil and air from natural and anthropogenic sources. Arsenic is available in both inorganic and organic forms and different oxidative states [4, 5]. However, Arsenite (As(III)) and Arsenate (As(V)), the inorganic As (iAs) of trivalent and pentavalent oxidative states, respectively, are the main toxicologic interests. Humans are commonly exposed to iAs through naturally occurring As contaminated ground water. Exposure from food, smelter's emission, mining, and through commercially used As compound applied in timber, pesticide, and semiconductors are also reported [6, 7].

Arsenic is widely recognized as a potent toxicant and carcinogen. Studies have also reported an association between As exposure and non-malignant illnesses affecting multiple organ systems of the body [7, 8]. The intrauterine and early childhood periods are the most biologically sensitive windows for chemicals that may impair growth and organ development. Arsenic readily crosses the placenta in both humans and animals [9, 10]. However, the effects of As on early human development are not well studied. A few epidemiological studies, mainly cross sectional or ecological in designs, have reported associations between As exposure and adverse pregnancy outcome and impaired infant health [11-17]. The findings so far available are inconclusive. Therefore, this thesis is based on rigorous epidemiological studies that analysed the association between prenatal As exposure and foetal loss, size at birth, infant morbidity and mortality of the offspring.

Arsenic poisoning: Bangladesh and worldwide

Arsenic contamination of tube-wells, the main drinking water source, is one of the major public health concerns in Bangladesh. Bangladesh is one of the poorest countries in the world with a per capita gross national income of 480 US$. About 40% of its 150 million population live under extreme poverty (<1$ per day) [18]. The country has a high prevalence of child and maternal

malnutrition, and the highest proportion of low birth weight babies (about 36%) [19], making these groups potentially more vulnerable to toxic exposure from the environment.

The country has significantly achieved the target of providing bacteria-free water by installation of millions of tube-wells. Although some tube-wells reportedly were used already in the 1940s, mass-scale installation of tube-wells was initiated by the Department of Public Health Engineering (DPHE) and the United Nations Children's Fund along with other international and non-governmental organizations (NGOs) from the 1970s and onwards. It is estimated that about 6 - 11 million tube-wells have been installed in Bangladesh [6].

Surprisingly, As was not in the list of chemical substances for drinking water analysis routinely performed by the promoters of tube-wells. In Bangladesh, the presence of As in tube-well water above the local drinking water standard ($\geq$ 50 μg/L) was first reported in the early 1990s [20-22]. British Geological Society (BGS) with help of DPHE conducted a water survey in the late 1990s in all but one districts of the country. With a total of 3,534 tube-well water samples collected, it was found that about 46% of the samples had As concentrations above the WHO guideline value (Figure 1). It is estimated that about 35 - 57 million individuals in Bangladesh are exposed to high levels of As, causing one of the greatest natural calamities in the history of mankind [2, 6].

Arsenic contamination is also a global public health problem. Worldwide, an increasing number of aquifers with high As contents are identified (Figure 2). Argentina, Chile, China, Hungary, India (particularly West Bengal), Mexico, Taiwan, and parts of the USA have also identified problems with As contaminated ground water [2, 6, 7]. The extent of contamination and people affected are noticeable in many countries in the world. It is estimated that more than 100 million people worldwide are exposed to high levels of As through drinking water [1].

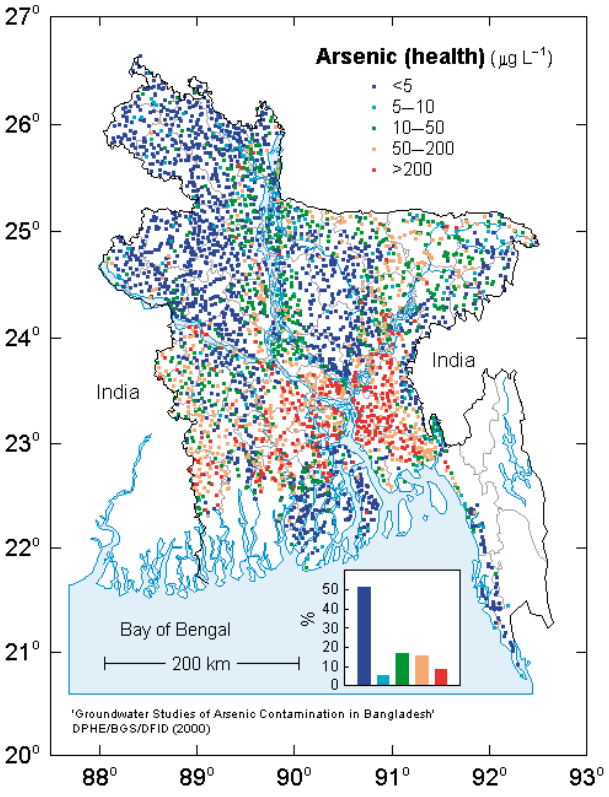

Figure 1. Map showing concentrations of arsenic in ground water in Bangladesh [6].

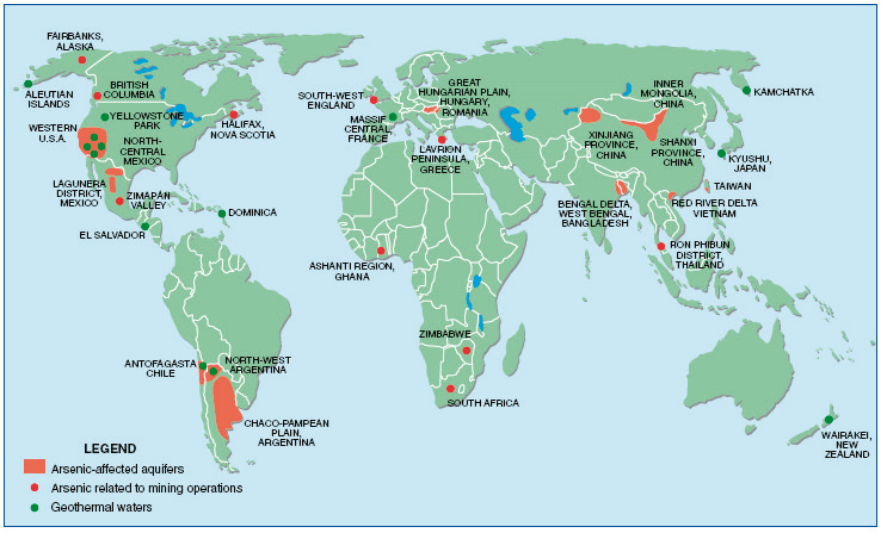

Figure 2. Documented areas of As-affected groundwater related to natural contamination or mining [6].

Health effects of arsenic

Characteristic skin lesions such as melanosis, leukomelanosis and keratosis are the hallmarks of chronic arsenicosis. Melanosis, often raindrop-like pigmentation, may occur anywhere on the body but typically on unexposed part. Leukomelanosis follows the same distribution, and may present even in the absence of melanosis. Keratosis is small corn-like elevations found on the palms, fingers and on the soles [6, 7, 23, 24]. Development of skin lesions usually depends on the duration and level of As exposure of the individual. Individual susceptibility and genetic factors are also influencing the occurrence of skin lesions.

Arsenic is one of the first chemicals that was recognised as a carcinogen. Skin cancers were reported in patients who had been treated with As compounds hundreds of years ago. Scientific evidence has been given that As is associated with cancer in a variety of sites, such as skin, lungs, liver, urinary bladder and kidneys. Scientific reports come from Argentina, Taiwan, Chile, China and recently from Bangladesh [7, 8, 25-30].

Non-cancerous health effects like Blackfoot disease, diabetes, hypertension and chronic obstructive pulmonary disease have been reported from many As affected areas [7, 23, 31-34].

Arsenic and reproductive health effects

Reproductive toxicity is manifested as adverse effects or abnormalities in gamete production, reproductive cycle, fertility, gestation, parturition, and/or lactation, and pregnancy outcomes (spontaneous abortion, still birth). Other effects on the developing organism may be visible as prenatal and early postnatal death, structural abnormalities, altered growth, and functional deficits [35]. Since the report of malformation caused by As in the early 1970s, a substantial number of experimental studies have been carried out to explore the teratogenic effect of iAs. Other reproductive end points such as foetal death, growth retardation and perinatal mortality have also been investigated [36]. A number of reviews are available where these effects are described in detail [37-39].

Arsenic has been suggested to be a reproductive and developmental toxicant in humans and therefore the toxic manifestation may cover a wide range of effects. However, this thesis focuses on epidemiological evidence for prenatal As exposure and adverse pregnancy outcomes (spontaneous abortion, stillbirth, perinatal death, and size at birth) and also on infant morbidity and mortality of the offspring. Animal studies will also be discussed in short.

Figure 3 presents a simplified illustration of how prenatal As exposure may affect the foetus and the offspring. Women are exposed to As mainly through contaminated ground water through tube-wells, which has been considered the major source of contamination. Exposure of As may also take place through the food chain.

After As intake by pregnant women, the body tries to detoxify by a process of metabolism in order to prevent harmful effects. The nutritional status of women, which is closely related to the socio-economic status (SES), may influence the detoxification process by repeated reduction and methylation of iAs absorbed [40]. There is currently also a socio-economic gradient in the exposure to As contaminated drinking water in Bangladesh.

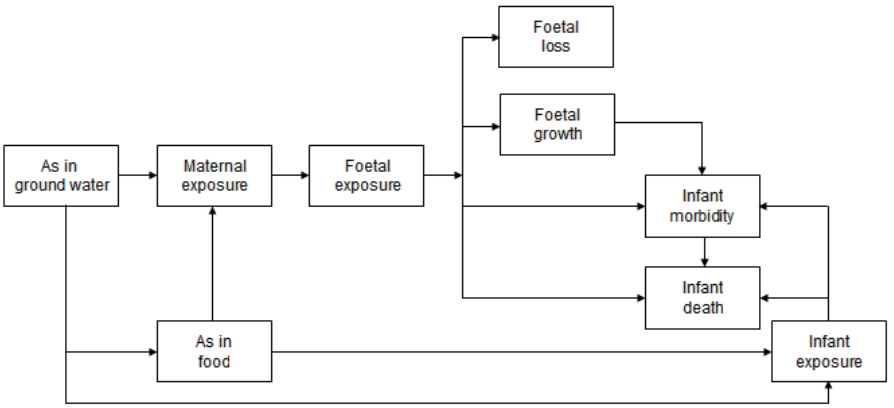

Figure 3. Conceptual framework of arsenic exposure and potential effects on foetus and infant. As: arsenic

Arsenic readily passes through the human placenta, and give rise to as high concentrations of As in cord blood as in maternal blood [9, 41]. In contrast to the free passage of As through placenta, passage of As through mammary glands is limited, and small amounts of As are excreted in breast milk [42, 43]. Therefore infants who are exclusively breastfed are reportedly protected from As exposure. This finding has also been supported by findings showing that urinary As concentrations in infants of As exposed mothers were very low [44]. However, weaning food and other liquids are often introduced at around 6 month of age that may expose the infant to high level of As from environmental sources. Therefore, the As related effects in infant life may be attributed to intra-uterine exposure as well as direct exposure during infancy.

Arsenic and its methylated metabolites are accumulated in placental and foetal tissues, and subsequently exert toxic effects. This may result in foetal loss, and may also influence foetal growth, and later infant morbidity and potentially increase the risk for infant death. One outcome, e.g. infant mor-

bidity, may (partly) be on the causal pathway to infant death. Below we will present the current knowledgebase regarding prenatal As exposure and effects on pregnancy outcome and infant health. Effects on immune function will also be discussed.

Arsenic, pregnancy outcome, infant survival

Drinking Water

A large number of studies have been conducted during the last few years in Bangladesh, West Bengal, Chile, China and other As affected areas. Most of these studies have been related to cancer risks, but an increasing number of reports have also been published about the potential association between As exposure through drinking water and different reproductive end points, particularly pregnancy outcome.

A case-control study with As and other trace element concentrations obtained from regular public tap water examinations, reported a non-significant increase in spontaneous abortion with high As contents along with other detectable elements in water [45]. An ecological study in Hungary indicated an increased occurrence of spontaneous abortions in relation to higher As exposure through drinking water; however, the full report was not published [46].

Two cross-sectional studies in Bangladesh, with retrospective assessment of outcome data, have reported two- to three-fold increase in spontaneous abortions and stillbirths in women exposed to As through tube-well water [11, 15]. The study conducted by Ahamad et al. included 192 randomly selected women, and compared the adverse pregnancy outcome of the group selected from a village with mean As concentrations of 240 μg/L with the outcome of the group from a village with low As concentration in the drinking water (mean of 20 μg/L) [11]. The proportions of spontaneous abortion and stillbirth were 68.8 and 53.1 per 1000 births, respectively, in the high As area, while the proportions for the same outcomes in the low As area were 23.7 and 23.7 per 1000 live births. In the study by Milton et al. 533 women were included, and he reported odds ratios for spontaneous abortion and stillbirth to be 2.5 (95% CI: 1.5 – 4.3) and 2.5 (95% CI: 1.3 – 4.9), respectively, for women who used tube-well water with As concentrations ≥ 50 μg/L in comparison with women who had As concentrations <50 μg/L in tube-wells [15].

In contrast to the above mentioned reports from Bangladesh, a study that used outcome data collected through a food supplementation program reported an association between As exposure and malformation but no association with other negative pregnancy outcomes [47]. In a recent ecological study in Bangladesh, Cherry et al. reported a significant increase in risk of stillbirth (OR 1.8, 95% CI: 1.14 – 2.86) among women with high As content in tube-well water (> 50 μg/L) [12].

In a cross-sectional study conducted in West Bengal, India that included 202 women information on reproductive events and infant death was collected through interviews [16]. There was no association between As exposure and spontaneous abortion. However, the risk of stillbirth was six times increased (although with a wide confidence interval) among women who were exposed to As in drinking water with concentrations >200 μg/L in comparison with women who had water As levels < 50 μg/L during pregnancy (OR 6.07, 95% CI: 1.54 – 24.0).

A few studies have suggested a negative association between As exposure and birth weight. Yang et al. observed an overall 30 g reduction of birth weight in As exposed area in Taiwan, where the household members used well water with As concentrations that varied from undetectable levels to 3590 μg/L [17]. In Chile, a non-significant difference of 57 g of birth weight was found when comparing two areas - Antofagasta that was served by drinking water with a fairly high As concentration (30-40 μg/L), and Valparaiso with drinking water low in As (<1 μg/L) [13]. A recent study, based on only 52 pregnant women, reported a significant association between hair As concentrations and birth weight. However, neither was the study population profile provided nor was it clear whether the analyses were adjusted for important covariates such as SES and women's anthropometry [48].

Overall, most of these studies have been ecological or cross sectional in design, and exposure and outcome assessments may be questioned, exposure was not assessed during critical periods of development, important covariates were missing, and no dose response data were available.

Other sources of exposures

A non-significant elevation of spontaneous abortion risk (RR 1.33, 95% CI: 0.94 – 2.04) was observed in an area in Sweden close to a Copper smelter that emitted significant amounts of As [49]. A series of studies were carried out in the Rönnskär smelter in northern Sweden, and several reproductive outcomes were reported (malformations, spontaneous abortions, and birth weight) and those were related to environmental pollutants including As [50-52]. In a case-control study in USA an association between ambient air As levels (above 100 $\mu g/m^3$) and stillbirth was reported (OR: 4.0; 95% CI: 1.2; 13.7) [53].

Animal studies

Teratogenic and embryotoxic effects of As have been reported from several animal models such as hamsters, mice, rats, rabbits as well as other species [7, 37, 38, 54, 55]. The commonly investigated abnormalities are neural tube defect (anencephaly, exencephaly), anophthalmia, exophthalmia and other urogenital defects. Experiments have been carried out with iAs and its metabolites administered through intravenous or intra-peritoneal routes [7, 37,

56]. The doses used were high in comparison with the As levels humans commonly encounter from natural sources. The timing and amount of exposure was chosen with an intention to cause the most severe form of toxicity. Therefore, most of the time malformation was associated with maternal toxicity. Some studies did not document any severe malformation in spite of high doses of ingestion of arsenicals in single or multiple doses [57, 58]. However, resorption and growth retardation of foetus have been reported in maternally toxic single dose [36].

In the last few years several animal studies have been carried out with environmentally relevant doses that humans may encounter. In a study with environmentally relevant doses of inorganic As an increased number of neonatal deaths were reported including impaired brain development and responsiveness in a new environment [59]. Ingestion of As(III) treated water by rats caused reduction of gonadotropic hormones and also deposition of As in the genital tract including the ovaries [60]. Arsenic has been found to cause meiotic aberration of oocytes, decrease cleavage rate of zygotes and morula formation in mice [61]. In a recent study in mouse, maternal oral treatment with As was reported to increase the rate of neural-tube defect significantly without maternal toxicity [62].

The route of administration and the dosage used in animal models were inmost cases not on the same level as human subjects commonly encounter from the environment. Also, there is a lot of differences in As metabolism between humans and animals. Therefore, epidemiological studies are needed to evaluate As toxicity in humans [63].

Arsenic, immunologic effects, morbidity

In- and ex-vivo studies with animal models have reported immunotoxic effects of As. In several studies it has been shown that As exposure suppressed the IgM and IgG antibody–forming cell response, it decreased interleukin-2 mRNA expression, inhibited antigen driven T-cell proliferation and macrophage activity, and it suppressed contact hypersensitivity responses [64-67]. In vitro-study reported that human macrophage is the target of inorganic As and can alter differentiation features of macrophages [68]. In a study in Mexico, an increased concentration of As in urine was associated with reduced proliferative response to phytohemaglutinin stimulation, CD4 subpopulation proportion, and interleukin-2 secretion levels in children [69]. These immunologic effects may potentially account for a high burden of infectious diseases in childhood, and so far no human study has evaluated the association of As exposures with childhood infections.

Epidemiology of adverse pregnancy outcomes and infant morbidity and mortality

Adverse pregnancy outcomes such as spontaneous abortion, stillbirth, and infant mortality are high in low income countries. Table 1 presents the occurrence of different pregnancy outcomes including infant mortality in the demographic surveillance area in Matlab, Bangladesh. Although some reduction of neonatal mortality has occurred, the most significant reduction has taken place among post-neonatal deaths.

Table 1. Proportion of pregnancy outcomes and infant mortality recorded in the Matlab Health and Demographic Surveillance in Bangladesh

Year	Spontaneous* abortion	Stillbirth*	Neonatal** mortality	Post-neonatal** mortality
2002	85	26	35	16
2003	95	25	32	12
2004	71	26	32	11
2005	70	24	31	10
2006	74	27	27	8

*per 1000 pregnancies; **per 1000 live births

Spontaneous abortion

Pregnancy involves a complex interaction between genetic, endocrine, immune and neurological systems of the woman's body. Any factor, either endogenous or exogenous, may disrupt these systems and eventually lead to pregnancy loss before the expected outcome takes places. Spontaneous abortion is the most common adverse pregnancy outcome and is usually defined as unintended loss of pregnancy before 20 or 28 weeks of gestation. Prospective studies based on daily urine specimen examination reported spontaneous abortion rates from 22 to 31% [70, 71].

More than 80% of spontaneous abortions take place in the first trimester, and about half of these results from chromosomal anomalies, usually knows as aneuploidy [72, 73]. After the first trimester, spontaneous abortion rates decrease, and when occurring usually attributed to an array of other factors.

The aetiology of spontaneous abortion is diverse and not quite understood, and the death of the embryo or foetus always precedes the spontaneous expulsion. Therefore, finding the cause of abortion involves ascertainment of the cause of foetal death. This may arise from genetic factors (chromosomal anomalies, mutant genes), infectious agents, uterine abnormalities, and other maternal (age, diabetes, hypertension) and paternal factors [74]. Environ-

mental factors such as exposure to heavy metals (mercury, lead), radiation and other toxic intake (caffeine, tobacco) may also play an important role in the occurrence of spontaneous abortions [70, 75-77].

Stillbirth

Death of a foetus in *utero* is always a shocking experience for the mother as well as for the whole family. Currently stillbirths contribute to about one third of all foeto-infant mortality and more than a half of the periantal mortality, especially in low income countries. Each year about 4 millions of stillbirths occur worldwide and about 99% of these foetal deaths take place in low income countries [78]. In contrast to infant mortality, the causes of stillbirths are usually not caught by any vital statistics, and there are always difficulties in determining the cause of stillbirth. Aetiology and risk factors differ between types of stillbirth. A macerated stillbirth is often associated with insults that occur in utero during the antenatal period, while fresh stillbirths may suggest problems in the care during labour and at delivery [79].

The cause of stillbirth can be divided into foetal, placental and maternal factors. There are a number of risk factors related to stillbirth such as advanced age, a bad obstetric history, low socio-economic status, high maternal weight and short statured women, nulliparity and grand multi-parity [80-82]. Poor quality of intra-partum and delivery care also increase the risk of stillbirth, particularly in low income countries.

Morbidity

Diarrhoea

Diarrhoea is usually defined as three or more loose stools in 24 hours that are sufficiently loose to take the shape of a container. Any number of loose stools containing blood is also considered as diarrhoea [83]. Diarrhoea is a major burden of illnesses among children younger than 5 years of age in low- and middle-income countries. Diarrhoea still remains a significant cause of childhood mortality [84]. It is estimated that about 22% of about 10 million annual deaths of under five children is contributed by diarrhoeal diseases. Persistent diarrhoea is related to about 50% of these fatalities [84].

The incidence of diarrhoea episodes in low income countries is reportedly 3 -5 episodes per child-year in children under five years of age [85, 86].

Poor sanitation and poor quality of water, both related to poverty, are associated with an increased risk of diarrhoea. Host immune functions contribute to the occurrence of diarrhoea as well. This is evident in HIV infection,

where diarrhoea often is found as the first clinical symptom of AIDS. Lack of exclusive breast feeding is also associated with the occurrence of diarrhoea during infancy.

Acute respiratory infections (ARI)

ARI is the most common group of illnesses during infancy, and implies infections affecting the respiratory organs that create short-term illness of varying degree of severity. ARI includes both upper respiratory tract infections (URTI) and lower respiratory tract infections (LRTI). LRTI include bronchitis, bronchiolitis and pneumonia or any combination of the three. The common acute infections of the upper and lower respiratory tract range from a simple cold or cough, otitis media, sore throat, laryngitis to bronchitis, bronchiolitis, and pneumonia. Within the group of disease conditions included in ARI pneumonia represents the main cause of death among children younger than the age of five years. About 75% of all pneumonia deaths occur during infancy [87].

Pneumonia represents inflammatory conditions of lung parenchyma. Most of the pneumonias can be detected using a simple protocol by counting breathing frequency and evaluating whether chest-indrawing is present in children who have cough and/or difficult breathing. WHO has developed age-specific definitions of fast breathing or tachypnea which are ≥ 60 in infants younger than 2 months, ≥ 50 from 2 to 11 months and ≥ 40 breaths per minute from 12 to 59 months [88].

The risk factors for pneumonia include poverty, low parental education, environmental factors such as smoking, smoke produced by fire-woods and crowding. Factors related to the child itself include lack of breastfeeding, low-birth weight, and malnutrition (deficiency of vitamin A and zinc).

Birth weight, neonatal and infant mortality

Birth weight is an important determinant of morbidity and mortality in childhood and also associated with chronic diseases in adulthood [89-91]. Birth weight is the function of both duration of gestation and intrauterine growth. Low-birth-weight (LBW) is defined as birth weight <2,500 g, and may be caused by premature delivery (<37 weeks of gestation) and/or intra uterine growth retardation (IUGR). The proportion of LBW is very high in South Asia, specially in Bangladesh [19].

Intrauterine growth depends on multiple factors including maternal nutrition, food and micronutrient intake during pregnancy, physical activity, oxidative stress related to toxic exposure and also on other environmental exposures in an interplay with genetic predisposition [92]. In low income countries poor maternal nutritional status including a short stature, low calorie intake and

inadequate weight gain during pregnancy are risk factors for LBW. Other important risk factors include teenage pregnancy, repeated pregnancies, infectious diseases, stressful conditions including high work load and family violence.

Infant mortality has substantially declined in the world. However, this decrease is mainly due to decreased mortality in the post-neonatal period [93]. The reasons for this worldwide reduction are improved immunization rates, use of oral rehydration therapy during diarrhoea, management of pneumonia and malaria at community level, vitamin-A supplementation, and other interventions conducive to reduce deaths in that age group [94].

Among the 4 million neonatal deaths in 2000, 99% occurred in low- and middle-income countries [95]. South Asia has the highest number of deaths, while the highest rates are found in Africa. Latin American countries have been successful in reducing neonatal mortality rate up to 40-50 percent since the early 1990s. In Asia progress varies widely with reduction ranging between 10 – 40%, in Sub-Saharan Africa rates have actually increased [78, 96].

Risk factors for neonatal death include poor maternal health, poverty, and lack of maternal education, adverse social conditions, and inadequate care during pregnancy, delivery and in the immediate postpartum period [97, 98]. Pregnancy complications, i.e. prolonged or obstructed labour, abnormal foetal position, and hypertensive diseases were found to account for 30%, while premature labour accounted for 27% of perinatal mortality in Matlab from 1987 to 1993 [98]. In the post-neonatal period the main causes of mortality are pneumonia, diarrhoea, malaria and also HIV/AIDS [98]. The important risk factors for death after the neonatal period are poverty, lack safe water and sanitation, crowding, indoor air pollution, low practice of exclusive breast feeding, and malnutrition [99, 100].

Rationale for the studies of the thesis

Arsenic exposure has been found to cause developmental and reproductive toxicities in animal models. Epidemiological studies so far conducted have suggested associations between As exposure and different reproductive end points. Despite indications of negative effects of As on pregnancy outcome and infant survival in several studies, there is lack of strong evidence to support these findings conclusively. Several of the previous studies had relatively weak designs and used retrospective assessment of outcomes. Variation in water consumption and the possibility of temporal variation of As concentration in tube-wells have increased the uncertainty of exposure assessments. Further, water As concentrations may not fully reflect the total

exposure if exposure through food chain also takes place. In addition to limitations in exposure assessments, some of the published studies have not managed confounders satisfactorily. There are limitations when drawing inferences from animal studies. Route of administration and dosage used in animal models are often not relevant to what human subjects commonly encounter through the environment. Also, there are major differences in As metabolism between humans and animals. Thus, epidemiological studies are needed to evaluate As toxicity in humans.

Therefore, in order to study the potential health effects of prenatal As exposure on foetus and offspring this research project

- Included two cohorts of pregnant women from the community
- Collected individual As exposure information based on water and urinary As concentrations
- Obtained prospectively collected outcome data that was enabled by a health and demographic surveillance system and also by including an As study component in the prospective cohort of a community-based food and micronutrient supplementation trial.
- Collected and carefully adjusted for potential confounding factors.
- Included large sample sizes that gave sufficient power to estimate also lower risks and make some stratified analyses possible.

Aim of the thesis

The overall objective of this thesis was to analyse the possible effect of As exposure in pregnant women on foetal and infant health through epidemiological studies in Matlab, Bangladesh, where people have been using tube-well water for more than three decades, and where As contamination in tube-wells is common.

Specific objectives

(i) Evaluate the association between individual As exposure via tube-well water during pregnancy and foetal loss and infant mortality in a cohort of pregnant women and their offspring identified within a demographic surveillance system.

(ii) Evaluate whether individual As exposure assessed by urinary As concentrations during pregnancy is associated with foetal loss, perinatal and infant mortality of their offspring in a cohort of women who participated in a food and micronutrient supplementation trial.

(iii) Analyse the association between individual prenatal As exposure based on urinary As concentrations and size at birth of their newborns in a prospective cohort of women who participated in a food and micronutrient supplementation trial.

(iv) Analyse the association of prenatal individual As exposure assessed by urinary As concentrations in a cohort women who participated in a supplementation trial and lower respiratory tract infection and diarrhoeal disease morbidity in their infants.

Methods

Study site

The study area is located in Matlab, 53 km southeast of the capital Dhaka, Bangladesh. Matlab is one of the 464 Upazillas (sub-districts) in the country. It is a flood-prone low-lying delta, situated where the river Meghna joins the confluent streams of two rivers - the Brahmaputra and Ganges. The ground-water in Matlab is highly affected by the historic natural As laden soils transported by the rivers from the mountains in the north. About two-third of the tube-wells in the area had As concentrations more than the local drinking water standard ($\geq$ 50 µg/L), while about 70% had As levels above the WHO guideline value (10 µg/L, Table 2).

Table 2. Arsenic concentrations of tube-wells in the study area (2002-2003) [101].

As concentration µg/L	Number of tube wells	Percentage	Cumulative percentage
< 1	2234	17	17
1-9	1559	12	29
10-49	1099	8.3	37
50 – 149	1471	11	48
150-299	3021	23	71
300-499	2651	23	91
500 – 999	1192	9.0	99.6
$\geq$ 1000	59	0.4	100
Total	**13,286**	**100**	

The major occupations in the area are agriculture, fishing and day labour while a substantial proportion of inhabitants is landless. About 85% of the population is Muslim and the rest is mostly Hindu.

International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), has been running a health and demographic surveillance system (HDSS) in a population of about 220,000 in 140 villages since 1966 in the area. HDSS is divided into two parts: the ICDDR,B service area (Figure 4), where ICDDR,B provides health services to children less than five years of age and the mothers of reproductive age, and the Government service area,

where health services are provided through the government facilities. ICDDR,B service area is divided into four administrative blocks and each block has a sub-centre providing 24 hour service by paramedical staff. All field and sub-centre activities are supervised from a central office and hospital in Matlab township.

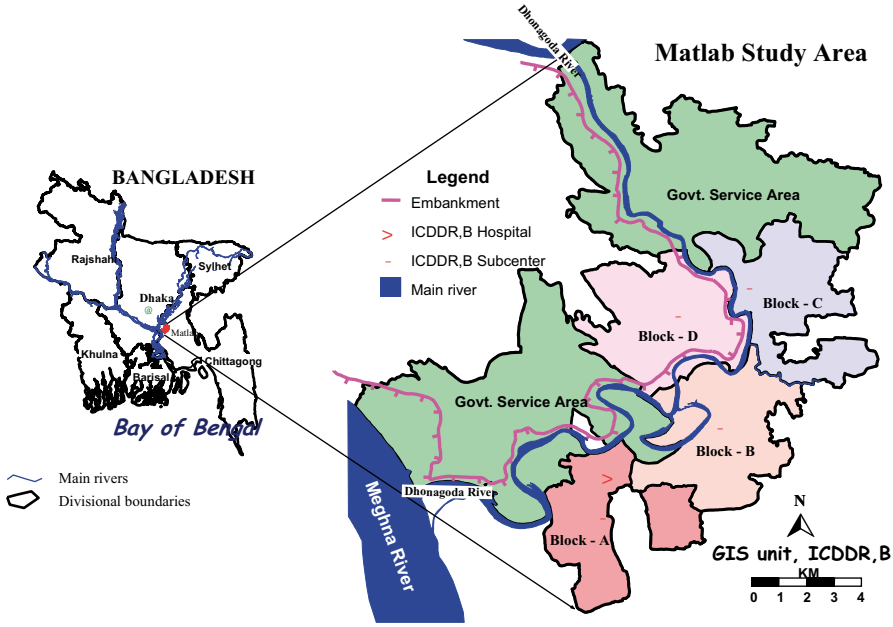

Figure 4. Map of Bangladesh and Matlab study area

Study design and subjects

The studies of this thesis are based on different assessments of As exposure as discussed in the Introduction. When water As concentration was used in the historical cohort study (paper I) it reflects only the exposure from drinking water. In the prospective cohort approach (paper II-IV) urine As exposure was analysed that reflects exposure from drinking water sources and also from food.

The studies were accomplished by taking advantage of the ongoing HDSS, and also of two other concurrent studies carried out in Matlab in 2001 – 2004. One was the Arsenic and Health Consequences, Matlab (AsMat) study, which included a cross sectional survey to detect As induced skin lesions, and also analysed water from all tube-wells in the area for As concentration. The other one was a randomised trial, the Maternal and Infant Nutrition Interventions, Matlab (MINIMat). MINIMat was a community based randomised food and

micronutrient supplementation trial. The study included pregnant women where pregnancy was identified by urine pregnancy tests and where these women and their offspring were followed up prospectively. Table 3 illustrates study designs and related information in short.

Table 3. Designs, samples, exposure assessments and outcomes in the four papers of the thesis

	Study design	Sample size	Data bases	Exposure	Outcomes
I	Historical cohort	29,153 pregnant women and their offspring	AsMat, HDSS	Arsenic in drinking water	Foetal loss, infant death
II	Prospective cohort	2,924 pregnant women and their offspring	MINIMat, HDSS	Urinary arsenic	Foetal loss, infant death
III	Prospective cohort	1,578 pregnant women and their newborns	MINIMat, HDSS	Urinary arsenic	Size at birth
IV	Prospective cohort	1,552 infants and their mothers	MINIMat, HDSS	Urinary arsenic	Incidence of LRTI and diarrhoea

AsMat: acronym for study on arsenic and health consequences in Matlab. MINIMat: acronym for Maternal and Infant Nutrition Interventions in Matlab. LRTI: lower respiratory tract infections. HDSS: health and demographic surveillance system.

The historical cohort study included pregnant women who were recorded by the HDSS in 1991-2000. Out of 63,992 pregnant women identified, 29,134 women had available information on water source and data on As concentrations in tube-well water used by the women during pregnancy, and were therefore included in the analyses to evaluate the association between water As concentrations and foetal loss and infant death (**Paper I**).

The prospective cohort studies were based on the pregnant women identified by the MINIMat trial for a full year period from February 2002 to January 2003. The cohort study established a system to collect urine samples two times during pregnancy for As analyses- at or around gestation week (GW) 8 and 30. Outcome data were taken from the MINIMat study, which collected information prospectively. The prospective cohort study is the basis for three analyses and papers in this thesis: 2,924 pregnant women and their newborns evaluated for foetal loss, perinatal and infant mortality (**Paper II**); 1,578 mothers and their newborns evaluated for size at birth (**Paper III**); and 1552 mothers and their offspring analysed for lower respiratory tract infections and diarrhoea during infancy (**Paper IV**, Table 3).

Health and Demographic Surveillance System

ICDDR,B has been running a HDSS since 1966 and collects longitudinal data on vital events such as marriage, birth, death, and migration through Community Health Research Workers (CHRWs) by monthly home visits. In 1978 a community based family planning and health service programme was initiated in the ICDDR,B service area. In 1986 a maternal and child health programme was initiated and thereafter gradually strengthened. An ARI intervention programme was initiated in 1988 and included both active and passive surveillance that was continued up to 2005. As a pioneering research organization in the field of diarrhoea, ICDDR,B conducted many field trails on oral rehydration therapy, vaccines and other socio-behavioural projects related to diarrhoea. Therefore, the knowledge on symptoms and signs of ARI and diarrhoeal diseases is expected to be higher in the population than in other parts of the country.

CHRWs are the grass root level workers, and each worker covers on average 400 households corresponding to a population of about 1,800 – 2,000. CHRWs provide services to married women of reproductive age and children less than 5 years of age. In addition to collection of demographic information of vital events they also collect selected child and maternal health information. The HDSS also conduct periodic censuses to update the socio-economic databases that include data on possession of asset, land, and income of the family.

Arsenic study in Matlab, AsMat study

This cross-sectional survey was conducted to determine the prevalence of As related skin lesions and the level of As contamination of tube-wells in the study area in 2002-2003 [101]. About 180,000 inhabitants who were above 4 years of age were identified from the HDSS databases with an additional inclusion criteria that they should be drinking water from water sources in Matlab at least once per week. In total, the field workers screened 166,934 individuals for As induced skin lesions and also obtained drinking water histories (type and location of all water sources used since 1970). The information on water history was validated through survey data collected by HDSS in 1974, 1984, and 1996, where information on drinking water sources was available. A follow-up field team visited each household and measured the As concentrations in all tube-wells by field kits (Merck, Darmstadt, Germany). In accordance with the national standard of safe As level, all tube-wells with an As concentration $\geq$ 50 μg/L were painted red and the others were painted green. Duplicate water samples were collected from all tube-wells for subsequent analysis of total As by atomic absorption spectroscopy (AAS). The AsMat study provided drinking water history and As expo-

sure information for each calendar year from 1970 up to time of interview date of study participants.

Maternal and Infant Nutrition Interventions, Matlab (MINIMat) trial

In the MINIMat trial (trial registration ISRCTN16581394) women were randomised into different food and micronutrient supplementations during pregnancy. The trial interventions were not used in the analyses of this thesis.

Pregnancies were identified by CHRWs, who asked the woman whether she had had her menses since last visit. If a woman reported that her Last Menstrual Period (LMP) was overdue by 2 weeks or more or that she was pregnant after a continuous period of amenorrhea, she was offered a urine pregnancy test. Pregnant women were invited to participate in the supplementation trial. In the sub-centre women underwent ultrasound examination for gestational age assessment and were, after informed consent, randomised into different interventions. The individual woman was randomly allocated to be invited to daily food supplementation with an early start (from 1st trimester) or to be invited later, at the usual program start (from 2nd trimester). In addition, she was randomly and double-masked allocated to daily capsules from wk 13 of either 30 mg iron and 400 μg folate, 60 mg iron and folate, or multiple micronutrients (15 micronutrients including iron and folate).

The prospective cohort studies of As exposure and different outcomes were nested into the MINIMat trial (Paper II – IV). Starting in January 2002 women with a pregnancy positive test were requested to donate a urine sample for As analysis. In addition to this urine sample, collected around gestational week 8 (GW8), urine samples were also obtained around week 30 (GW30) during an antenatal contact at a health centre. To cover a full year of potential variations in As exposure and outcomes studied, we included women identified as pregnant from 1 February 2002 to 31 January 2003.

Pregnancies were followed up prospectively and information on pregnancy outcome (foetal loss, live birth) was collected. A birth notification system was established and birth anthropometry was measured within 72 hours after birth. Information on mother's weight, height, and socio-economic information was collected. The cohort of MINIMat infants was followed up prospectively, and morbidity information was collected by monthly household visits, that included a one-week recall of disease episodes each time.

Exposure assessment

Water arsenic

Field workers of the AsMat study collected duplicate water samples from all functioning tube-wells in the study area. To obtain the water sample the tube-well was pumped 30 stokes and subsequently water was stored in two 20 ml polyethylene vials. The sample containers were marked with IDs and transported and kept at – 20^0C in the Matlab laboratory until analysis. The sample vials contained acids to prevent precipitation of iron and co-precipitation of As. The concentration of As in water was determined in duplicate by hydride generation atomic absorption spectrophotometer (HG-AAS, Shimadzu AA6800, Shimadzu Corporation, Kyoto, Japan) at the ICCDR,B laboratory in Dhaka [102]. The detection limit of analysis was 1 µg/L. Internal quality was controlled by using standard reference materials, while inter-laboratory comparison was also made with the laboratory at the Karolinska Institutet, Sweden. As exposure for individual pregnancies was based on the concentration of As in the tube-well water used by the women during the year of pregnancy (Paper I).

Urinary arsenic

Arsenic exposure was assessed by concentration of iAs and methylated metabolites in urine at around gestational week GW 8 and GW 30 (Paper II, III and IV). Although the half-time of As in the body is in the order of a few days only, it is likely that women had reached a steady-state level of excretion of As and its metabolites through urine due to continuous exposure via drinking water. In early pregnancy the urine samples were collected at the women's home and in late pregnancy samples were collected at the health facility during antenatal contact. When collected at home, the samples were chilled with cooling blocks, and transported to Matlab laboratory where they were stored frozen at -70^0C. The details of urine sample collection and temperature maintenance during transportation has been described elsewhere [103].

The sum of the inorganic As and methylated metabolites in urine was determined using hydride generation (HG) atomic absorption spectrophotometry (AAS) in Sweden [103, 104]. The detection limit of the HG-AAS method was 1.3 ± 0.27 µg/litre. We also participated in inter-laboratory comparisons of As metabolites in urine to verify the analytical accuracy [105]. In order to compensate for variation in the dilution of urine, caused by variation in fluid intake, time of sampling, temperature and physical activity, we adjusted the obtained concentrations by specific gravity (the average being 1.012 g/mL). Specific gravity adjustment of urine dilution is less influenced by muscle mass and nutritional status than is the more commonly used creatinine adjustment [106-108].

Assessment of outcomes and covariates

Outcomes

Early foetal loss or spontaneous abortion was defined as loss of a foetus within 28 weeks of pregnancy excluding "menstrual regulation" (vacuum aspiration within 10 weeks following a missed menstrual period) or induced abortion.

Late foetal loss or stillbirth was defined as birth of a dead foetus after 28 weeks of gestation. An *infant death* was defined as death of a live born child before 12 months of age.

A *neonatal death* was defined as death of a live birth within 28 days.

Perinatal death was defined as a stillbirth or death of a neonate within 7 days of birth.

Post-neonatal death was defined as death of an infant after 28 days but before 12 months of age.

Size at birth: About 40% of deliveries took place at health facilities and birth anthropometry was taken by the attending nurse (either sub-centre level or at Matlab Hospital). A birth notification system was established by the MINIMat study, and for women who delivered at home MINIMat staffs were notified and a trained paramedic measured the birth anthropometry mostly within 72 hours of birth. We included the adjusted birth anthropometry in the analysis compensating for the variation in measurement time as describe elsewhere [109].

Birth weight was taken by electronic scales (SECA, Hamburg, Germany) with a precision of 10 g.

Birth length was measured with a locally made wooden scale with a precision of 1 mm.

Circumferences (head and chest) were measured by a tape with precision of 1 mm.

Morbidity: Definition of morbidity was based on reported symptoms and signs based on interviews of mothers. The recall period was 7 days.

Lower Respiratory Tract Infection (LRTI) was defined as presence of cough and/or difficult breathing with rapid respiration

Severe LRTI was defined as presence of cough and/or difficult breathing including chest indrawing.

Diarrhoea was defined as three or more loose stools or passage of blood in stool in 24 hours.

Loss to follow up was considered if a woman either migrated out from the HDSS area or withdraw from participation.

Covariates

Covariates for Paper I was retrieved from HDSS databases. Information of women's age, gravidity, education, socio-economic status by asset quintiles, gestation age at outcome, year of outcome were available.

For the Paper II – IV, the available covariates were women's age, gravidity, parity, education, socio-economic status by asset quintiles, gestational age, toxic exposures (tobacco or betel nut chewing) and women's anthropometric status.

Gravidity was defined as number of pregnancies including the present one, and *parity* as the number of live or dead children before the current pregnancy.

Educational status was assessed by number of years completed at school.

Asset quintiles Economic status was assessed by generating scores through principal component analysis based on household asset, housing structure, land occupation and income. The generated scores were then indexed into quintiles, where 1 represent the poorest and 5 the richest [110].

LMP: Last menstrual period date was determined by recall during the pregnancy identification interview at the time of monthly household visits.

Gestational age at pregnancy outcome was measured by subtracting the LMP date from date of pregnancy outcome and then expressed in weeks.

Women's anthropometry: Women's weight and height were measured during the visit to health facilities at enrolment into the MINIMat study (usually at gestational week 9).

Weight was measured by electronic scales (SECA, Hamburg, Germany) with a precision of 100 g

Height was measured with locally made wooden scales with a precision of 0.1 cm

Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$.

Cause of death information was collected through “verbal autopsy" method that used a standardized interview with the parents [111].

To determine the confounding effect of *seasonality* outcome months were categorised into pre-monsoon (January – May), monsoon (June-September) and post-monsoon (October-December) periods. In addition women’s *geographical location* was extracted for evaluation.

Statistical analyses

Arsenic exposure, measured by water As concentrations, was divided into quintiles and the first quintile corresponded relatively well to the WHO guideline value of 10 µg/L [3]. Urine As concentration was used as a continuous variable to assess the association with birth size. However, exposure was divided into quintiles to determine the association of exposures with foetal loss, infant death and morbidity (LRTI and diarrhoeal diseases). First quintile was used as a reference category.

Covariates were evaluated for association with exposure and outcomes. In the unadjusted analysis associations were determined by Spearman’s correlation coefficient, $\chi 2$, analysis of variance, Wald test or by non-parametric test appropriate for the type of data being analyzed.

Potential confounders, associated with exposure and outcome at $p \leq 0.20$ significance level were identified, and those found to change the effect estimation by 5% or more were included in the final multivariate model. However, to assess the robustness of the significant effect estimate observed, all important biological factors were also included in the model. Stratified analysis was also performed, as described in the description of the individual paper.

Effects of As exposure on outcome were assessed by multivariate regression models, appropriate for the specific study outcome evaluated.

Description of design and methods in paper I-IV

Paper I

This study evaluated the effect of As exposure on foetal and infant survival in a cohort of pregnancies identified by the HDSS in 1991-2000. Out of 63,992 pregnant women identified, 29,134 women had available information on water source and data on tube-well water As concentration for the year of pregnancy and were included in the analysis.

Arsenic exposure, reflected by drinking water history and analysis of As concentrations in tube-well water used by women during pregnancy, and came from a separate survey (AsMat study) conducted in 2003-2003.

Data on vital events, including pregnancy outcome (early and late foetal loss) and infant mortality, were collected by the monthly surveillance and visit of CHRWs at the household levels.

In the analysis following covariates were available for adjustment: women's age, gravidity, education, socio-economic status assessed by asset index, and geographic location of residence. The study also extracted the seasonality of pregnancy outcome and year of birth.

The risk of foetal loss and infant death in relation to As exposure was estimated by Cox proportional hazards model. Arsenic exposure was divided into quintiles, and 1st quintile corresponded relatively well with the WHO guideline value of 10 µg/L. Exposure was also divided into the levels less than 50 µg/L and more than or equal to 50 µg/L to determine the effect size related to the current Bangladesh drinking water standard.

Paper II

This prospective cohort study was carried out during 2002-2004. Spontaneous abortion was evaluated in relation to urinary As concentrations in early gestation (2,924 women); and stillbirth, perinatal and infant mortality in relation to the average of urinary As concentrations at GW 8 and GW 30 (1,725 women).

Women were followed up monthly for pregnancy outcomes (spontaneous abortion, stillbirth, live birth) and survival of infants. Cause of death information was collected through "verbal autopsy" method that used a standardized interview with the parents. Information on outmigration was also collected.

Information on women's age, gravidity, parity, education, and household assets were retrieved from MINIMat databases and also from HDSS databases. Last menstrual period date was determined by recall during the pregnancy identification interview at the time of monthly household visits. Gestational age at pregnancy outcome was measured by subtracting the last menstrual period date from date of pregnancy outcome and then expressed in weeks. Season of birth was categorized into pre-monsoon (January – May), monsoon (June-September) and post-monsoon (October-December) periods. In addition women's geographical location was extracted for evaluation.

Women's weight and height were measured during the visit to health facilities at enrolment into the MINIMat study (usually at gestational week 9). Body mass index (BMI) was calculated as said before.

Logistic regression analysis was used to analyze the association between As exposure and spontaneous abortion and stillbirth. A time-to-event approach by Cox proportional hazards model was employed to determine the risk of perinatal and infant death.

Paper III

This prospective cohort study was nested into MINIMat study and included women who were enrolled from 1 February 2002 to 31 January 2003. Out of 1,697 women with singleton birth where sizes at birth were measured, 1578 had urine As concentration data at both GW8 and GW30, and included in the analysis.

Exposure was assessed by average of iAs and methylated metabolites in urine collected two times during pregnancy, one at early and the other at late gestation.

Outcome information (birth weight, length, head and chest circumferences) was collected by the MINIMat study prospectively following standardised methods [112].

Covariates available for evaluation were women's age, parity, education, socio-economic scores by asset quintiles, women's anthropometry (weight, height and BMI); and gestational age at birth and sex of infant. History of betel-nut chewing and/or tobacco smoking was obtained. Season of birth was categorized to pre-monsoon (January–May), monsoon (June-September) and post-monsoon (October-December) periods.

Association between average As exposure and size at birth was assessed with a least-square linear regression model analysis evaluating any linear

association over the full range of exposure. In addition exposure and outcome data were examined by plotting scatter graphs including Loess's moving fitted-average line. The Loess line indicated a negative dose-effect of As exposure on size at birth in the lower range of exposure (<100 µg/litre) after which the line levelled out and no further negative dose-effect was observed. The suggested pattern of dose-effect in the graphs was statistically tested by modelling size at birth as a function of As concentration (continuous variable), level of exposure (categorical variable with exposure of As <100 µg/litre coded = 0 and ≥ 100 µg/litre coded = 1), as well as a variable capturing the interaction between these two variables. Coding the variable "level of exposure" to 0 provided an estimation of the dose-effect of As in lower range of exposure. We also evaluated the dose-effect of As at the higher level of exposure by reversing the codes of the "level of exposure" variable and constructing a new interaction variable. The analytical strategy used in this study has been used elsewhere [113, 114].

Paper IV

In this cohort study, 1552 mother-infant pairs of MINIMat study were included in the analysis when having a minimum of 1 postnatal monthly visit and had urinary As concentrations available from both early and late gestational periods. As each mother- infant was followed up every month in infancy and each visit included a 7-days morbidity recall, an infant had the probability of contributing 84 days in the study base. The mean sum of person-days of observation was 75. In total 115,872 person-days of observation were contributed by 1,552 infants.

A birth notification system was developed for the MINIMat study, and mother-infant pairs were followed up by monthly home visits. Information on symptoms related to lower respiratory tract infection (LRTI) and diarrhoea, two of the most common morbidities during infancy, was collected by repeated 7-days recalls. Mothers were asked about cough and/or fever and any breathing difficulty in the preceding 7 days. If a woman's answer was positive, she was further inquired about presence of rapid breathing and chest-indrawing. The mother was also inquired about passage of loose or watery stools including frequency or passage of any stool with blood.

The birth notification system enabled measurement of birth-anthropometry within 72 hours. Data on newborn sex, birth weight and length practices was collected. Information on feeding including breast feeding and anthropometry of infant was also collected by monthly home visits.

The risk of LRTI and severe LRTI and diarrhoea incidence in relation to average prenatal As exposure (mean As concentrations of early and late gestation) was evaluated by Poisson regression.

Ethical considerations

The main ethical issue was related to the detection of high level of As in drinking water, as well as in urine of pregnant women. The retrospective cohort study included pregnant women who were registered in HDSS in 1991 – 2000 and had water As concentrations in tube-well water assessed by AsMat study in 2002-2003. However, the AsMat Study included a mitigation option that was implemented in collaboration with Bangladesh Rural Advancement Committee (BRAC) – the largest non-government organization in Bangladesh. High priority was given to the households with As induced skin lesion or pregnant women. Tube-wells with As levels ≥ 50 µg/L were painted red and those < 50 µg/L green in accordance with the Bangladesh government program. Pregnant women were advised to drink water from the green tube-wells. A series of village-information meeting were also conducted as part of the study. In the meeting, discussion took place about choice of other possible mitigation options such as filtration, rain water harvesting, pond-sand filter and deep tube-well water. All these options were also implemented in the area. We were not able to inform individual mothers about the level of As exposure measured in urine due to delayed analyses of those samples abroad.

These collaborative studies had been approved by the ethical review committee of ICDDR,B, Bangladesh. Approvals from ethical committees were also obtained from and Karolinska Institutet in Sweden.

Results

General characteristics of study participants

The mean age of women enrolled in the retrospective cohort study was 27 (Standard deviation (SD) 6) years. About one quarter had their first pregnancy, while 12% had their 5th pregnancy or more. On average they had attended formal schooling for 3 years. About half of the women had not attended any school.

The mean age of women enrolled in the prospective cohort study (n = 2,924) were 26.5 (SD 6) years. About 30% had their first pregnancy, while about 15% of women had their 5th pregnancy or more. The mean number of years of attendance in a formal school was 5 (SD 4). About 25% of the women had not attended any school. Among the 1,725 women who had urinary As concentration in GW8 and GW30, mean weight and height measured at GW8 was 45 (SD 7) kg and 150 (SD 5) cm, respectively. About one-third of these women were malnourished (BMI < 18.5 kg/m^2).

Exposure to arsenic

Water arsenic levels

A major proportion of women were using tube-well water with high As concentrations. In total about 70% of pregnant women were exposed to As concentrations more than the local drinking water standard ($\geq$ 50 µg/L), while about 80% were exposed to As more than the WHO guideline value ($\geq$10 µg/L). The mean water As concentration was 239 µg/L (SD 209) (10th percentile below 1 µg/L; 90th percentile at 513 µg/L) indicated a highly skewed distribution of water As (Figure 5). Women's education, socio-economic status, and order of pregnancy were associated with As exposure.

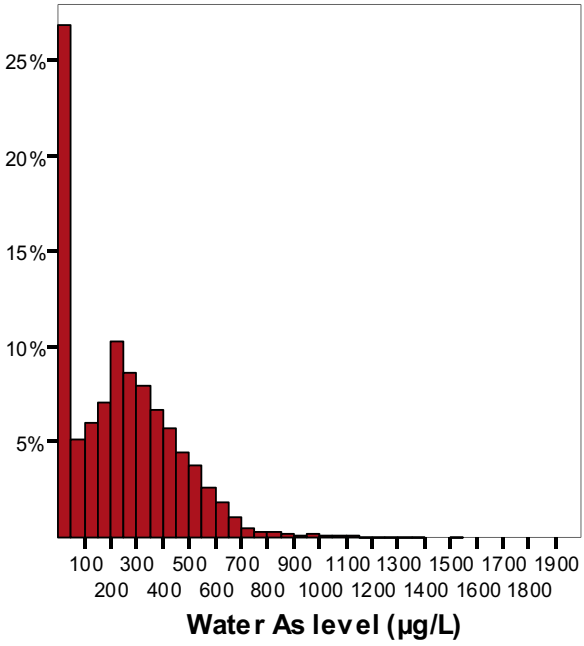

Figure 5. Distribution of arsenic concentrations in tube-well water used by pregnant women in the cohort 1991 – 2000 in Matlab, Bangladesh

Urinary arsenic concentrations

The median urinary As concentrations in GW8 was 80 µg/L (lowest: 1 µg/L; highest: 1253 µg/L).The mean concentration was 154 (SD 176) µg/L, demonstrating the highly skewed data. The median and mean concentrations at GW30 were 82 µg/L and 171 (218) µg/L, respectively (lowest: 2 µg/L; highest: 3,384 µg/L). The median and mean average urinary As concentration in early and late pregnancy combined (average of GW8 and 30) were similar; 94 µg/L and 163 (174) µg/L (lowest: 5 µg/L; highest: 2,019 µg/L).

In general the concentrations of urinary As in GW8 and GW30 were similar (Spearman's correlation coefficient: 0.61). However, there was a marked intra-individual variation in urine As concentrations measured in early and late gestational age. In 198 women urinary As concentrations decreased by ≥ 50 µg/L, while in 255 women the concentrations increased by ≥ 50 µg/L between GW8 and GW30.

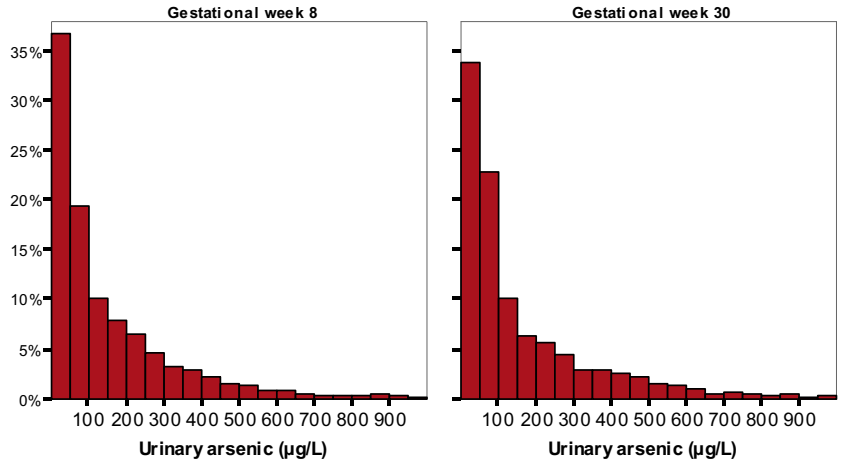

Figure 6. Distribution of urinary arsenic concentrations in GW8 and GW30 of women participating in the prospective cohort study in Matlab, Bangladesh

Outcomes

The rates and proportions of pregnancy outcomes are presented in Table 4. Early foetal loss was higher in the prospective cohort study, which most likely reflects the efficient case detection if pregnancies were identified early by urine pregnancy tests. Neonatal and infant mortality were lower in the prospective cohort, reflecting the improvement of maternal and infant health that has taken place.

Table 4. Pregnancy outcomes and neonatal and infant mortality rates of the different papers

Outcome	**Historical cohort (Paper I)**	**Prospective cohort (Paper II-IV)**
Induced abortion[a]	38[a]	30
Early foetal loss[a]	55[a]	94
Late foetal loss[a]	28[a]	18
Neonatal mortality[b]	33 [b]	19
Infant mortality[b]	54[b]	26

[a] per 1000 pregnancies, [b] per 1000 live births

The mean gestational age at birth was 39.3 (SD 1.7) weeks. Eleven percent of infants were born preterm (<37 weeks). The mean birth weight was 2,681 (SD 401) g and 32% had a low birth weight (<2,500 g).

The incidence rates of LRTI and severe LRTI were 2.4 (95% CI: 2.2 - 2.5), and 1.9 (95% CI: 1.8 - 2.1) episodes/person-year, respectively. The incidence of diarrhoeal diseases was 3.9 episodes (95% CI: 3.7 - 4.2) per person-year.

Associations with foetal and infant survival

In the historical cohort study, the risk of foetal loss and infant mortality was significantly increased by 14% (Relative risk (RR) 1.14; 95% CI: 1.04 - 1.25) and 17% (RR 1.17, 95% CI: 1.03 - 1.32), respectively, for women who were exposed to As levels ≥ 50 µg/L in comparison with women exposed to As levels < 50µg/L in tube-well water. Significant dose response of As exposure to risk of infant death was also observed (*P*-value for linear trend = 0.02).

When applied to the prospective cohort study design with exposures assessed by urinary As concentrations, the above findings were confirmed. Women with urine As concentrations at the fifth quintile level (249 – 1,253 µg/L) in early gestation had 62% increased risk of spontaneous abortions occurring before 120 days of gestation (Odds ratio (OR) 1.62; 95% CI: 1.04, 2.55) in comparison with women who had As concentrations at the first quintile level (< 33 µg/L). The relative risks were about 3 and 5 times higher for perinatal (RR 3.01, 95% CI: 1.07 - 8.45) and infant mortality (RR 5.01; 95% CI: 1.41 - 17.84), respectively, for women with urinary As concentrations at the 5th quintile (268 – 2,019 µg/L) in comparison with women who had As concentrations below 38 µg/L (1st quintile). Significant dose-response associations were also observed for spontaneous abortion, perinatal and infant mortality.

Associations with size at birth

This study evaluated the association between individually assessed As exposure in pregnant women and size at birth (n = 1,578). While there was no dose-effect association over the full range of As exposure a significant dose effect was found with birth weight in the lower range of exposure. In this range (0 - 100 µg/L) of exposure there was a 1.68 g reduction in birth weight for each 1µg/liter increase in urinary As concentration. The dose-effect levelled out and no additional decrease in birth weight was observed when exposure exceeded 100 µg/L. We also observed 0.05 mm and 0.14 mm decrease of head and chest circumference, respectively, for 1µg/liter increase of As in the exposure range <100µg/L concentration in urine.

Associations with LRTI and diarrhoea morbidity

Arsenic exposure was significantly associated with risk of LRTI and diarrhoea morbidity. In comparison with exposure to average urinary As at the first quintile level the risks of LRTI and severe LRTI were increased by 68% (RR 1.68; 95% CI: 1.35, 2.07) and 52% (RR 1.52; 95% CI: 1.20, 1.94), respectively, for women who had urinary arsenic concentrations at the 5th quintile level. The corresponding increase for diarrhoeal diseases was 19% (RR 1.20; 95% CI: 1.00, 1.41).

Discussion

The studies of this thesis were conducted on rural Bangladeshi women with a backdrop of low-socio-economic conditions, high prevalence of malnutrition, and wide use of As contaminated tube-well water for drinking and cooking purposes. The analyses focus on epidemiological associations between prenatal As exposure and foetal loss, size at birth, and infant morbidity and mortality of the offspring. The studies have demonstrated detrimental effects of As on early human development. The magnitude of exposure of the population in Bangladesh and some other low-income countries increases the public health significance of our findings. However, we observed increased risks at the relatively low levels of exposure that are found in drinking water also in other parts of the world, including parts of Europe and North America, indicating that the findings may be of relevance for pregnant women and their children even in those areas.

In this community based studies we observed women who were exposed to high As levels during the entire period of gestation. In **Paper I**, where As exposure was assessed by water As concentrations in tube-wells used by pregnant women, we observed a weak association between As exposure and foetal loss. However, we found a significant dose-dependant association between As exposure and infant mortality and most of the risk increase was at the level of local drinking water standard (10 - 49 μg/L). In **Paper II –IV** exposures were assessed by urinary As concentrations in pregnancy, which reflect As intake both from drinking water and food. The findings observed in the Paper I were confirmed in the prospective cohort study (**Paper II**), and we found significant dose-dependant associations between urinary As concentrations and spontaneous abortions, perinatal and infant mortality. The risk estimates were also more pronounced. In **Paper III**, significant negative dose-effects were found with birth weight, head and chest circumference at a relatively low level of As exposure (<100 μg/L in urine). No further increase in negative effects was shown at higher levels of As exposure. In **Paper IV**, for the first time we have also shown an association between prenatal As exposure and LRTI and diarrhoea morbidity of the offspring.

The studies that form the basis for my thesis were population based, they included large sample sizes that gave opportunity to demonstrate even

smaller increases in risk that still may be of public health significance, the assessment of individual As exposure included use of biological markers, and outcome data were prospectively collected as part of a health and demographic surveillance system (HDSS) and a community based nutrition trial with a large pregnancy cohort (the MINIMat trial). Selection and information biases in the historical cohort study were minimized by combining outcome data from the regular monthly household surveillance and exposure information from the cross-sectional survey of As concentrations in all tube-well with interviews about drinking water history on the individual level. In the prospective cohort study, As exposure was assessed by concentration of iAs and methylated metabolites in urine that reflected exposure both from drinking water as well as other sources including foods.

Methodological considerations

Internal validity

The validity and reliability of the studies of this thesis were based on quality assurance systems inbuilt into HDSS, and AsMat and MINIMat studies. All data collected in the field were checked and rechecked for missing values and/or inconsistencies. The AsMat and MINIMat studies were carefully designed and implemented. Data collection instruments were developed and finalised after sufficient pre-testing. Field implementation manuals were in place in time, where all processes of measurements were described. Field workers were efficiently trained for interviews and other methods of data collection as well as for collection of biological samples. In addition, a quality control team worked independently to re-assess parts of data collected. Regular meetings with field workers took place at Matlab and sub-centres and refresher training was arranged following a pre-set schedule.

Exposure assessment

The historical cohort (Paper I) study included pregnant women recorded by HDDS from 1991 to 2000, while exposure data came from a later cross-sectional study that collected information on lifetime drinking water use year by year and also measured the water As concentrations of tube-well water used by the pregnant women. The validity of the exposure assessment in this paper should be discussed in relation to two assumptions – that there was no temporal variation in water As concentrations in the tube-wells and that the pregnant woman had only one drinking water source during the year of her pregnancy, the one she had reported in the interview with the field workers. Such limitations have also been present in all earlier studies that based exposure on water As concentrations [11, 15, 16]. There are only a few studies on temporal variation of As in ground water. In well-water in northern Argen-

tina and Western Nevada in the United States the water As concentrations remained at about the same levels over a period of 10 – 20 years [115, 116]. Also, a small temporal variation of water As concentrations in tube-well water was reported in Bangladesh [6, 117]. Uncertainties in exposure assessment have often been related to the potential variation in water consumption and use of multiple drinking water sources. The use of drinking water from tube-wells is almost universal in rural areas in Bangladesh including the study area. Other sources of safe water such as bottle water are not common. Therefore, water As concentrations should provide a valid measure of exposure at the population level. There is increasing evidence that the population also is exposed to As through the food chain, especially from rice and vegetables caused by the wide use of ground water for irrigation [118, 119].

In paper II – IV, we used biological markers of As exposure – the urinary As concentrations. As that is absorbed from the gastrointestinal tract is excreted in urine within 1 – 2 days. With sustained exposure As levels rise into a steady state level. Therefore, urinary As concentration is considered a valid indicator of individual exposure. There are other biological markers such as As concentration in blood, hair and nail that have been used in different studies. Measures of blood As only reflects exposure in the very recent past, as As is found to clear from the body within a few hours. Therefore, blood levels do not appear to be a reliable indicator of chronic exposure of low dose As. Measurements of As in hair and nail may be useful indicators of past As exposure as As tends to accumulate in those tissues. However, analysis of hair may be misleading due to the ability of hair to adsorb external contamination through the surface.

Outcome assessment

Studies of early foetal loss have always a degree of uncertainty due to inadequate assessment of pregnancy loss close to conception. The proportion reported in Paper I was 5.8%. A monthly surveillance system that relies on women's reports is not able to identify some early pregnancies that end in foetal loss within some weeks, especially since such losses are often not noticed by the women themselves. It is recognised that spontaneous abortion rates depend on data collection instruments [120], with the highest rate if pregnancies are identified by repeated hormonal test. Usually such rates vary from 10 to 15%, although much higher rates (22 – 31%) also have been reported [71, 121].

We defined early and late foetal loss based on gestational age. The assessment of gestational age is based on reported LMP date. The monthly visits by HDSS staff make it easier to get correct information on date of last menses, and the experience from the field work indicates that only very few women failed to give exact date of LMP. We have related LMP based as-

sessment of gestational age with the corresponding data based on ultrasound and found a very good agreement (Paper II – IV), and thus we assume that it is unlikely that gestational age data have any major errors.

Measurement of size at birth was mostly collected within 72 hours after birth. An adjustment of birth anthropometry was also done if birth weight was collected after 72 hours [109]. Cause of death was systematically collected by verbal autopsy method, which has been recommended as a valid tool of cause of death assignment in areas where many deaths take place at home [111]. Infant morbidity was based on the reported symptoms, and was collected during monthly home visits, covering symptoms of respiratory tract infection and diarrhoea during the last week. The morbidity interview used a standardised methodology that has been used in many other population-based studies in Matlab and elsewhere, and there are no reasons to believe that any misclassification of morbidity should be differential in relation to As exposure.

Confounding issues

To deal with the confounding issues we defined criteria for identification of a confounder beforehand to guide which variables to be included in data collection and later used for adjustment, if needed. However, in order to assess the robustness of the findings we also included the relevant important covariates that were found to be associated with outcome of interest.

The observational design of the studies call for cautious interpretation of the effect of As on different outcomes studied. We have probably managed confounding in a satisfactory way. The possibilities of residual confounding should however be considered. In Paper I, information on maternal anthropometry and exposure to other toxic agents (e.g. tobacco smoking and betel-nut chewing) was not available. This was also the case when evaluating the association with spontaneous abortions in paper II in the full cohort of women. However, these covariates were available in the sub-sample of women who participated in the MINIMat study. Women in the study area do not use tobacco but chewing of betel-nuts is common. We did not observe any confounding related to these habits. Similarly, in the subsample where women's nutritional status was available, the effect estimates were not changed after adjustment with BMI.

In the analyses of all the four studies, socio-economic indicators came out as important confounding factors. We used asset score or quintiles of these scores as measures of SES, derived from household possession of assets, housing structure, land occupation and income. To assess whether asset quintiles represented the SES, we evaluated each of the components used to derive asset scores. When asset quintiles were included in the model, no

individual asset was found to change the effect estimation, and therefore obviates the presence of residual confounding due to SES.

We did not measure other concurrent elements either in water or in urine. Whether these unmeasured elements (e.g. manganese, lead, cadmium and mercury) have confounded the results could not be excluded. The concern arises from the fact that the metals like lead, mercury, and cadmium are also recognised as reproductive toxicants. So far knowledge is relatively limited about the co-existence of other metals in this setting. Several studies have reported the presence of manganese in the lower range of As exposure in tube-well water [6, 122]. However, in a subsample, where water manganese was available, we did not observe any association with size at birth (unpublished data). Future studies should address these issues and also explore the interaction of As exposure with other elements on reproductive outcomes.

Selection and information bias

Systematic errors in selecting subjects and in ascertaining exposure and outcome may spuriously increase or decrease the effect estimates. In these population-based studies, we managed to keep non-participation low and thereby achieving a good representation of the target population. In paper I selection bias was minimised by including all women who were living in the area at the time of the As survey that produced data on water As concentrations during pregnancy. It is unlikely that pregnant women living in the area 1991-2000 but later unavailable in the As survey should represent a potential bias of results. In paper II-IV, all women identified by urine pregnancy testing over a full calendar year period were invited to participate in the study. In paper II 2,924 out of 3,081 pregnant women were included in the analyses. In paper III and IV 155 out of 1,866 live-born infants available mother-infant pairs were lost to follow-up and 119 had no urine sample either at GW8 or at GW30 or on both occasions. It is unlikely that this relatively limited non-participation had any association with As exposure levels.

Information bias usually results from either imperfect definition of study variables or flawed data collection procedures. The design of these studies and process of measurement of exposure and outcome data probably prevented the occurrence of information bias. At time of water history interviews (paper I) interviewers as well as respondents were unaware of the water As level – that was tested the following week. In the prospective pregnancy cohort data collectors as well as participating women were not aware of the urine As concentrations, which were analysed in the laboratory at a later stage. These procedures may have eliminated biases in interviews and reporting. Also in the laboratory the staff was unaware about any As or outcome information on the individual when performing the analysis of the sample.

External validity and generalization

The findings from this thesis are relevant all over Bangladesh, as high As levels in tube-well water are common in almost all parts of the country. Also, socio-demographic and cultural conditions are similar to other rural areas in the country. The outcomes of interest in this thesis have also been investigated in other parts of Bangladesh as well as in other countries, e.g. in Chile, Hungary, India, Taiwan [11, 13 -17]. The increased risk of foetal loss, low birth weight, infant morbidity and mortality observed in these papers were in line with results of studies conducted in different parts of the world [11, 13-17, 46]. Arsenic exposure in utero and prominent pulmonary effects in young adults in Chile also support our finding of prenatal exposure associated with morbidity in infants [123].

We have not explored the role of susceptibility factors such as nutritional status and methylation patterns in this thesis. However, in the stratified analyses we observed similar increases in risks due to As exposure by BMI groups and SES groups. In addition, no interaction was observed with BMI and SES in Paper I – IV. This increases the possibility that the findings presented are possible to generalise also to As-exposed populations with better nutritional status of women and less poverty. Also, the risk increase was found already at relatively low exposure levels, which increases the relevance of our findings to most countries worldwide where As contamination is reported.

Relation to other research

There are so far relatively few epidemiological studies focusing the association between prenatal As exposure and negative reproductive outcomes and child health of the offspring. To the best of our knowledge, no earlier study has assessed individual exposure during pregnancy with a biological marker of exposure. Earlier studies in Bangladesh by Ahamad et al. and Milton et al. reported higher odds ratios of foetal loss than what we observed in Paper I – II [11, 15]. However, those studies were ecological or cross sectional in design, outcome was assessed retrospectively, exposure was not assessed during pregnancy and no dose-response association was observed. In contrast to this another cross-sectional study in West Bengal, India, reported much higher risk of stillbirth in the group using water with more than 200 µg/L, but with a very broad confidence interval. However, they did not find any association with spontaneous abortion and infant mortality [16]. The level of risk of infant mortality demonstrated in Paper I was similar to those found in other studies that have evaluated infant death risks [14 -16]. The strong association of prenatal As exposure on infant death in Paper II may be due to higher precision in exposure and outcome assessments in that study.

A recent study, based on only 52 pregnant women, reported a significant association between hair As concentrations and birth weight. However, neither was the study population profile provided nor was it clear whether the analyses were adjusted for important covariates such as socio-economic status and women's anthropometry [48]. Further, hair As concentration may be influenced by external use of As contaminated water. Several studies in Bangladesh have reported increased risk of infant death, higher blood pressure and impaired cognitive development at relatively low As exposure levels supporting our findings of negative effects at low levels of exposure [124, 125]. Our report of significantly shorter head circumference may be in line with the reported adverse effect of As exposure on cognitive development in childhood, as found in studies in Bangladesh and elsewhere [125, 126].

To our knowledge there are no earlier epidemiological studies on prenatal As exposure and lower respiratory tract infection and diarrhoea in infancy of the offspring. Some studies have reported As related respiratory symptoms and signs such as chronic cough, bronchitis and bronchiectasis, in relation to drinking water with high As levels [31, 127, 128].

The exact mode of action of As in relation to these findings is not known but may involve oxidative stress, interference with hormones, especially glucocorticoids and estrogens, perturbation of DNA methylation, increased telomerase activity, and modulation of signal transduction pathways [7, 59, 129-132]. Arsenic may also cause aberrant placental vasculogenesis and placental insufficiency, as shown in animal models [133]. A number of studies have reported increased levels of oxidative stress markers in urine in populations exposed to high levels of As [7, 35, 134-137]. Considering the available knowledgebase, it is believed that As induced oxidative stress plays an important role in developing reproductive toxicity. Recently attention has been focused on certain chemicals that may produce the hazardous effects due to the ability of these substances to mimic or block endogenous hormones. Chemicals with this type of effects are called endocrine disruptors. There is growing body of evidence that this may have significant public health implications, as these chemicals may be capable of affecting the synthesis, secretion, transportation, binding, action and elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development and/or behaviour through neuro-endocrine axis [35]. Animal studies have documented As to be an endocrine disruptor, altering hormone-activated gene transcription mediated by the closely related steroid receptors already at very low doses of exposure [129, 138]. However, these aspects have not been studied in this thesis.

Causality

One question is pertinent whether the observed associations between As exposure and early human development and infant health are causal. The commonly used criterion as proposed by Hill was first published as part of the first Surgeon General Report on Smoking and Health. The guideline remains as a cornerstone in making inferences on causal relationship. However, we did not included criteria such as coherence, specificity and analogy, as there is much disagreement on usefulness of these points [139], Table 5.

Experimental evidence: To determine the causal association, evidence emanating from a randomized controlled trial is considered gold-standard. However, except the beneficial interventions (e.g.vaccine trial), in most cases in epidemiological studies randomization is not feasible or ethically acceptable. However, few epidemiologists have extended this view whether the disease has been produced in animal models. Considering this point, there is good evidence that prenatal As exposure affect the offspring. However, this was not available for morbidity outcomes.

Table 5. Evidence in favour of causality

Criteria	Paper I		Paper II		Paper III	Paper IV
	Foetal loss	Infant mortality	Early foetal loss	Infant mortality	Birth weight	Morbidity
Experimental evidence	++	++	++	++	++	-
Temporality	++	++	++	+++	+++	+++
Strength of association	+	++	++	+++	+	+++
Dose response	-	++	+	+++	++	+++
Biological plausibility	++	++	++	++	++	++
Consistency	+	++	+	++	++	-

-: no evidence; +: some evidence; ++: good evidence; +++: strong evidence

Temporality: The right temporal sequence of events should be present. Under all circumstances it should be possible to demonstrate that exposure preceded the outcome of interest in order to support the inference that the relationship is causal. In an epidemiological study it is often difficult to judge whether the disease process remains in subclinical phases or has an insidious onset. There are also problem of reverse causality that may indeed change the exposures. In Paper I, where As in tube wells was measured by a later cross sectional survey this evidence is not present. However, in Paper II – IV exposure was assessed by assessments of urinary As concentrations prior to the occurrence of study outcomes making the argument in favour of maintaining temporality good or strong.

Strength of association: The strength of association was not strong in the case of foetal loss. However, the effect estimates on perinatal and infant mortality, and also on infant morbidity were good or strong.

Dose-response: the observation of a straightforward dose-response relationship between exposure and risk of an outcome is regarded as evidence of a cause-effect relationship. We observed this kind of relationship between As exposure and perinatal and infant death. However, the dose-response association was less evident regarding foetal loss. Although a dose-dependant association was observed, it did not continue across all levels of exposure.

Biological plausibility: A biologically plausible chain of the events also supports a causal association between prenatal As exposure and adverse pregnancy outcomes. There are biologically plausible explanations for each link in the chain of events as described in the pathway of As induced toxicity (Figure 3).

Consistency: We have observed consistency of results across different epidemiological studies from a wide range of geographical areas, e.g. Bangladesh, Chile, Taiwan, and West Bengal, India. This consistency supports that the observed associations reflect causal effects of prenatal As exposure on adverse pregnancy outcomes and health during infancy of the offspring.

In summary, there is good evidence that consumption of As contaminated tube-well water in pregnancy can cause adverse pregnancy outcomes and negatively affect infant health of the offspring, i.e. increase the risk of lower respiratory tract infections and diarrhoea, as well as increase the risk for infant death (Table 5). Underlying factors such as socio-economic background and nutritional status may interplay in the development of the disease outcome.

Public health implications

The observed As induced effects on early human development has severe public health implications considering the extent of exposure and the relatively high effect estimates on foetal loss, infant morbidity and mortality. The risk for adverse effects was also observed at the level the Bangladesh drinking water standard is judging as safe, which further stresses the importance to change of local drinking water standard (<50 μg/L) and adopt the WHO guideline value immediately.

We estimate that more than 10% of the current infant mortality in Maltab, Bangladesh, is attributed to As exposure during pregnancy (relative risk:

1.17 at drinking water level more than 50 mg/L). The excess risk increase in the study indicates that 10,000 to 19,000 out of the 250,000 estimated annual infant deaths in the country might be attributed to As exposure (exposure prevalence: 25 to 50% at drinking water level more than 50 µg/L in the country). This estimate is conservative considering the strong effects found when exposure was assessed by a biological marker. The negative effect of birth anthropometry is also important, since these effects were shown at a relatively low exposure level that may be of relevance for pregnant women in many parts of the world, including parts of Europe and the USA. The negative effect of As on birth weight we report is of similar size as has been reported by cigarette smoking during pregnancy [140]. This magnitude is also similar to the maximum of what can be achieved by food supplementation in pregnancy of malnourished women, albeit the latter is an improvement [114]. In all findings of this thesis, most risk increase was observed at a relatively low level of exposure. Therefore, As exposure may be considered a significant reproductive health hazard in a worldwide perspective.

Conclusions

In this thesis we investigated effects of As exposure of women in pregnancy on the health of their pregnancies and offspring. The outcomes included in this study are common, e.g. spontaneous abortions. Lower respiratory tract infection, often pneumonia, and diarrhoeal diseases are the two most important global threats to infant health and survival. Perinatal deaths and death during infancy are catastrophes for mothers and families and still very common in low-income countries. In summary, we may draw the following conclusions from this thesis:

- The findings revealed that As exposure in pregnancy is associated with increased risk of foetal loss, and perinatal and infant mortality of the offspring.
- Prenatal As exposure is associated with size at birth already at low exposure levels.
- The study results also reveal that prenatal As exposure is associated with increased risk of lower respiratory tract infection and diarrhoea in the offspring.
- For most findings the evidence of causal associations was good or strong.
- The public health consequences of the findings are severe, and highlight the importance of reinforced mitigation activities, where women of reproductive age should be prioritised.

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