Human biomonitoring survey
assessment of prenatal exposures to mercury
using biomarkers in cord blood, maternal urine and hair

Site-specific protocol
Ghana

2017

Contents

1. Introduction and background

1.1 Mercury and its health effects

Mercury is recognized by WHO as one of the top 10 chemicals or groups of chemicals of major public health concern. Its toxicity to human health has long been known, and the toxic effects of different forms of mercury extensively studied *(1)*.

Elemental and methylmercury are toxic to the central and peripheral nervous systems. The inhalation of mercury vapour can produce harmful effects on the nervous, digestive and immune systems, lungs and kidneys, and may be fatal. The inorganic salts of mercury are corrosive to the skin, eyes and gastrointestinal tract, and may induce kidney toxicity if ingested.

All humans are exposed to some level of mercury. Most people are exposed to low levels, often through chronic exposure (continuous or intermittent long-term contact). However, some people are exposed to high levels of mercury that can cause acute poisonings.

Fetuses are most susceptible to mercury. Methylmercury exposure in the womb can result from a mother's consumption of contaminated fish and shellfish. It can adversely affect a baby's growing and developing brain and nervous system, which leads to disorders of cognitive functions, memory, attention, language, and fine motor and visual-spatial skills later in life *(2, 3)*.

Human biomonitoring (HBM) is an effective and reliable tool to assess cumulative exposure to environmental pollutants and is an essential element in a strategy aiming to integrate health and environmental policies. Biomonitoring data directly reflect the total body burden (or biological effect) resulting from all routes of exposure, and inter-individual variability in exposure levels, metabolism and excretion rates. Determination of mercury levels in human tissues, such as hair, blood, nails, milk and urine, is recommended for assessing population exposure to mercury and its compounds *(4)*. The results of biomonitoring-based surveillance can be used for planning and assessing the effectiveness of risk prevention measures.

The basic intent of this document is to provide guidance for countries in constructing a national protocol for the monitoring of human exposure to mercury. This document was developed based on the outcomes of an international experts meeting held in Bonn, Germany on 24–25 June 2015 *(5)*. A number of other meetings and expert discussions provided important input to this methodology development.

The protocol comprises recommendations on survey design, recruitment and fieldwork, dealing with biological materials, data management and communication, and ethical considerations for the pilot survey in Ghana.

1.2. Minamata Convention

The Minamata Convention (6) to whicj Ghana is signatory, is a global legal instrument aiming at reducing mercury emissions from all sources and to regulate its use in order to safeguard humanity and the environment. The Convention regueres the health sector to identify population groups at risks of mercury exposure to Human biomonitoring is recognized as the most effective tool for evaluation of cumulative human exposure to mercury and identification of exposed population groups.

Since the period of in-utero development is the most vulnerable stage, in terms of long-term adverse neurodevelopmental effects of mercury, characterization of prenatal exposure is critical for

evaluating the public health impact of mercury, and for assessing the public health benefits of reducing exposure. A harmonized approach is necessary to ensure provision of reliable and comparable results at national, regional and global level.

1.3. Scientific evidence and international consultations

This document is based on scientific information on mercury biomonitoring and health effects collected by WHO, including the following: *Guidance for identifying populations at risk from mercury exposure* (2008)*(4)*; *Mercury and Health* fact sheet (2016)*(1)*; *Mercury exposure and health impacts among individuals in the artisanal and small-scale gold mining community* (2014)*(7)*; documents on the work of WHO in coordinating the development of standardized protocols for HBM surveys on mercury, and planning pilot testing in volunteer countries, under the mandate of the Parma Declaration commitments to reduce early life exposure to environmental pollutants *(8)*; and the *Report on information on harmonized systems for measuring mercury body burden* (2011)*(9)*.

In April 2012, at a meeting in Catania, Italy, WHO experts discussed the overall approach, biological matrices and indicators for assessment of prenatal exposure to mercury for development of a harmonized approach to mercury HBM *(10)*. Women who had just delivered a child were agreed as the target population, and scalp hair, cord blood and urine as the matrices for assessment of prenatal exposure to mercury during last three months of pregnancy. *(10)*. The approach proposed by the experts was agreed by the representatives of WHO European Region Member States at the Second Extraordinary Meeting of the European Environment and Health Task Force (EHTF), The Hague, Netherlands, 31 May–1 June 2012 *(11)*.

The discussion continued during a number of forums including: the special session “Protecting human health from negative impact of mercury: from science to policy” at the International Conference on Mercury as a Global Pollutant (14–19 June 2015, Jeju, Korea)*(12)*; the session “Human biomonitoring as an instrument for assessment of exposure to mercury” at the meeting of representatives of the European Member States “Health sector involvement in the implementation of the Minamata Convention” (24–25 June 2015, Bonn, Germany)*(6)*; the international technical experts workshop “Harmonized approach to biomonitoring of human exposure to mercury” (26 June 2015, Bonn, Germany) (unpublished minutes); and during the session “Exposure assessment and health effects” organized by the National Institute for Minamata Disease, Japan, WHO Collaborating Centre for Studies on the Health Effects of Mercury Compounds at the Fifth Conference on Prenatal Programming and Toxicity (14–16 November 2016, Kitakuyshi, Japan)*(13)*.

International Ethical Guidelines for Health-related Research Involving Humans (2016) prepared by the Council for International Organizations of Medical Sciences (CIOMS) in collaboration with WHO laid the basis for the ethical requirements included in the protocol *(14)*.

2. Aims and approach of the survey

The primary objectives of the survey are to provide the data needed for the development of a global mercury monitoring plan, and baseline data on prenatal exposure to mercury in different population groups. The specific objectives of the survey in Ghana are the following:

- extend the knowledge on baseline levels and sources of human exposure to mercury;
- characterize the level and distribution of prenatal in the general population potentially exposed to methylmercury in fish, in the Accra-Tema Metropolitan Area of Ghana;

- compare biomarker values with health-based thresholds to assess risks of methylmercury in general population group;
- provide data for baseline assessment of exposure to mercury globally;
- test the applicability of a harmonized approach to assessment of pre-natal exposure to mercury in Ghana given ethnic and cultural considerations;
- assist Ghana in the implementation of the Minamata Convention and development of effective measures to prevent the negative impacts of mercury on human health, and especially in vulnerable groups;
- provide analytical tools to monitor progress towards implementation of specific goals of the Minamata Convention (the WHO methodology involves assessment of exposure in a set of participants at a certain moment in time; such a cross-sectional survey is expected to be repeated at regular intervals, e.g. every five years).

The objective of this protocol is to provide a uniform framework for all activities and tasks associated with the collection, analysis, assessment and reporting on prenatal exposure to mercury.
The approach will be applied to the survey in Ghana.

Through expert recommendations and technical meetings, the following approach developed by WHO has been accepted and applied for the survey in Ghana: :

- Recruitment will be conducted during antenatal visits and exceptionally at maternity hospitals.
- Participants will be enrolled using a set of defined inclusion and exclusion criteria (legal adults, living in the catchment area of the hospital, live birth, etc.).
- A standardized questionnaire will be administered to participants to assess potential sources of exposure.
- The survey will use non-invasive sampling only (maternal hair, urine and cord blood); standard operating procedures (SOPs) for no risk sampling are provided by WHO.
- National surveys will involve a capacity-building component, to enable analysis of samples in domestic laboratories; methodological support will be provided by WHO, its temporary advisers and reference laboratories.
- Proficiency test and duplicate quality control samples will be analysed in reference laboratories to confirm data reliability.

The protocol has been developed to be applicable in Ghana for assessment of general population exposure to mercury.

3. General principles

The following underlying principles should be considered when applying this protocol to developing a national protocol for monitoring of exposure to mercury:

- Sampling of biological material (hair, cord blood and urine) should not harm or pose an undue burden on recruited women.
- Safeguarding the confidentiality of information should be assured.
- Ethical standards, including prior informed consent, should be respected.
- The protocol should be practical, feasible and sustainable.
- Emphasis should be placed on proficiency.
- Quality assurance of results should be independently confirmed.

- Selection of sample size and hospitals should be justified
- All measures should be taken not to affect health service provision.

3.1. Roles and responsibilities of WHO and participating countries

Both WHO and Ghana implementing institutions (Ghana Health Service, Ministry of Helath and CSIR Water Research Institute) have roles and responsibilities in the application of the protocol.

The role of WHO in the protocol application is:

- to submit and get approval of the Ghana site-specific protocol from the WHO Research Ethics Review Committee (ERC) and to communicate modifications of national protocols to the ERC, requesting approval before their implementation;
- to organize a training for national coordinators and laboratory analysts on the survey design and implementation;
- to develop and provide Ghana with training materials and SOPs for sampling of biological material, mercury analysis, and creation of national databases, as well as to develop and provide an eligibility screening form and a questionnaire to be completed by the survey participants;
- as an owner of data collected in the national pilot surveys in Ghana, to gather the data from Ghana and to store them in a consolidated global database; to analyse the data gathered through the survey implementation, and to report on the level and distribution of the exposure to mercury at national, regional and global scales to interested governmental and nongovernmental stakeholders (including experts and academia) at an international level;
- to provide technical assistance to Ghana, if necessary, including in implementation of the survey, interpretation of results and risk communication;
- to update the protocol on a global level before each round of mercury HBM, if necessary;
- to coordinate the quality control process to ensure the quality of laboratory analysis of mercury in participating countries.

The role of Ghana in the protocol application is:

- to adapt the WHO protocol to national realities and to obtain approval from national ethics committees;
- to communicate any modifications in the WHO protocol to WHO before the survey implementation;
- to fully comply with the protocol principles when implementing the mercury HBM survey;
- to train the field staff involved in the survey implementation including, but not limited to, interviewers, maternity hospital staff, those responsible for collecting biological samples, those responsible for the storage and transportation of biological samples, laboratory analysts, those responsible for data handling and database creation, etc.;
- to collect data on exposure to mercury in target population groups; to fully comply with WHO SOPs on analysis of mercury in human scalp hair, cord blood and urine including non-invasive sampling procedures;
- as an owner of the national data, to collect and store the data in a national database;
- to analyse national data on the level and distribution of exposure to mercury and to report the data to interested governmental and nongovernmental stakeholders at the national level;
- to report on the application of the protocol and to submit the national protocol to WHO;
- to report to WHO on results obtained in the survey, conducted according to the WHO protocol.

4. Developing a national protocol

The WHO master Protocol served as a basis for the development of the national protocol to meet the aims of the survey in Ghana and WHO global survey.The national coordinator is responsible for overall planning and implementation of the survey in the country, assisted by the appropriately trained scientific and clinical field and laboratory staff from the Water research Institute of the Council for Scientific and Industrial Research and Ridge Regionla Hospital and Tema General Hospital. In particular, the national coordinator will assure that the survey meets all national ethical requirements for studies involving human subjects. No modifications in terms of the survey design and ethical requirements are made in the WHO Master Protocol. Additional information to respond to WHO requirements to site-specific protocols is included in this protocol.

5. Survey design

5.1. Sources of mercury in Ghana and selection of a sampling site

There are a number of mercury sources in Ghana that were revealed in previous investigations. Potential sources of human exposure to mercury in Ghana include the following:

- **Artisanal and small scale gold mining (ASGM)**

In ASGM Hg is used in amalgamation of gold. Several studies have been conducted to explore the biomarkers of human exposure to Hg. In one study, data from approximately 700 hair samples and 800 urine samples from mining areas in the northern part of Ghana shows that the majority of the urinary mercury levels fall between 0.5 and 50 ug/L (median values across studies were 1 to 30), the latter representing the WHO guideline value for occupational exposures. The hair mercury values generally fell below 10 ug/g (median across studies was 1-2 ug/g) (Basu et al., 2015).
In terms of ecological biomarkers, mercury levels have been reported in water (range: from below detection limits to 50 µg/L), sediment (range: from below detection limits to 48.85 µg/g), soil (range: from below detection limits to 185.94 µg/g), collected from sites across Ghana in proximity to ASGM areas (Basu et al., 2015).

In addition to these abiotic samples from ASGM, mercury levels have also been found in fish and seafood (mean range: from 0.004 to 0.896 µg/g). Though most fish contain detectable levels of mercury, the concentrations are generally below Food and Agricultural Organization/World Health Organization (FAO/WHO) and U.S. EPA guideline values (0.5 and 0.3 µg/g, respectively) (Basu et al, 2015; Obiri S, Dodoo D.K. Armah F.A et al., 2010).

- Fish & sea food

Sixty percent of animal protein in Ghanaian diet comprises fish & sea food. Ghana produces 400,000 meT fish annually (MOFA, 2010) of which 75% is consumed locally (Sarpong et al, 2005). Annual per capita fish consumption is estimated as 20-25kg, a figure close to 10 kg higher than the world's average of 16 kg (FAO, 2006). Mean daily fish consumption is estimated as 78g/day (Co: Japan - 1828g/day (Hata, 2007).

Accra is a coastal town where fishing is a major occupation as the city is boundered on the south by the Atlantic Ocean. The country is also endowed with several rivers inland, from which fish are obtained for human consumption. Fish consumption is therefore rife in the country. Annual per capita fish consumption in Ghana is estimated as 20-25kg, close to 10 kg higher than the world average of 16 kg (FAO, 2006). Fish consumed in Accra is obtained from the ocean as well as from inland fresh water bodies. Hg in fish and seafood is present in the organic form of Hg: methyl

mercury. There is a data gap however on the contribution of methyl mercury in fish (from marine sources) and sea food to the body burden of Hg from around the country.

Though a number of studies have measured mercury in fish from across Ghana, we are unaware of a market-based study designed to increase understanding of population-based exposures. Existing studies are usually localized and/or focused on a specific exposure source such as mining.

In a review of fifteen studies on mercury in 65 species of freshwater and marine fish and shellfish (n = 1305 samples total, nine studies sampled freshwater fish (31 species) and shellfish (1 species), and six studies sampled marine fish (28 species) and shellfish (5 species). All mercury concentrations were presented as wet weight concentrations; dry weight concentrations were converted to wet weight assuming 80% moisture content when the moisture content was not listed (Laar et al, 20110). Total mercury concentrations in fish and shellfish were presented in this review, since many studies lacked data on methylmercury concentrations. Although the U.S. EPA and FAO/WHO guidelines were developed specifically for methylmercury in fish (0.3 µg/g and 0.5 µg/g, respectively) [US EPA, 2001, FAO, 2013), fish mercury is often >90% methylmercury (Spry et al 1991). Figure 1 displays the mean concentrations of total mercury in marine and freshwater fish by trophic levels.

- Food crops

In addition to fish, foodstuffs consumed by the general population originate from the coast (maize, cassava) and from the hinterland (plantain, yam, cassava and other root crops). Due to the widespread use of Hg in amalgamation of gold by small scale miners in the central, eastern, western and northern parts of the country, it is plausible that some of the foodstuff originating from the hinterland is contaminated with Hg. Foodstuff and fish contaminated by Hg is ingested in the form of methylmercury. Some of the foods may also be contaminated with inorganic Hg. Food crops constitute most of the staples consumed in the country with some for export. Limited biomonitoring is suggestive of crop contamination in some areas. In the mining areas of the north, the mean range of levels in edible plants was 0.003 to 3.421 µg/g). The level that has been found in some other areas in the following crops is in the stated ranges, namely cocoyam: 0.002-0.005ug/gm, cassava: 0-0.56ug/kg, plantain: 0-0.65ug /kg, and mushroom: 28-31ng/g dry weight (Norom et al., 2012).

- Electronic Waste

The largest e-waste recycling dumpsite is located in Accra. Hg is present as a component of cathode ray tubes & fluorescent tubes and hence may be released into the atmosphere where such items form a component of electronic waste (e-waste). Some preliminary human biomonitoring shows levels of Hg in urine & blood higher among controls than among e-waste workers (E-waste workers-blood: .034ppm, urine: below detection limit); Controls –blood: .095 urine: .005ppm) (Caravanos et al., 2013). Another study in Ghana (Basu et al 2013, unpublished) found among 58 e-waste workers mean blood levels of 1.8 ug/L and mean urine levels 0.2 ug/L. In both studies, the values are very low.

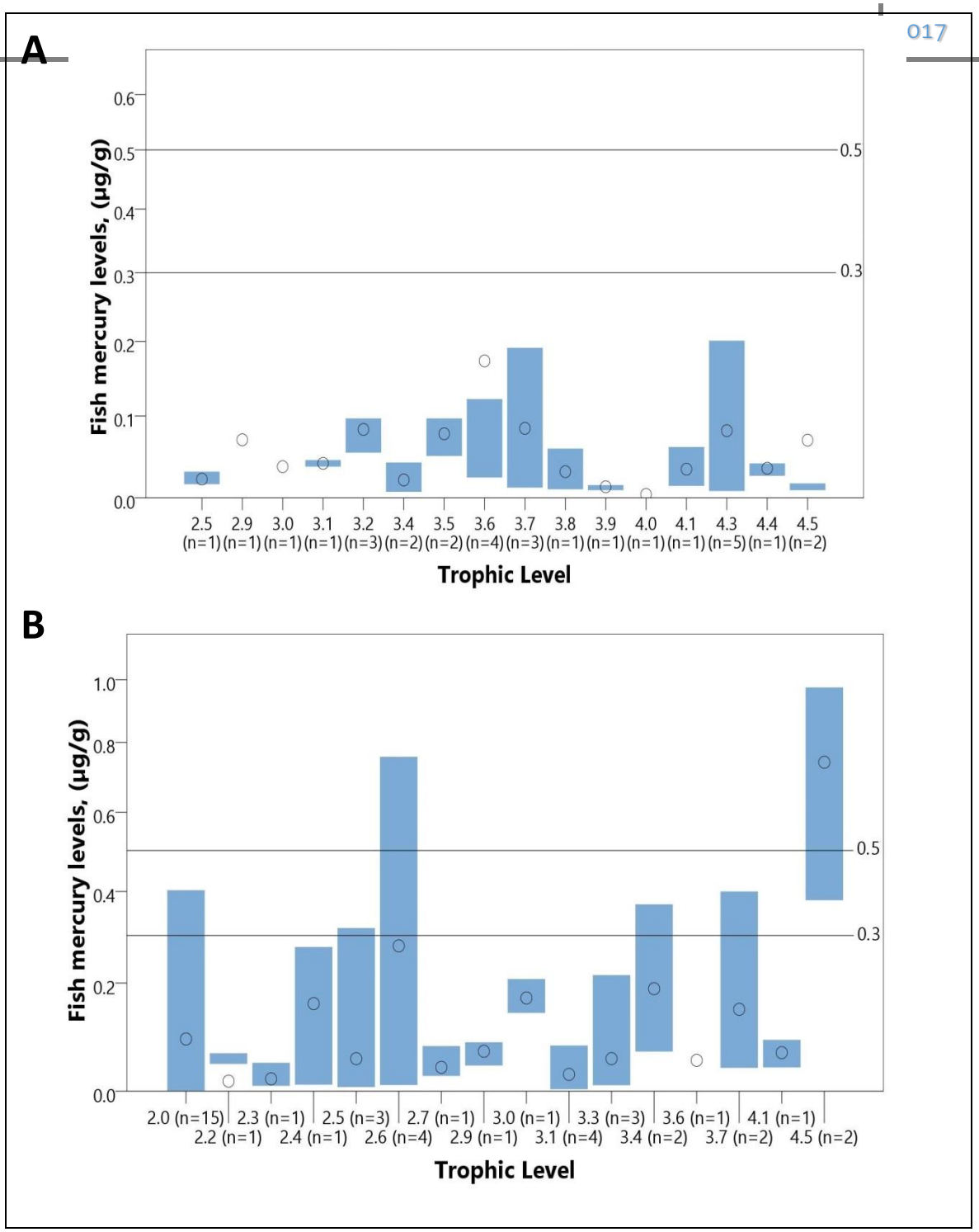

Figure 1. Mercury in marine (A) and freshwater (B) fish in Ghana. Bars represent range and dots represent means for fish at each trophic level (n=number of studies) across Ghana. For values below detection limit, standard protocol of dividing the detection limit by $\sqrt{2}$ was followed. Where minimum and maximum measurements were not available for a study, the mean value is included in the overall mean for the trophic level, but no minimum or maximum was included in calculations. All studies except for two sampled in districts or regions with ASGM, but only one explicitly sampled at or close to ASGM site.

Fig 1: Mean concentrations of total mercury in marine and freshwater fish by trophic levels

- Cement Production

The country has three cement factories. However, a data gap exists regarding levels of Hg in cement products & the production process.

- Hg- containing skin lightening creams and soaps

Hg- containing skin lightening creams and soaps are used in the country. These pose an increased risk of skin cancer; however a data gap exists on human biomonitoring on the effects of these substances.

- **Hg use in dentistry**

Mercury is used in dentistry as a component of amalgam used for restoration of teeth. Some synthetic materials have been introduced, but mercury amalgam is still largely used. An assessment of the occupational practices and fish consumption patterns as well as body burden of Hg in a dually exposed population of dental professionals found the following: Hg levels in biomarkers of elemental Hg (urine) and methylmercury (hair and blood) were measured were higher than the general US population. Geometric means (95% CI) were 1.28 (1.19-1.37) µg/l in urine, 0.60 (0.54-0.67) µg/g in hair and 3.67 (3.38-3.98) µg/l in blood. In multivariable linear regression, personal amalgams predicted urine Hg levels along with total years in dentistry, amalgams handled, working hours and sex.

The following criteria were applied to identify sampling sites:

- There are sources of population exposure to mercury;
- Predicted level of exposure can pose a risks for exposed population groups;
- Medical service for pregnant women are well developed and there are hospital with qualified staff to provide specific medical service if required;
- Availability of sites to enable wide involvement of scientists for field work to decrease as much as possible additional burden to mid-wives in hospitals;
- Size of population is big enough to enable collection of estimated number of sample during the survey period.

Based on analysis of data above, costal area of Accra city has been selected as a sampling area and general population with high fish consumption as a target population group.

The survey will be carried out in the Accra and Tema metropolitan areas of the Greatear Accra Region of Ghana. These two metropolitan areas are cosmopolitan with people from all parts of the country and beyond, migrating in and out. The two metropolitan areas have a combined population of 2,251,251 (1,848,614 from Accra and 402, 637 from Tema) (Ghana Statistical Service, year, 2010).

5.2. Overview of the survey design

The survey involves mothers of newborn children recruited during antenatal visits, or, as an exemption, at maternity wards in hospitals if it was not possible to recruit during antenatal visits. The randomized clustered design of the survey allows assessment of prenatal exposure to mercury in the general population and in exposure hotspots, such as areas contaminated by industrial emissions or areas with high levels of consumption of contaminated foods (for example, fishing communities for methylmercury exposure).

It is very important to involve the community and local representatives in the survey from an early stage, so as to ensure support for the survey and proper communication of healthy behavioural

habits to pregnant women to prevent avoidable exposure, if necessary. The proposed community involvement strategy is in Annex 5.

This document provides a detailed description and sample size justification for the general population

The proposed survey design includes a limited set of biomarkers. Affordability and feasibility were important biomarker selection criteria as the survey is intended to be applicable in the majority of countries.

5.3. Target population

The target population is mothers who have just delivered a child.

Women should be recruited during antenatal care visits. Participants will comprise women who will be contacted at the antenatal clinic during the last 4 weeks of pregnancy ie from the 37th week of gestation. Women in the fertile age (WIFA) are estimated to constitute 23.2% of the total population of Ghana (GHS Facts & Figures, 2009) and is therefore estimated as 527, 468 women out of this population. In cases where women do not have an antenatal care visit during the two weeks before delivery, they can be contacted in maternity hospitals shortly before or after the birth.[1] The following criteria should be applied to determine whether a woman can be recruited and consent given at the time of delivery:

- low level of stress (no fear at childbirth)
- normal development of the childbirth process
- satisfactory physiological condition of the mother
- satisfactory physiological condition of the fetus
- no severe pain
- no emergency signs *(15)*.

Women should be contacted and provided with information leaflet during ante-natal visit (Annex 1). Survey interviewers (scientists from WRI) should briefly describe the objectives of the survey and ask the women if they are interested in participating. If a positive answer is provided, the interviewer, using an eligibility screening form (Annex 2) should conduct a brief interview to check the eligibility of the candidate. If eligibility is confirmed, the interviewer should explain the purpose of the survey, specific activities and risks, and present the informed consent form (Annex3). It is preferable to get women consent agreement during antenatal visits. If consent is provided, the interviewer should then collect exposure information using the standardized questionnaire (Annex 4), obtain medical and anthropometrical data from the medical records, and collect a sample of scalp hair (following relevant SOPs (that can be made in hospital after delivery). Samples of urine and cord blood should be collected by the medical personnel (mid-wives) in due to rules and procedures in the maternity ward (following relevant SOPs).

Since the survey aims to characterize prenatal exposure to mercury, maternity hospitals are the preferred recruitment venue due to the availability of medical records and because they may be the easiest place for sampling hair, cord blood and urine, especially in those countries where mothers spend several days in hospital after delivery. However, collection of hair and urine samples, and interviews can also be conducted in other settings, such as at home within two weeks after the delivery.

[1] No more than two weeks after delivery.

It is important to collect all relevant information on factors that may affect exposure to mercury (e.g. age, nutritional habits, occupation, socioeconomic status, education and use of chemicals and/or mercury-containing equipment at home).

5.4. Selection of hospitals and number of participating mothers

5.2.1. Number of survey participants in the general population

A minimum sample size was calculated for the combined populations of Accra and Tema metropolis as 384 mothers based on a confidence limit (absolute precision) of 5%, a hypothesized proportion of outcome factor in the population estimated as 50% and a design effect of 1.0 for a random sample. In order to account for contingencies such as errors due to incomplete responses to questionnaires, inaccurate responses etc., the sample was further increased by 5% to bring the required minimum sample size to 400 mothers.

This sample size will allow estimates to be computed with a precision of 5% at 80% confidence level for *each of the two metropolitan areas* but allow for a precision of 5% at 95% confidence level for estimates on the *combined populations* of Accra and Tema metropolis.

5.4.2. Selection of hospitals

The following criteari were applied to select hospitals:

- provision of medical service to target population;
- highly qualifird medical personal;
- a number of personal allowing not to affect health service due to survey;
- a number of deliveries allowing recruitments of 400 women during the period of the survey (3 month).

Mothers participating from the two purposively selected hospitals (Ridge Regional Hospital and Tema General Hospital) will be selected proportionately based on the average number of deliveries per month conducted at each hospital). Both public health facilities under the Ghana Health Service and have highly qualified staff. Hospitals are located in the areas selected for sampling based on previous analysis of mercury sources in Ghana. The rate of deliveries in hospitals allows collection of estimated number of samples during the pilot survey period.

With average monthly deliveries at Ridge and Tema General Hospitals over the last 5 years (2011-2015 computed as 760 and 537 respectively, the minimum sample sizes of mothers to be interviewed at the two facilities therefore works out proportionately as 234 and 166 respectively.

5.5. Criteria for enrollment of mothers

All women attending the antenatal clinic who meet the inclusion criteria (as above) will be invited to participate, until the predetermined number of participants for the facility has been reached.

With regard to the selection of potential participants, the recommended inclusion criteria are as follows:

- women at least 18 years of age (legally adult);
- live birth;
- normal term delivery (at least 37 weeks of pregnancy);
- singleton pregnancy;

- living in the catchment area of the maternity hospital (general population) or in the selected survey area (high-exposure group) for the last three years and for most of the time during the last three months of pregnancy (spending not more than two weeks outside the area);
- hair at least 3 cm in length on the back of the head.

Immigrants should not be excluded as long as they have sufficient language ability in the interview language(s) and meet the other eligibility criteria.

A potential occupational exposure will not be considered an exclusion criterion.

The recommended exclusion criteria are as follows:

- women younger than 18 years old;
- delivery before 37 weeks of pregnancy;
- still-birth or delivery of a lifeless child;
- not a singleton pregnancy (twins, triplets, etc.);
- living in the catchment area of the maternity hospital or in the selected high-exposure area for less than three years before delivery;
- living outside the selected high-exposure area for more than two weeks during last three months of pregnancy;
- having hair shorter that 3 cm on the back of the head;
- not having sufficient language skills to understand information about the survey, the informed consent and other relevant information;
- women with mental disorders.
- women with hepatitis C, malaria, HIV and other contagious conditions, according to the relevant national regulations;
- women having lacerations during child delivery;
- women having complicated pregnancy.

However, it is recommended that the above list be followed as closely as possible to ensure the international comparability of data.

5.6. Project follow-up: medical surveillance of people with high mercury concentrations

The main objective of the HBM survey is to generate data on the levels and distribution of prenatal exposure to mercury, in connection with different potential sources of mercury exposure, and to develop a global plan for mercury monitoring.

Elimination of mercury sources is the most important follow-up measure to reduce exposure and the associated health risks. In order to reduce exposure from industrial or environmental sources, the authorized governmental regulatory authorities, for example environmental protection agencies, need to be involved. For the reduction of exposure to methylmercury, public and individual advice, including dietary recommendations and guidance, based on the available scientific knowledge *(19)*, should be made available to exposed groups. Monitoring of fish contamination with mercury should be established to control potential exposure.

The health impacts of mercury depend on its form and the level of exposure. Exposure to mercury vapours can cause acute and chronic kidney disorder. People chronically exposed to high concentrations of inorganic and organic mercury develop neurological symptoms.

However, it is unlikely that such clinical cases would be detected through the HBM survey.

Existing resources of both hospitals will be used to provide medical follow-up if necessary and upon women's request. It includes:

- examination of mother by urologist (if high level of mercury observed in urine);
- examination of mother by neurologist (if high level of mercury will be observed in hair);
- consultation with nutritionist, if necessary;
- examination of neurological development of a child during post-natal visits and in hospitals if necessary.

An individual medical follow-up should be considered on a case-by-case basis, only for mothers with a confirmed high level of mercury. Additional investigation of potential sources of exposure should precede risk communication and planning of protective measures.

Neurological and cognitive development surveillance could be considered for children delivered by mothers with a very high concentration of mercury, within the first control at three months from delivery. It could be framed within the usual surveillance programme for newborns.

The national survey coordinator is responsible for contacting mothers with high mercury concentration and/or their doctors and advising on neurological examination of a child.

6. Recruitment and fieldwork

The processes of recruitment and fieldwork are described briefly in this section; to understand the processes in detail it is essential to also consider the respective SOPs (Annex 4).

6.1. Fieldwork management

Fieldwork is the responsibility of the participating country. Each country decides on the organization of the fieldwork, including the following:

- using the standardized methodological documents provided by WHO as a starting point to prepare the SOPs, fieldwork manual and other documentation in a national language;
- training field personnel and supervising their work;
- selecting maternity hospitals;
- obtaining necessary permissions from regional and local authorities;
- liaising with the local community, identifying and engaging local representatives to promote the survey;
- developing information leaflets for maternity hospitals and for survey participants;
- informing the recruited women, administering informed consent and conducting interviews;
- collecting, storing and shipping samples to the respective laboratories;
- entering the data into a data file and performing preliminary data cleaning;
- analysing national data or submitting the data to a WHO-affiliated data analysis centre;
- communicating the results of the survey to the participants and national public health authorities.

Participating countries can deploy experienced fieldwork personnel or ask the maternity hospitals to perform the work. If the latter option is selected, regular personnel of maternity wards will likely perform the survey tasks in addition to their normal duties, which may adversely affect their performance. To ensure the adherence of hospital staff to the survey protocol, sufficient training, quality assurance and quality control measures must be in place.

Fieldwork will be managed taking into consideration the following steps and processes:

- A request will be made for ethical approval. Once received, an official application will be made to the Director General of the Ghana Health Service for permission to collect data in the identified health facilities. He will in turn provide letters informing the Greater Accra Regional Director of Health Services, and medical directors of proposed study facilities of the impending study, seeking their collaboration. Gynecologists /doctors in charge / senior nurses of the maternity services in the facilities will subsequently be informed by the respective medical directors of the facilities.
- A fieldwork manual and Standard operating procedures (SOPs) will be developed for various stages of the study using the standardized methodological documents provided by WHO as a starting point. The SOPS will cover the following: selection of participants and recruitment, sampling of biological samples, transporting, processing and storage of samples and sample reception. SOPs will also be developed for data handling and data quality control procedures.
- Pilot testing and evaluation of the SOPs.
- Training and supervising of field personnel.
- Developing information leaflets for attendants to antenatal clinics / maternity departments the potential survey participants ;
- Informing, administering informed consent and conducting interviews for potential participants;
- Collecting samples and storing them in a fridge prior to transfer to the WRI laboratory
- Development of a log template to document samples collected.
- Shipping samples to the respective laboratories for analysis (WRI in Ghana) and quality control (Research Centre for Toxic Compounds, Czech Republic)
- Entering survey data in a data file and performing preliminary data cleaning;
- Analysing data and submitting data to a WHO-affiliated data analysis centre;
- Communicating the results of the survey to the participants (as needed) and the national public health authorities namely the Ministry of Health and Ghana Health Service.

Experienced fieldwork personnel, namely, scientific staff of Water Research Institute and midwives from the maternity department of survey facilities will perform the fieldwork.
The fieldwork personnel (comprising research assistants from WRI) will:

- contact women during antenatal visits;
- provide information on survey and administer consent;
- carry out interviews and checking in the questionnaires;
- obtain hair samples;
- organize samples conservation before transportation to the laboratory;
- samples labelling and preparation of samples accompany forms;
- collect medical information.

Midwives in hospitals will obtain the cord blood and urine samples.

To ensure the adherence of study personnel to the survey protocol, there will be sufficient training, quality assurance and quality control measures in place.

Also, at least two of the midwives trained with the survey team will be present during each shift to ensure that the cord blood is obtained following delivery of the participant's child. The work of medical personal in hospitals will be coordinated by chief doctors authorized by the Public Health Ghana (see community involvement strategy, Annex 5).

To decrease a burden of work in the framework of survey, hospital medical staff responsibilities will be limited by cord blood sampling due to restricted entrance in maternity words.
The project budget include honorarium for the field and hospital staff involved in the project (Annex 7).

6.2. Timing of the survey

Exposure patterns, such as fish consumption, may vary by the season. To avoid a seasonal bias, sampling should either take place during fall 2017. The former approach may not be feasible for this relatively small survey involving a limited number of maternity hospitals. Therefore, it is advisable to conduct all data collection activities in a specific season.

In the case of a comparison study, sampling in the general population and in the high-exposure group should take place in the same season to allow for the comparison of results.

It is envisioned that this survey will be repeated at regular intervals to monitor trends in exposure. Combining data from several data collection rounds would also increase the power of the statistical analysis of exposure determinants. Follow-up surveys in the same country should use the same schedule (be conducted in the same season) to ensure data comparability. The baseline survey may produce important information on exposures and lead to policy interventions aiming at reducing exposures. Since new policy measures would require substantial time to take effect, conducting a follow-up survey is recommended.

6.3. Recruitment, interview, medical data collection and biological sampling

The recruitment of participants starts with distribution of an information leaflet. All efforts should be made to provide information about the survey to women during antenatal visits, and to make the information leaflets available for women to take home. This would give time to reflect on taking part in the study and would reduce the burden of consent process just before or after delivery. The leaflet can also be provided before or shortly after delivery.

The leaflet should give information on the survey's objectives, its scope, benefits for the women themselves, and the communication of the results. It should also provide information on the inclusion and exclusion criteria.

The interviewers, scientific staff of WRI, will be present at the maternity hospital.

A female fieldworker might generally be a better choice to contact women shortly after delivery. The fieldworker should introduce themselves, and do the following:

- handover the information leaflet (unless it was made available to the woman during one of her antenatal visits), briefly describe the survey and ask whether the woman is interested in participating;
- conduct the screening interview and administer the informed consent form;
- collect the data on exposure, socioeconomic status, etc. using the questionnaire (it is preferable to do this in an interview rather than to leave the questionnaire with the woman for self-administration);
- collect a hair sample;
- arrange for the collection of urine and cord blood samples, strictly following the procedure recommended by WHO for sampling (note: if the recruitment is conducted after the delivery, it may be necessary to collect cord blood and urine samples prior to recruitment; if

the woman is not eligible or does not agree to participate, the collected biological samples should be immediately discarded; samples must not be delivered to the analytical laboratory and analysed prior to obtaining informed consent; samples will be collected in the hospital and stored before shipment to an analytical laboratory; the national coordinator of the survey should ensure that only samples from consenting women are shipped to the analytical laboratory for an analysis);

- obtain medical data on the woman and her child, including ICD-10 codes of diseases and conditions during pregnancy and delivery: nephropathies (N00-N16); polyneuropathy and encephalopathy (G50-G99); complications of labour and delivery (O60-O75) and delivery (O80-O84); and basic anthropometrical measurements of the infant (weight and height); such information could be used in the further analysis of the data on mercury concentration in biological matrices and the questionnaire data, and to facilitate formulation of exposure- and risk-reduction recommendations.

The following will be documented:

- Number of women asked to participate
- Number of these women fulfilling the inclusion/exclusion criteria
- Number of women agreeing to take part
- Number of women whose data are used in analysis.

6.4. Questionnaire

The template questionnaire developed by WHO is fully applicable in Ghana.

Preliminary questionnaire versions in national languages will be pilot tested prior to the main survey during training. As pilot testing is an essential part of developing the questionnaires and national survey protocols, it should be conducted as early as possible.

Screening interviews and obtaining consent (annexes 2 and 3) have to be done prior to administering the questionnaire.

The main questionnaire (Annex 4) can be used to interview the participants at the time of hair sampling. Completion of this questionnaire takes about 30 minutes, if administered by an interviewer. Section A comprises personal information, anthropometric data, ethnic origin, educational level of the family and socioeconomic status. Section B focuses on potential exposure pathways to mercury, and is divided into four parts: (1) occupational exposure, (2) exposure in the residential environment, (3) personal care and lifestyle (e.g. smoking behaviour), and (4) food and beverage consumption.

Personal interviews conducted by trained interviewers are the most commonly used method to collect data on behavioural and nutritional exposure factors. However, this method has a tendency to under-report socially undesirable behaviours (for example smoking). This is known as the “social desirability” bias. On the other hand, interviews have the advantage over self-administered surveys that any misunderstanding can be resolved immediately, which leads to higher data completeness and quality. Training of the interviewers is essential to ensure that the interviews are conducted in a standardized way. The training of interviewers has been shown to improve their performance, particularly in reducing under-reporting of pertinent information.

The participating country is in charge of generating a file with data from the questionnaire and assuring data quality using a template developed by WHO. National survey coordinators are

responsible for developing SOPs for data handling and data quality control procedures, and for conducting pilot testing and evaluation of these procedures prior the beginning of a national survey.

The national coordinator should retain questionnaires from all the respondents until the end of the study and they should be kept for future reference. Retention of all records should conform to national requirements and international norms concerning confidentiality. The national coordinator should complete a summary of information form about mothers donating samples. They should also provide scanned copies of the questionnaires to WHO upon request.

6.5. Training of fieldwork staff

To ensure standardization of processes, the training must be strategic and organized as far in advance as possible. Training should involve a range of fieldworkers engaged in survey implementation, including interviewers, hospital staff, those responsible for collecting samples, those responsible for sample transportation and laboratory analysists.

A train-the-trainer approach may be the most cost-effective, which utilizes the exchange of knowledge and experience to minimize costs.

Establishing a technical help desk during the survey, starting from the moment the general protocol is adapted to the national situation, might increase consistency and promote adherence to survey protocols. The help desk could be available through a central website supporting the survey. It should provide answers that have been formulated by experts in the field in a timely manner.

If trained hospital personnel conduct interviews in addition to their regular duties, then additional motivation might be needed.

It is envisioned that the survey will involve reference laboratories. Technical assistance in the form of training of laboratory technicians will be provided to individual countries when necessary upon their request.

6.6. Quality control measures

Quality control with respect to fieldwork and training of the project staff is a responsibility of the national coordinator. It is in the interest of all partners involved that the fieldwork is controlled and checked.
To enable high quality data collection the following steps will be taken:

- Detailed guidelines will be developed as the "Fieldwork Manual"(Section ...). The Fieldwork Manual will describe all steps of field work and provide SOPs for all essential steps.

- Paying particular attention to quality of training and ensuring that the necessary competencies required for interviewing in the appropriate manner are imparted to field workers, midwifes and laboratory staff.

- Before the start of the survey, the criteria for quality targets as well as how to deal with errors will be defined. Both aspects have to be part of the interviewer training,

- Everyone involved in field work will keep a *log-book in which p*ositive and negative experiences will have to be written down and exchanged regularly with supervisors and ther

team members to allow learning from each other. Those experiences that are considered worthwhile will be communicated to the WHO

- ***All*** samples will be labelled with the participant ID code and sampling date immediately after collection. For traceability of the sample throughout the study, and to be able to link the sample with the information provided by the study participant, all documents related to the samples (questionnaires, registries, etc.) will be labelled with the same sample ID code immediately Additional quality control measures encompass *internal and external quality controls to ensure that* fieldwork is performed in a harmonized and correct way. *For example,*

- *Internal quality controls*: *check lists will be used* to facilitate internal and external control of the field work. Internal quality control means that each step of field work is controlled, mostly by the staff member who will perform or has performed the fieldwork him/herself..

- External quality control - or field visits - refers to the checking of work of the field team members by supervisors. Field visits by supervisors once to twice a week to avoid errors will be used.

C*heck lists* will be used to facilitate internal and external control of the field work.

7. Biological material

7.1. Overview of biomarkers for assessment of exposure to mercury

Justification for the selection of biomarkers of prenatal exposure to organic and inorganic mercury
In population-based HBM surveys, non-invasive matrices are preferred for assessing exposure to mercury in order to maximize the response rate. The selection of biological matrices for assessing human exposure depends on the mercury compounds (organic vs. inorganic), exposure pattern (chronic or acute) and time of sampling after the exposure *(4)*.

Maternal scalp hair
Exposure to methylmercury is reflected in the level of mercury in scalp hair *(4)*. Once incorporated into hair, mercury does not return to the blood, providing a good long-term marker of exposure. Mercury in maternal hair (close to the scalp) is a proxy of fetal mercury exposure *(20)*. Mercury concentration in 3 cm of scalp hair taken close to the scalp shortly after delivery reflects the exposure of the fetus during the last three months of pregnancy. However, the concentrations of mercury in hair can change to a certain extent due to the changing growth rate of hair *(21)*.

Hair-mercury concentrations can be affected by several factors, including hair colour and variable growth rates *(20)*. Previously conducted studies have shown that total mercury in maternal hair is a predictor of long-term neurotoxic effects in children *(22)*, despite some studies reporting inconsistent results, particularly when assessing the effects of exposure to low mercury levels *(23)*.

Mercury levels in populations consuming a very small amount of fish are normally below 0.5 µg/g in hair; in populations with moderate fish consumption total mercury in hair varies from below 1 to 2 µg/g; while people with frequent consumption of fish (once or more per day) may have mercury levels in hair exceeding 10 µg/g. The United States Environmental Protection Agency (US EPA) reference dose of 0.1 µg methylmercury per kilogram of body weight per day corresponds to approximately 1 µg/g mercury in hair in people with low fish consumption.

More recent calculations resulted in an adjusted biological limit corresponding to 0.58 µg/g in hair, the validity of which is supported by recent studies of developmental neurotoxicity at exposure levels close to the background *(24)*.

A tolerable limit proposed by WHO corresponds to a hair-mercury concentration of approximately 2.5 µg/g, which takes into account the possible compensation for methylmercury toxicity by beneficial nutrients in seafood. Due to the ease of collection and handling, maternal hair-mercury level is one of the most widely used biomarkers of prenatal exposure to methylmercury in population studies.

Cord blood

In contrast to hair, the presence of mercury in blood represents short-term exposure to organic and inorganic mercury, and does not provide information on long-term exposure and its variations *(4)*. Total mercury concentrations in cord blood are proportional to methylmercury concentrations in hair. As a biomarker of prenatal exposure, mercury in cord blood is preferable, as it provides information on both the exposure of mothers and prenatal exposures of their children *(25)*. Mercury in cord blood may have a stronger association with neurobehavioural deficits in the child compared to mercury in maternal hair *(26)*. Concentrations of total mercury in cord blood of individuals who do not eat fish are normally in the range of 0.5–5.0 µg/L. In cases of high fish consumption, values higher than 10 µg/L are frequently occurring. The reference value for mercury in cord blood based on the US EPA's reference dose is 5.8 µg/L. Mercury levels in cord blood and hair are recommended biomarkers of prenatal low-level methylmercury exposure due to its selective transfer through biological barriers such as blood, hair and placenta *(27–29)*. Cord blood is a non-invasive matrix, but should be collected by the nurse after birth.

Maternal urine

Urine is the matrix of choice for assessing exposure to inorganic and elemental mercury *(30, 31)*. In an occupationally non-exposed population, the number of amalgam surfaces was found to be associated with urinary mercury *(32)*. In the general population, urinary mercury can be elevated also due to high fish consumption, as a consequence of demethylation and excretion of inorganic mercury and partially also due to limited excretion of methylmercury through urine. Urine is a non-invasive matrix, is easy to collect and is commonly used to assess exposure to elemental and inorganic mercury, particularly in occupational health settings where biomonitoring of random spot urine samples is routinely practiced.

Due to wide variability in urinary excretion rates among individuals, as well as the great temporal variability in urine composition within individuals *(33)*, the results should be expressed per gram of creatinine or adjusted for the specific gravity. Concentrations of total mercury in urine of non-exposed individuals are normally in the range of <0.1–5.0 µg/L. In cases of non-occupational exposure to inorganic and elemental mercury, values of up to 10 µg/L have been reported, while workplace exposures can result in levels higher than 50 µg/L. The health-based German HBM I,[2] which corresponds to the concentration of total mercury in urine below which adverse health effects are not expected, is 7 µg/L, or 5 µg/g creatinine; the German HBM II value that corresponds to the concentration above which there is an increased risk of adverse health effects in susceptible individuals of the general population is 20 µg/L, or 25 µg/g creatinine *(34)*.

[2] These values are based on the German Environmental Surveys (GerESs), nationwide population surveys that have been carried out in Germany periodically since the mid-1980s.

7.1.1. Choice of the matrices for the survey and sample collection

The literature provides adequate evidence that mercury in maternal hair (close to the scalp) is an appropriate biomarker of fetal mercury exposure *(26)*. Moreover, this biomarker has been used to show an association between prenatal mercury exposure and long-term neurotoxic effects in children *(22)*.

Human hair has the advantage of being a non-invasive matrix that is easy to collect through a simple procedure that requires minimal training of survey personnel. Hair samples can be transported and stored in a zipper bag or a paper envelope at room temperature *(35)*. Hair samples have been used extensively in studies of methylmercury exposure from fish consumption *(36, 37)*.

Once incorporated in the hair, mercury remains there, providing information on exposure during the hair growth period. Most mercury in hair is in the form of methylmercury, especially among populations that consume fish. It is an accurate and reliable method to measure methylmercury intake levels. The relevant SOP for analysis of mercury in hair, provided by WHO to the national coordinators, describes in detail the place on the head for collecting hair samples, the amount of hair to be collected and the principles of sample storage.

Cord blood can be collected by the nurse after birth and does not cause any pain to the mother or baby. Mercury levels measured in cord blood reflect exposure of the fetus to mercury and its compounds. A detailed description of the collection of cord blood is given in the relevant SOP for analysis of mercury in cord blood, provided by WHO to the national coordinators. The procedures described in this SOP are only suitable for mercury.

Urine is another non-invasive matrix, which is easy to collect. Urinary concentrations of pollutants, including mercury, can be influenced by the composition of urine. Therefore, creatinine levels or special gravity should be measured as well. The results for primary biomarkers are expressed as adjusted for the creatinine content or special gravity measurement results. Urine collection is described in detail in the SOP for analysis of mercury in urine, provided by WHO to the national coordinators.

For the collection of cord blood and urine samples, appropriate containers should be used to prevent background contamination. Prior to sample collection, the batch of containers for urine and blood should be tested for the presence of interfering chemicals. The containers for the collection of cord blood should contain ethylenediaminetetraacetic acid (EDTA) to inhibit blood coagulation.

Based on analysis of biological matrices non-invasive biological matrices have been selected for assessing exposure to mercury due to the ease of collection and handling and to maximise the response rate. Maternal hair and urine as well as cord blood have been selected as biomarkers of choice. Cord blood is considered non-invasive as it will be collected by the nurse after birth when the child has been completely separated from the placenta.

Also a non-invasive method that is being recognized as a cost-effective and low-tech method that is conducive for work in resource-limited settings. The selection of biological matrices for assessing exposure depends on mercury compounds (organic vs. inorganic), exposure pattern (chronic or acute) and time of sampling after the exposure (UNEP and WHO, 2008).

The biological samples will be transferred to the laboratory of the CSIR / WR in Accra where the samples will be stored: the urine and blood samples at < 20^0C and the hair and dried bloodspots at room temperature until transfer to laboratories for analysis. These processes will be supervised by the laboratory officer at the CSIR trained to undertake the study.

7.2. Obtaining samples, transportation and concervation

In preparing samples for transportation, the national coordinator or fieldworker must ensure that samples will not be destroyed or lost during transportation and that any person coming into contact with them will not be infected.

Hair samples

Hair from which sample is to be obtained should be over 3 cm in length. This is necessary in order for the 3 cm closest to the scalp to be used for analysis. Two locks of hair are obtained from the nape of the head. This corresponds to a minimum of 200 grams weight To facilitate the cutting of hair close to the scalp, the locks of hair are immobilized with adhesive tape placed away from the root of the hair. With a pair of scissors with blunt tips (to avoid injury to the scalp), cleaned with cotton wool soaked in alcohol, the hair is then cut. An arrow is drawn on the adhesive tape with the tip pointing towards the root of the hair.The individual hair sample is placed in a Ziploc bag,placed in a paper envelope and labelled with the participant's ID code and sampling date immediately after collection. Hair samples do not require any special transport conditions; they will be transported at room temperature in a paper packaging. The corresponding log listing all samples sent in the package and information concerning any event that occurred during sampling which could affect the sample will be included with the samples.

Hair samples do not require any special transport conditions; they can be transported at room temperature. However, it should be checked that the corresponding documents, including a sheet listing all samples, is sent in the package and information on any event that occurred during sampling that could affect the sample, has also been included.

Urine Samples

Spot urine samples will be obtained by asking paticipants to void urine while at the ANC, into 50 ml bottles. These should have been pre-cleaned with nitric acid to eliminate metal contamination. Urine will be stored in the fridge at 4^0C until it is transferred to the laboratory. The sample will be labelled with participant's ID code and sample date.

Cord Blood Samples

Cord blood will be obtained via ex-utero collection of blood i.e. following delivery of the placenta and clamping of the umbilical cord. 10 ml of blood obtained and transferred into a polypropylene metal-free tube (pre-cleaned with nitric acid) or Royal Blue Cap Tube with K2 EDTA trace metal free tubes (purchased from BD by WHO). The blood is then stored at minus _< 20^0C until analysis. An SOP will be used to guide the step by step processes in obtaining and storing the blood.

7.3. Preparation of samples

A specific form will be used to document the sampling, labelling, processing and shipping of the samples.

Detailed instructions for hair sampling and sample pre-analytical treatment can be found in the SOP for analysis of mercury in hair.

The cord blood samples should be aliquoted (at least two aliquots) to enable mercury analysis in the national laboratory and the reference laboratory.

Urine samples should be aliquoted (at least three aliquots) to enable mercury and creatinine analysis in the national and the reference laboratories.

7.4. Analysis of samples

A closed vessel microwave system (Ethos D, Milestone S.r.l., Sorisole, BG, Italy) will be employed for the digestion of human urine and hair samples using nitric acid.
Mercury will be determined by the Cold Vapour –Atomic Absorption Spectrophotometer (CV-AAS) technique. Concentrations obtained will be converted to µg/L for urine and µg/kg for the hair samples
The CV-AAS method provides the analytical procedure necessary for the determination of (Hg) by AAS-VGA in cold vapour mode. The procedure for digestion of samples prior to mercury determination is summarized below.

Digestion of Hair, Urine and Blood Samples

i. Weigh 100 mg of human hair sample into a 50 ml flat bottom flask.
ii. Weigh 2 mls of urine sample into a 50 ml flat bottom flask.
iii. Pipette 200 µl of blood sample into a 50 ml flat bottom flask by first homogenizing it.
iv. Weigh 50 mg of two hair reference material samples (e.g. NIES or IAEA) into two separate 50 ml flat bottom flasks.
v. Pipette 1 ml of urine reference material if available, into a 50 ml flat bottom flask.
vi. For hair and blood samples ONLY, add 1 ml of distilled water to each.
vii. Set the heating mantle/block to 240 °C.
viii. Prepare a mixture of HNO_3 and $HClO_4$ in a ratio of 1 : 1.
ix. Add 2 mls of the above mixture (HNO_3 : $HClO_4$) to each of the samples to be analyzed.
x. Add 5 mls of concentrated H_2SO_4 to each of the samples to be analyzed.
xi. Now put all the samples on the heating mantle for 20 minutes with their covers open.
xii. After this, make the samples up to the 50 ml mark with distilled water and proceed to measure the concentrations with the CV-AAS using 10 mls for each determination.
xiii. To check for contamination, select some tubes randomly, fill with distilled water and analysed for Hg.

In the event that hair, urine and blood are to be analysed at the same time, run/analyse urine first followed by blood and lastly hair samples in that order.

7.5. Standardization

Results of the measurements must be analytically comparable between laboratories. To ensure this, each national survey must follow the SOPs for sampling and analytical methods, and develop procedures for quality assurance and quality control that cover the pre-analytical phase. The availability of appropriate reference materials (samples with a certain level of mercury) supports

internal quality assurance. External quality assurance should be done through international inter-laboratory comparison investigations (ICI).[3]

A proficiency test of the analysis of total mercury in cord blood and urine can be organized by WHO, using freeze-dried samples as well as a mirror analysis of 20 samples of each biological matrix.

7.6. Storage of samples remaining after the mercury analysis

Biological samples collected during the survey will be used only for this survey purpose and will be destroyed as soon as mercury analysis is completed.

7.7. Laboratory quality control

i. Quality Control in the Laboratory

A. Operator competence: Analyst should be competent, having been trained by the most senior Technical Officer.

B. Analysis of blank: To assess contamination, analyse blank and travel blank samples together with real samples.

C. Maintenance of Control Chart: Prepare one control standard, analyse and plot on the control chart. If the control standard is above the action limit (+/- 3 σ), prepare fresh control standard.

D. Analysis of duplicate: For a batch of 5 samples, duplicate one sample.

E. Method accuracy: Analyze a certified reference material *(NIEHS & IAEA obtained from Josef Stefan Institute)* and run along the samples

ii. Quality Assurance / Quality Control- Before and during field sampling

Sample bottles are rinsed with the sample in question before sample collection. Field equipment are calibrated before use. Samples are preserved with ice in insulated boxes and nitric acid.

Duplicate samples are analyzed alongside the samples. Travel blanks are prepared in the laboratory and taken to the field unopened throughout the trip and handled the same as other exposure samples. The travel blanks used are deionized water sample which are treated as samples during analysis. They are used to identify possible errors or contamination in sample collection and analysis. Samples are properly labeled, kept in ice chests and transported to CSIR- WRI laboratory in Accra.

iii. Quality Assurance / Quality Control – During Analysis in the laboratory

Blanks are run concurrently with the samples for quality assurance. Blank samples in the laboratory are freshly prepared distilled water and deionized water lacking parameters of interest to make sure they are free of contamination during sample handling. When the blank reads a value, it is subtracted from the sample concentration. All Instruments are calibrated.

[3] ICI is a measure to harmonize analytical methods and their application so as to improve the comparability of analytical results. ICI is carried out before the laboratories begin to analyse the samples.

Duplicate samples are analyzed and compared through repeatability and the acceptable concentration is 2 times the standard deviation. Standard Reference Material is analyzed alongside the samples.

Control samples are also analyzed together with samples. For internal data check, analytical results are checked by computing the ionic error balance. Results with ionic balance error > 10% are rejected.

8. Data management, analysis and evaluation

8.1. Data management

Data generated during the fieldwork will need to be further processed and merged in order to allow for final evaluation and results. A database will combine the laboratory data files and the questionnaire database. The database is constructed as a matrix with one row per subject and all separate variables in columns. The data from each participant are identified by a unique identity number (ID number). Please see the following example:

ID number	Variable name	Matrix	Biomarker	Unit	Data source
XXXXX	HM_HG	Hair Blood Urine	Total mercury	ng/mg	Lab result

Ideally, the data will be stored in a uniform format in all participating countries. WHO will develop and provide the data management and storage format. Information on the structure of the database, including variable names, formats, units and rules for handling missing values or values below the limit of quantification, will be included in a codebook.

However, each participating country, in consultation and agreement with the project coordinator in WHO, will choose its software for database management and statistical analysis based on the following criteria:

- suitable for importing data from external data files provided by chemistry laboratories (most commonly Excel or Access files);
- allows input of the questionnaire data;
- sufficient database management functionality;
- capacity to perform statistical analyses;
- possibility to deliver external databases to a WHO database.

Based on experience in other multicentre studies, statistical analysis programs like R, SPSS or SAS meet these criteria and are thus recommended.

Data processing will be conducted in each participating country, while statistical data analysis can be conducted either at the national level or at WHO. The participating countries will transfer the data to WHO for creation of a database at the global level, and analysis of levels and distribution of exposure to mercury at national, regional and global levels.

8.2. Statistical analysis

8.2.1. Data analysis at the national and the international level (recommended approach)

It is recommended that participating countries conduct statistical analyses at the national level and submit anonymized data for statistical analysis to a central database. The aim of a statistical analysis at the international level is to assess associations between biomarker values and predictors such as age, gender, fish consumption habits, etc. in a pooled dataset. However, in some cases WHO can make its own statistical analysis based on data provided by the national coordinator.

Data analysis will involve descriptive statistics and regression analysis. At the descriptive statistics stage, response rates and distributions of parameters will be evaluated, outliers identified and checked.

The regression analysis stage will involve analysis of biomarker data in relation to predictors. The associations will be studied using univariate and multiple regression models.

8.2.2. Data evaluation

The interpretation and evaluation of the HBM results will be dealt with in separate steps. Some of the questions that the HBM survey aims to answer are outlined below:

- Are the observed levels of exposure important/significant in terms of health risk?
- Are elevated exposure levels associated with specific types of exposure source?
- How are specific biomarkers distributed among defined/selected survey population strata/subgroups of the general and exposed populations?
- What is the spatial variability in exposure levels in participating countries globally?

Additionally, it will be valuable to compare the results of the HBM survey with existing data available in the literature.

9. Communication

Communication campaigns aim to promote awareness, encourage stakeholder involvement, maximize recruitment and retention, ensure transparency and openness towards stakeholders, and to safeguard translation into precautionary and preventive policy. Apart from providing information to the survey participants, the survey leaders have to provide targeted information to the general public, policy-makers and public health professionals.

Effective communication can help to raise awareness in the population and to stimulate preventive action at the population and individual levels. At the same time, it is important to avoid inducing anxiety in survey participants when corrective actions are not warranted at the individual level.

Three periods of extensive communication campaigns are identified: prior to and at the onset of the sampling period, during the survey, and at the results dissemination stage.

9.1. Communication prior to the survey

Measures to enhance recruitment should start before the recruitment itself begins. The recruitment process has two main goals: (1) to recruit individuals that adequately represent the target population; and (2) to recruit a sufficient number of participants to meet the sample size and power requirements. Therefore the initial campaign should start as soon as the protocol is ready.

In order to meet these goals, it is necessary to make sure information leaflets are tailored to the target population. The briefing of policy-makers in the health sector and religious leadersshould start at the same time.

It is important that the participants have sufficient opportunities to ask questions, to encourage uptake and to reduce withdrawal from the survey. The survey information leaflet and other materials should have contact details (including name, address, telephone number and email address of the survey coordinators) and be available for participants. Links to the survey website with a description of the survey, answers to frequently asked questions (FAQs) and information on sources of funding can also be provided.

The information leaflet and informed consent form should provide a brief summary of the survey and its aims, in plain language understandable for a non-professional audience. The leaflet and consent form should also explain what participation means in practice: how long it takes, where it takes place and what it involves. The following list is not exhaustive but gives an idea of the main topics to be covered:

- nature and aims of the survey;
- promise of confidentiality, that the participant's responses will not be linked to their name or any other identifiable information;
- description of what participation means in practice (when, where, who, what);
- inclusion and exclusion criteria for participating in the survey;
- possible risks, inconveniences or discomforts that could reasonably be expected to result from the survey;
- possible benefits for participants (if relevant, as there might not be any direct benefits);
- participating country's institutional contact details;
- information about how the survey leader obtained the potential participant's contact information;
- information about what will happen to the results;
- explanation that participation is always voluntary and that participants can withdraw at any time;
- explanation about how privacy and confidentiality will be maintained over the time data is stored;
- description of data storage in a biobank (if applicable) and possible uses of data in the future.

A withdrawal form should be prepared for any survey subject who decides that they would like to withdraw from the survey. Survey participants may withdraw at any time; they will be asked to confirm their withdrawal with a signature.

9.2. Communication during the survey

Communication should continue during the survey implementation, and it is important to react quickly and effectively to any upcoming questions.

To facilitate communication, it is recommended that a contact point be identified (with their name, phone number and email address) to receive and answer questions and queries, as well as to develop FAQs.

9.3. Communication of the survey results

Before communicating the result of the HBM survey, careful consideration needs to be given to the assessment of individual and population risks, based on the measured concentrations of mercury and the questionnaire data, as well as on the main goals of risk communication, taking into account different target groups and their needs. The level and distribution of mercury levels and the associated risk determine the main communication aims. For example, if the HBM survey reveals low exposure levels and low or negligible health risks, the main purpose would be to inform participants of the results and to use this as an opportunity to raise awareness and educate. Whereas, if the survey showed a high level of exposure to mercury, communication of results would include more information about health risks and risk-reduction measures, including on preventing exposure and promoting safer behaviours. It is critically important to distinguish between communication addressed to individuals and to the wider population (e.g. different approaches to risk assessment, recommended risk-reduction measures, and defining of responsibilities of individuals and relevant authorities, etc.) as well as to involve different stakeholders according to their roles and capacities.

In general, the fundamental goal of risk communication is to provide meaningful, relevant and accurate information in clear and understandable terms, targeted to a specific audience. It should facilitate understanding of complex technical issues – such as exposure to mercury, the associated health risks and risk-reduction measures – to bridge the gap between lay people and experts and to help people make more informed and healthier choices.

All stakeholder categories – including policy-makers, health-care professionals, the general public, local communities and individuals involved in the survey – should be included in the mercury risk communication. When communicating the results, consideration needs to be given to the meaning of HBM results, their interpretation at individual and population level, and their potential health relevance (health risk, predictive value of biomarkers, etc.), including communication about uncertainty. Furthermore, communication on available protective and preventive measures at individual and population level, especially in the case of observed high mercury concentrations, is an obligation.

It is crucial not only to prepare clear and understandable messages, tailored to the capacities and needs of the audience, but also to identify the most effective channels to communicate the message (e.g. through publications, mass media, scientific reports, leaflets, lectures, involvement of an expert or a recognized community leader, etc.). It is important to get support from central and local authorities and the medical community.

There will be a national dissemination workshop which will involve all stakeholders including representatives of sub-metros involved in study, health staff from the study facilities, representatives of Association of Pediatricians, relevant government MDAs (e.g. Fisheries, MOFA,), community & religious representatives. The findings will be shared with all these practitioners, researchers and the MOH, GHS and partners, to determine the way forward. The dissemination should culminate in an action plan on how to move the findings into policy review and implementable actions to address baseline situation.

9.3.1. Communication of the survey results to policy-makers, including government health-care and environmental protection bodies

Policy-makers, particularly in the health and environment sectors, should receive a summary of the HBM survey findings with recommendations on further steps and available risk-reduction measures. The summary should include information about the levels and distribution of exposure to mercury in a population, existing and projected health risk at population level, the main sources of exposure to mercury, as well as available and feasible actions and measures to reduce exposure and health risk. Ideally, a preventive action plan should be developed, with a proposed timeline and economic analysis of its implementation. Inclusion of information on good practices could be very useful to demonstrate the potential benefits of implementation of risk-reduction measures. The relevant policy framework at national and international level should provide a context for presenting the survey results and the proposed actions.

9.3.2. Communication of the survey results to the general population and communities involved in the survey

Risk-communication messages for the general public and communities should be formulated in a way that avoids misunderstandings and undue concerns. Prior to formulating risk-communication messages the population-level risks should be carefully evaluated, using all information available, and population groups at higher risk (of exposure and health effects) should be identified. A clear distinction needs to be made between interpretation of HBM results at individual and population levels.

The meaning of the HBM survey results should be clearly communicated, focusing on population groups at risk; it should include recommendations on reducing exposure to mercury and/or preventing health risks. An example of this could be fish consumption advice; as much as possible this should take into account local conditions (fish and seafood types, fishing patters, cultural aspects, etc.) and should be presented in the context of the health benefits of fish consumption.

The public perception of risks might affect the acceptability and the appropriateness of risk-reduction measures. Therefore, it is essential to ensure that the risk-communication process takes into consideration general public perceptions, for example of the risk of mercury exposure associated with fish consumption.

The most effective way to communicate risks is through mass media; for example, as an article in the newspaper, or a programme on regional or local radio and/or television. Involvement of topical experts can strengthen the message and support the recommended risk-reduction measures in certain cases. The use of mass media should allow the message to be presented in a manner understandable to a broad audience, and provide the opportunity to discuss the problem, answer questions and give clarifications. Information about the results of the HBM survey, including on the observed levels and distribution of mercury, should be put in the context of levels of mercury in the ambient environment and relevant safety levels, as well as any accidental mercury exposure, particularly of at-risk populations.

9.3.3. Communication of the survey results to health-care professionals

In cases where high concentrations of mercury are observed, communication prepared for health-care professionals should include general information on mercury and its health effects, the main

sources of exposure, principles of diagnosis and treatment, risk-reduction measures and vulnerable population groups, for example pregnant women. Identification of target groups for communication efforts among health-care professionals depends on the population groups at higher risk. These could be paediatricians, gynaecologists and obstetricians, occupational physicians, and general practitioners serving specific communities (artisanal and small-scale miners, fishing communities, etc.). Organization of training for health-care professionals can be considered to help gain support for implementation of risk-reduction measures.

9.3.4. Communication of the survey results to participants

Individual results should be provided to survey participants, except those who do not wish to know their results. In sensitive situations, experts in social sciences and communication might be consulted in order to understand public perceptions and to develop optimal communication strategies.

Prior to communicating the survey results to participants, the following measures are recommended in cases where a high level of mercury has been detected. First, the analysis should be repeated to exclude any mistakes, and to test the samples in a reference laboratory. The next step, after checking the quality of the measurements and confirming the result, is to evaluate risks using all available information on potential sources of exposure and the associated health risks.

When communicating risk-reduction measures, it needs to be remembered that they will differ in cases of exposure to methylmercury and inorganic mercury.

It is important to explain to participants the meaning of their results as clearly as possible. The results can be communicated to the survey participants through direct contact or through their family doctors.

Personal communication with individuals at high risk is the most effective way to discuss the problem and the recommended preventive measures and risk minimizing actions. Involvement of a family doctor and/or family members might be considered, subject to agreement of the participant. It is critical to be prepared to provide clear evidence-based answers to questions about the health effects and medical follow-up, to avoid any misunderstanding or exaggeration of the problem.

Communications at the country level and at WHO level should be coordinated and consistent. At the same time, it should allow customized, country-specific messages according to the local context (e.g. country characteristics, concerns). Confidentiality of personal data and testing results needs to be guaranteed. At the same time, all aggregated results can be made publically available, providing that no link can be made to specific individuals.

10. Ethics

The survey must adhere to the legal and ethical framework established by international directives, conventions and guidelines, and by domestic laws in participating countries.

Approval of a national ethical committee should be obtained before sampling starts.

The ultimate objective is to guarantee the optimal protection of the rights and dignity of every survey participant (data subject). Special attention should therefore be paid to:

- defining and explaining the specific, explicit and legitimate purposes of the survey to all actors involved;
- asking for written consent (informed, free, explicit, specific and documented) prior to the commencement of research. Informed consent includes:
    - the survey objective;
    - the targeted population and recruitment method;
    - possible risks and benefits to the participants;
    - approval of the survey protocol by an ethics committee;
    - the right to refuse consent or to withdraw consent at any time without giving reasons and without being subject to any form of discrimination;
    - the right to access personal results and the wish of participants to know or not to know their personal results;
    - the procedure for dealing with critically high biomarker values;
    - recipients of the survey data;
    - measures to assure the confidentiality of personal data;
    - the informed consent form will be read out to each of them individually outlining the risks and benefits of being interviewed, having haircut, obtaining urine and obtaining cord blood. They will be given the opportunity to decline to be interviewed or to discontinue the interview at any time, and /or to decline to provide urine or have a small amount of hair cut for examination.

When communicating results at the individual level, explaining their health significance (if known) is extremely important. When further evaluation or intervention is warranted due to a critically high biomarker value, communication at the individual level should involve professional counselling.

Some of the questions regarding practices and habits such as frequency of fish consumption and other dietary habits are personal. Should any participants not feel comfortable to share any personal or confidential information with us, they may decline to respond to those questions.

Participation will be completely voluntary. Those who give their consent will be asked to sign or put their thumbprint on the consent form to indicate their willingness to participate in the study.
Study participants will be assured that the information collected will be kept confidential and will be used in an aggregate form in the report. Also, in order to minimize participant's discomfort about the issues being discussed, all interviews will be conducted as much as possible in a room or private area in a space away from the remaining patients

All data collected will be secured in a locked cabinet in the Occupational Health Unit of the Ghana Health Service and WRI of the CSIR (laboratory results) respectively, and access will be strictly limited to the research team only. This includes the study investigators. The team leader will be responsible for ensuring that the data is stored in a safe and confidential place.

11. INSTITUTIONAL ARRANGEMENTS

This study will be a collaborative effort between the Ghana Health Service (Occupational Health Unit), the Environmental Protection Agency (CCMC), the CSIR- Water Research Institute and the School of Public Health (Biological Environmental & Occupational Health Department) as well as the WHO country office.

A country Study team will be formed with representatives from the above listed institutions in Ghana. The team will be led by the representative from the GHS and the laboratory component by the CSIR representative. The team will be responsible for the overall management of the baseline

study. Members will ensure that all activities outlined in the proposal are implemented. These activities include obtaining local research clearance and authorization, recruiting and training of personnel for the study, supervising data collection, including checking regularly for completeness of data collection, processing and analyzing data, writing a full report on the study and disseminating the findings.

The WHO European Office, Bonn will provide technical assistance and financial support.

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Annex 1. Study information leaflet

Mercury is a metal that may enter the human body, and can result in ill health. All people are exposed to some concentration of mercury because it is a global pollutant. Usually, people are exposed to low concentration of mercury that is not dangerous for human health. When a woman is exposed to high concentrations of this chemical during pregnancy, mercury can be transferred to the fetus, leading to an increase in the occurrence of certain disorders in the child. Testing of body fluids and samples (such as hair, urine, and cord blood) from mothers helps to identify exposure of the unborn child to mercury. This information allows assessing health risks to you and your child and to the population as a whole. Having this information we can provide an advice how to protect yourself and your child from negative impact of mercury and plan adequate measure in your region and in the country. There are different sources of mercury in Ghana such as fish, occupational exposure, releases into ambient air due to some industrial processes.

A survey is planned in collaboration with the World Health Organization (WHO) to determine how much mercury the unborn child is exposed to, by determining levels of the metal in pregnant women. In Ghana the survey is implemented by the Ghana Health Service (Occupational Health Unit), the Environmental Protection Agency (CCMC), the CSIR- Water Research Institute and the School of Public Health (Biological Environmental & Occupational Health Department) as well as the WHO country office.

Tests will be carried out in women in their last month of pregnancy, if they express interest in participating and meet some criteria. The survey will benefit our knowledge on the situation with exposure of Ghana population to mercury.

Participation in the survey is voluntary and your agreement will be asked to involve you in the survey. You have time to decide and inform us, better before you enter hospital for delivery. You will be asked to sign a consent form, answer some questions about you, your husband and the family life style (it will take around 30 min). We also will take a small amount of hair from back of your head that won't harm you. Mid-wife in hospital will take samples of cord blood and urine. It won't harm neither you no your child. All three samples will be analysed for levels of mercury. There is no risks posed by your participation in the survey.

Morover, you can withdraw your participation. You need just inform us about your decision before leaving hospital time without giving reasons and without being subject to any form of discrimination. It will not influence on medical care for you and your child.

We also would like to obtain some medical data such as date of birth, weight and height of your child, any defects at birth diagnosed by medical professionals, your chronic diseases of kidney (if any).

All data obtained during the survey are confidential. Only the national coordinator with know your name. All otherspecialists working in the project will know only a number given you for the survey purpose. Your personal data will not be made available to anyone. Any reports coming out of the study will be based on collective results of all participants.

All biological samples taken from you will be distroyd after the mercury analysis is complete. They wont' be used for any other purpose.

Approval for the survey will be given by the Ethical Review Committee of the Ministry of Health.

You will be informed about the survey results if you wish. Those who take part have a right to know if their personal results indicate the need for medical advice (in case of critically high mercury values).

If you seek further information /explanation on the above, you may call the survey national coordinator
Dr Edith Clarke, Ghana Health service
Phone number - 0302 660693.

Annex 2. Eligibility screening form

1. Are you at least 18 years of age?
   - ☐ Yes
   - ☐ No

   If no → not eligible, stop the interview politely

2. How many days ago was your delivery (if done after delivery)?

   _____ days

   If more than 14 days → not eligible, stop the interview politely

3. Do you live in [*the catchment area of the hospital*]?
   - ☐ Yes
   - ☐ No

   If no → not eligible, stop the interview politely

4. How long have been living in this area?

   _____ years

   If less than three years → not eligible, stop the interview politely

5. How many days during the last three months have you spent outside the [*catchment area of the hospital*]?

   _____ days

   If more than 14 days → not eligible, stop the interview politely

6. Sufficient language ability in the interview language? (assessed by the interviewer)
   - ☐ Yes
   - ☐ No

   If no → not eligible, stop the interview politely

7. Hair sampling possible (based on visual assessment – hair length of at least 3 cm on the back of the head)?
   - ☐ Yes
   - ☐ No

   If no → not eligible, stop the interview politely

8. Eligible for enrolment?
   - ☐ Yes
   - ☐ No

9. If eligible, consented to participate?
   - ☐ Yes
   - ☐ No

10. Participant gave written consent to (please mark all that apply):
    - ☐ Hair sample

☐ Urine sample
☐ Cord blood sample
☐ Access to medical records

11. Enrolled in the survey?

☐ Not eligible
☐ Eligible but not willing to participate

☐ Enrolled to participate

IF ENROLLED IN THE SURVEY

Name of participant:

_____

Home address:

_____

Date of admission to the hospital:

_____

Date of delivery of child:

_____

Annex 3. Informed consent form

The informed consent form should be signed by all recruited women.

Before requesting an individual's consent to participate in research, the researcher/interviewer must provide the following information, in language or other form of communication that the individual can understand:

1) that each individual is invited to participate in research, the reasons for selecting the individual and that participation is voluntary;

2) that the individual is free to refuse to participate and will be free to withdraw from the research at any time without penalty or loss of benefits to which they would otherwise be entitled;

3) the purpose of the research, the procedures to be carried out by the researcher and the subject, and the aspects of the protocol that are incremental, in that they would not be part of routine medical care;

4) for controlled trials, an explanation of features of the research design (e.g. randomization, double-blind), and that the subject will not be told of the assigned treatment until the study has been completed and the blind has been broken;

5) the expected duration of the individual's participation and the possibility of early termination of the trial or of the individual's participation in it;

6) whether monetary or other forms of material goods will be provided in return for the individual's participation and, if so, the kind and amount;

7) that, after the completion of the study, participants will be informed of the results;

8) any foreseeable risks, pain or discomfort, or inconvenience to the individual (or others) associated with participation in the research, including risks to the health or well-being of a subject's spouse or partner;

9) the direct benefits to participants expected to result from the research;

10) the expected benefits of the research to the community or larger society, or contributions to scientific knowledge;

11) whether and when and how any products or interventions proven by the research to be safe and effective will be made available to subjects after they have completed their participation in the research and whether they will be expected to pay for them;

12) any alternative, currently available, procedures or courses of treatment and their potential benefits and risks;

13) the provisions that will be made to ensure respect for the privacy of subjects and for the confidentiality of records in which subjects are identified;

14) the limits, legal or other, to the researchers' ability to safeguard confidentiality, and the possible consequences of breaches of confidentiality;

15) the nature and sources of funding of the research, the sponsors of the research, the institutional affiliation of the investigators, and financial incentives for the investigators;

16) whether it is planned that biological specimens collected in the research will be destroyed at its conclusion, and, if not, details about their storage and possible future use, and that participants have the right to decide about such future use, to refuse storage, and to have the material destroyed;

17) whether commercial products may be developed from biological specimens;

18) whether the researcher is serving only as a researcher or as both researcher and the subject's health-care professional;

19) the extent of the researcher's responsibility to provide medical services to the subject;

20) that treatment will be provided free of charge for specified types of research-related injury or for complications associated with the research, and whether there is any uncertainty regarding funding of such treatment;

21) whether the subject or the subject's family or dependants will be compensated for disability or death resulting from such injury;

22) that the research protocol has been approved by an ethics review committee.

Informed consent form

CONSENT FORM for participation in a human biomonitoring survey to assess exposure to mercury in Accra, Ghana, a research project carried out by carried out by GHS/EPA/SPHUG/WRI, CSIR, with technical assistance from the World Health Organization (WHO).

Dear Madam,

We would like to invite you to take part in a study aiming to assess exposure of you and your child to mercury. All people are exposed to certain levels of mercury from the environment, but in some cases, for example if a person consumes a lot of fish, lives nearby places where mercury has been used and produced, or works with mercury, exposure levels can be higher, and pose a risk to health. There are many sources of mercury in Ghana – contaminated fish consumption, industrial processes, exposure at working places. You can contribute to survey and to advise the government on protective measures. After the survey we can provide you with advice on nutritional and other behavioural habits, for example, what kind of fish and how ofter you can eat, if high mercury concentrations are revealed in your biological samples.

Purpose

. Measurements of mercury concentration in your hair, cord blood and urine allow assessment of your personal exposure to mercury, as well as of your child. You will receive individual results if you want to know about level of your exposure to mercury;
Together with you, 400 women will be involved in the survey.

Based on the results of the survey, we will provide data on your infant exposure to mercury in worm, assess the level and distribution of exposure in our city, identify how big is a risk to human health in Accra and advise the government and you personally on protective measures if needed.

Our survey is the WHO as a part of a global initiative aiming at the development of a global plan of assessment of exposure to mercury and characterization of the global and regional distribution of exposure. It is necessary to plan protective measures and evaluate the effectiveness of their implementation at a global, regional and country level.

Background

Mercury is present in air, soil and food and finds its way into the human body, disturbing biological processes and in some cases affecting our health. Usually, people are exposed to low level of mercury which isn't not a harm to our health. When a woman is exposed during pregnancy, mercury can be transferred to the fetus, and affect the developing organs and systems. Relatively high concentrations should be to impact you and your child. Analysis of biological material from you (such as hair and urine) and of cord blood helps to characterize to what level of mercury your child has been exposed furing last three month of pregnancy. This helps to assess health risks for you and your child and at the population level in Accra and to support policy interventions aiming at reducing pollution and protecting health. It can also be used to provide recommendations on how to protect you and your child from exposure to mercury and reduce risk in cases of detected higher mercury concentrations. You will be informed about that and all necessary medical service will be provided to you.

The methodology applied in the survey has been developed and is recommended by WHO. It enables an assessment of exposure to mercury during the last trimester of pregnancy, through measuring concentrations of mercury and its compounds in the cord blood, scalp hair and urine. The most valuable data to assess prenatal exposure to mercury can be obtained if samples of biological material are taken immediately after delivery. For that reason, we approach you before and now, during their stay at maternity hospitals. If you are interested in getting more information about your and your child's exposure to mercury, and you meet the eligibility criteria, you are invited to participate in the survey.

Procedures

To assess exposure to mercury, we will analyse its concentrations in cord blood, scalp hair and urine, following your agreement to provide this biological material. All samples will be collected in a non-invasive manner without harm for you and your child. A sample of umbilical cord blood will be collected by the midwife at birth; a sample of your hair will be collected by cutting a small strand of hair close to the scalp from the back of your head; and, a spot sample of your urine will be collected in the container provided by survey staff. We will also ask you to answer a questionnaire with a number of questions about your diet, home and work environment, lifestyle and health. This information will help us to learn more about potential sources of mercury. Completion of the questionnaire should not take more than 30 minutes. All procedures will be conducted by trained personnel, who will also be ready to answer your questions.

We would highly appreciate it if you allowed us to access the following information from your and your child's medical records: your child's weight and height at birth, as well as your diseases and conditions during pregnancy and delivery. This information, together with the analysis of mercury

concentrations, will allow the national survey coordinator to provide you with advice on how to minimize your exposure, if necessary.

Biological samples taken from you will be destroyed after the mercury analysis is completed. They will be used only for the purpose of this survey.

The national coordinator of the survey *Dr Edith Clarke, Tel : 0243 629870 and Ms Hannah Frimpong Adminstrator, Ethical Review Committee, Ghana Health Service (Tel:0302 681109*) may contact you once after mercury analysis is performed, but no later than within three months after the sampling, to provide advice on medical follow-up and on how to minimize risks to your health from exposure to mercury, if high concentrations are discovered in your samples. Please indicate below, whether you agree that we contact you on that matter by phone or email.

☐ Yes, I don't mind you contacting me later for additional inquiry and to provide advice

☐ No, I don't agree that you contact me later for additional inquiry and to provide advice

In any case, you have the option to withdraw your consent to further contact at any time.

Voluntary participation/discontinuing participation

You do not have to take part in this research if you do not wish to do so. Refusing to participate will not affect your treatment at this hospital in any way.

Also, if you decide to participate in the survey, you will be able to discontinue your participation at any time. All you have to do is to inform the researchers that you no longer want to participate. Furthermore, you can ask for all the samples that you have provided to be destroyed. If you decide to withdraw your participation and ask for the destruction of your samples, please do it before leaving the hospital. Withdrawing your participation will not affect your medical treatment or access to medical services in any way.

The results of the analyses that have already been completed will remain in the survey database and will be used in survey reports.

Benefits of the survey

The results from all survey participants will be analysed collectively to characterize exposures to mercury and to guide policy-makers to make informed decisions for the benefit of public health.

Your results will be compared to health-based guidance values, when they are available. If necessary, you will receive recommendations on how to reduce the level of a pollutant in your body or to avoid future exposures.

You can ask that your individual test results be sent to you or to your doctor. If you choose to have the results sent to your doctor, we will ask you to provide their name and address in writing.

You can also specify to not receive your results if you do not wish to know.

Costs

No costs associated with this study will be charged to the participants.

Possible risks

No risks are anticipated associated with participation in this survey. There is no health risk related to the collection of cord blood. The procedure will not have any influence on the normal delivery procedures. Possible inconveniences are limited to the time you will have to spend on providing the hair and urine samples and responding to the questionnaires. The questionnaires and medical records contain information that can be viewed as sensitive. However these data will be kept strictly confidential. We will use coding and anonymized data at the data analysis stage. Your personal information will only be available to authorized investigators.

Confidentiality

Researchers will process the information from the questionnaires and the samples. Your name and address will be replaced by a code. If the results of this study are published in a report or scientific journal your name will not be mentioned and no information that can identify you will be included in such a report or publication. All information will be treated confidentially in accordance with relevant privacy laws.

Information about the survey

You have the right to ask for additional information about the research project and the procedures described in this document. All reasonable requests for information will be answered by the principal investigator to the best of their knowledge. The researchers will inform you if and when any major changes in the procedures, risks or benefits of this study occur.

Information on the progress of the survey can be requested from the national coordinator *(provide contact details: name, title, position, organization, phone number, email address*). At the end of the survey (*indicate the date*), you will be informed about the biological samples that are still preserved and any potential future use of these samples if you would like to get this information.

Explanation of the principal investigator

The principal investigator is responsible for this research, to be carried out under the conditions described in this document.

Name of principal investigator: Dr Edith Clarke, Health Service, Ghana

_____

Other contact people: Dr Kwadwo Ansong Asante, Senior Research Scientists, Water Research Institute

_____

I have read the information leaflet about participation in the human biomonitoring survey and want to participate in the survey. I understand the potential risks and benefits of this survey and take part voluntarily in this study. I understand that the information will be kept strictly confidential and that

the survey was approved by the independent ethics committee of WHO and by Ghana Health Service Ethics Review Committee.
.

Mother's name (printed or written in capitals):

_____

Mother's signature:

_____

Child's first and last names (if given):

_____

Child's date of birth (DD/MM/YYYY):

_____

Communication of results
The quality checked human biomonitoring survey results, including concentrations of mercury in hair, urine and cord blood, are expected to be available no later than three months after the sampling. Please indicate below, whether and how you want to obtain your individual results.

[ ] I do not wish to receive my results.
[ ] I wish to receive my results at my home address:

_____

_____

[ ] I wish that my results be sent to my doctor.
Doctor's first and last name:

_____

Doctor's address:

_____

_____

Annex 3. Main questionnaire for participants

Name of participant	
Medical record number	
Identity number of participant	
Date of interview	Date (day/month/year): _ _/ _ _/ _ _ _ _
Date of child delivery	Date (day/month/year): _ _/ _ _/ _ _ _ _

A. Personal information

A.1. Mother of the child (survey participant)

A.1.1. What is your ethnicity (or nationality)?

.....

A.1.2. Have you had children previously?

O No

O Yes How many? _____

A.1.3. What is your education level? *Please select **ONE** answer.*

O Primary (completed primary school)
O Secondary (completed secondary/high school)
O Post-secondary (college, university)

A.2. Farther of the child

A.2.1. What is the education level of the farther? *Please select **ONE** answer.*

O Primary (completed primary school)
O Secondary (completed secondary/high school)
O Post-secondary (college, university)

A.3. Economic status of your household

A.3.1. How easy is it for you to cope financially? *Please select **ONE** answer.*

O Difficult, not always able to afford the necessities
O Income is limited but can afford the necessities
O Live comfortably, but no excess in disposable income
O Stable financial situation, able to afford high-quality products and services

B. Potential exposure to mercury

B.1. Occupational exposure

B.1.1. Before your maternity leave/pregnancy, did you have a paid full-time or part-time job?
(as an employee, employer or self- employed)

O No
O Yes

If NO, please go directly to section B.1.5.

B.1.2. Have you ever worked in the following industries or sectors? *Please mark all that apply.*

Industry type	Never	Less than 6 months	Between 6 months and 1 year	1–5 years	More than 5 years	Any time during this pregnancy
Chemical/petroleum	O	O	O	O	O	O
Metal smelting	O	O	O	O	O	O
Metalworking	O	O	O	O	O	O
Chloralkali plant	O	O	O	O	O	O
Chemistry laboratory	O	O	O	O	O	O
Dentistry	O	O	O	O	O	O
Waste management (general)	O	O	O	O	O	O
Electronic waste management	O	O	O	O	O	O
Artisanal and small-scale gold mining	O	O	O	O	O	O
Production of goods that contain mercury, such as traditional remedies, cosmetics, etc.	O	O	O	O	O	O

B.1.2.1. Please provide the name and address of the industrial enterprise where you were working before/during this pregnancy.

……..

……

B.1.3. In your job, did you have contact with the following substances? *Please mark all that apply.*

Substance	Don't know	Never	Less than 6 months	Between 6 months and 1 year	1–5 years	More than 5 years	Any time during this pregnancy
Metallic dust	O	O	O	O	O	O	O
Mercury	O	O	O	O	O	O	O
Amalgam	O	O	O	O	O	O	O

Pesticides	O	O	O	O	O	O	O
Fumes from burning coal	O	O	O	O	O	O	O
Fumes from burning electronic waste	O	O	O	O	O	O	O

B.1.4. If you have worked in any of the previously mentioned industries or have had exposures as listed in the previous questions (you answered YES to any questions in B.1.2–B.1.3), please provide additional information below. *Please mark all that apply.*

	Always	Occasionally	No
Did you change work clothes before entering your home?	O	O	O
Did you change work shoes before coming home?	O	O	O
Did you take a shower after your work shift before coming home?	O	O	O
Did you ever bring your dirty work clothes or other contaminated items home?	O	O	O
If you answered YES to the previous question – Did you wash your work clothes separately from any other clothes?	O	O	O

B.1.5. During your pregnancy, did your husband/partner or anyone else living in your household work in the following industries/sectors? *Please mark all that apply*.

Industry type	Yes	No	Don't know
Chemical/petroleum	O	O	O
Metal smelting	O	O	O
Metalworking	O	O	O
Chloralkali plant	O	O	O
Waste management (general)	O	O	O
Electronic waste management	O	O	O
Chemistry laboratory	O	O	O
Dentistry	O	O	O
Artisanal and small-scale gold mining	O	O	O

B.1.5.1. Please provide the name and address of the industrial enterprise where your husband/partner worked before/during this pregnancy.

……

……

B.1.6. During your pregnancy, did your husband/partner have regular occupational or hobby-related contact with the following substances?

Substance	Yes	No	Don't know
Metallic dust	O	O	O
Mercury	O	O	O

Amalgam	O	O	O
Pesticides	O	O	O
Fumes from burning coal	O	O	O
Fumes from burning electronic waste	O	O	O

B.1.7. If your husband/partner or any other member of your household worked at an industrial enterprise (you answered YES to any question in B.1.5–B.1.6), please provide additional information below. *Please mark all that apply.*

	Always	Occasionally	No
Did your husband/partner change work clothes before entering your home?	O	O	O
Did your husband/partner change work shoes before coming home?	O	O	O
Did your husband/partner take a shower after work, before coming home?	O	O	O
Did your husband/partner bring dirty work clothes or other contaminated items home?	O	O	O
If you answered YES to the previous question – Did your husband/partner always wash work clothes separately from any other clothes?	O	O	O

B.2. Residential environment

B.2.1. Where is your place of residence located?

O In the city
O In a rural area

B.2.1.1. In what neighbourhood or residential area do you live?

O *Please provide name of the city/village*: ……..
O *Please provide the name of the area*: ……

B.2.2. Are there any of the following in the vicinity of your home (up to 2 km)? *Please mark all that apply*

	Yes	No	Don't know
Metalworking business	O	O	O
Waste incineration plant	O	O	O
Cement production plant	O	O	O
Chloralkali plant	O	O	O
Municipal landfill	O	O	O
Landfill for industrial by-products/waste	O	O	O
Crematorium	O	O	O
Mining operation	O	O	O
Artisanal small-scale mining	O	O	O
Thermo-power plant	O	O	O
Electronic waste dismantling	O	O	O

B.2.3. What fuel or energy source do you mainly use for cooking and for heating inside your home? *Please mark only one fuel source for each*.

Fuel source	Cooking	Heating
Natural gas	O	O
Coal or charcoal	O	O
Electric power	O	O
Wood or biomass	O	O
Hot water or hot air from central heating system (district heating or central boiler for a multi-apartment building)	O	O
Kerosene	O	O

B.2.4. What is your main source of water for drinking and cooking? *Please select only one water source for each*.

Water source	Drinking	Cooking
Public water supply	O	O
Private well or spring	O	O
Bottled water	O	O
Surface water (river, lake, etc.)	O	O

B.2.5. Has a thermometer or any other device containing liquid mercury (like a sphygmomanometer) been broken in your home during the last two years?

O No
O Yes. If yes, how long ago? Please specify below:
  O Less than 30 days ago
  O from 30 to 90 days (three months) ago
  O From 91 days to 6 months ago
  O More than 6 months ago but within the last 2 years
O Don't remember/don't know

B.2.6. Has an energy saving fluorescent lamp been broken in your home during the last three months (90 days)?

O No
O Yes. If yes, how many days ago? _____days
O Don't remember/don't know

B.2.7. Has anyone worked regularly with metals in your home in the last three months (e.g. soldering metals as part of do-it-yourself and hobby activities)?

O No
O Yes
O Don't know

B.3. Personal care and lifestyle

B.3.1. Do you have any dental amalgam fillings (dark-coloured fillings)?

O No
O Yes. If yes, how many amalgam dental fillings do you currently have? .....
O Don't know

B.3.2. Do you often use chewing gum or habitually chew (leaves/tobacco, etc.)?

O No
O Yes

B.3.3. Have you ever smoked cigarettes or other tobacco products in your life time?

O I have never smoked. *Go to question B.3.5.*
O I used to smoke, but quit prior to this pregnancy
O I was smoking during this pregnancy

B.3.4. How often did you smoke, on average, before and during pregnancy?

Frequency	Before	During
Did not smoke	O	O
Smoked less than once per week	O	O
Smoked at least once per week, but not every day	O	O
Smoked daily	O	O

B.3.5. How often did you drink alcoholic beverages during this pregnancy?

O Never
O At least once per month
O At least once per week

B.3.6. Do you regularly use skin-lightening products?

O No
O Yes

B.3.7. Did you use skin-lightening products during this pregnancy?

O No
O Yes. If yes, how often? *Please specify below*:
    O At least once per day
    O At least once per week
    O At least once per month
    O Less that once per month

B.3.8. Do you regularly use traditional remedies/medicines that may contain mercury (containing cinnabar)?

O No
O Yes

B.3.9. Did you use traditional remedies/medicines that my contain mercury (cinnabar) during this pregnancy?

O No
O Yes. If yes, how often? *Please specify below*:
  O At least once per day
  O At least once per week
  O At least once per month
  O Less that once per month

B.4. Food and beverage consumption

B.4.1. How often do you eat the following foods? *Please mark each category.*

Type of product	At least once per day	At least once per week	At least once per month	Less than once per month
a. Any type of fish/shellfish/sea weed (such as tuna in salad or sandwich, pizza, prawn cocktail, etc.)	O	O	O	O
a.1. Fish from shop	O	O	O	O
a.2. Shellfish from shop	O	O	O	O
a.3. Seaweed	O	O	O	O
a.3. Locally produced seafood (any type)	O	O	O	O
b. Cereal and grain products (any type)	O	O	O	O
b.1. Rice and rice products from shop	O	O	O	O
b.2. Bran and germ	O	O	O	O
b.3. Locally grown rice				
c. Meat and meat products (any type)	O	O	O	O
c.1. Game meat	O	O	O	O
c.2. Edible offal (liver, kidney, etc.)	O	O	O	O
c.3. Chicken	O	O	O	O
d. Vegetables and mushrooms	O	O	O	O
d.1. Wild mushrooms	O	O	O	O
d.2. Leafy vegetables from shop	O	O	O	O
d.3. Legumes from shop	O	O	O	O
d.4. Root vegetables from shop	O	O	O	O
d.5. Locally grown vegetables (your own or purchased at a local market)	O	O	O	O
g. Herbs collected locally	O	O	O	O

(including in herb teas)				

B.4.2. How often did you eat the following types of fish during the last three months?

Types of fish	At least once per day	At least once per week	At least once per month	Less than once per month
a. Swordfish, tuna	O	O	O	O
b. Oily fish (sardines, herring, mackerel, salmon, etc.)	O	O	O	O
c. Whitefish, cod, haddock, plaice	O	O	O	O
d. Freshwater fish (trout, perch, others) from shop	O	O	O	O
e. Freshwater fish locally caught	O	O	O	O
f. Shellfish	O	O	O	O
g. Seaweed	O	O	O	O
h. Canned fish	O	O	O	O

Annex 5. Community involvement strategy

Very big community is involved in the investigation in Ghana. However, the community needs to be involved in all stages: prior to the survey, during its implementation and in survey follow-up, especially if risk-reduction measures are to be implemented.

Given that In Ghana, high density population areas will be involved in the survey with population size around 2 000 000. Community involvement strategy focus on involvement of local medical staff, family doctors, policy-makers from health sector and religious leaders.

Community involvement will be beneficial and is necessary:

- to enable planning of the survey to take into account community needs;
- to ensure support for project implementation from the local authorities and population, and get a higher response rate for the survey; this will positively influence the reliability of survey results;
- to create a sense of participation and co-ownership, and to build trust towards the survey and the survey field staff;
- to increase acceptance of the survey results;
- to strengthen community knowledge and skills to understand the problem and implement risk-reduction measures;
- to ensure implementation of risk-reduction measures if they are needed.

Several steps will be taken to ensure community involvement.

1. Learn more about the community

The survey will be implemented in general population of Accra and Tema. That means that the community involved in the survey is quite diverse:

on level education;
on religion;
on occupation;
on customs and ethnic characteristics; for example, in some ethic groups cutting hair is prohibited;
on nutritional habits.

Community involvement is quite complex task and special attention should be taken to build links with religion leaders***.***

2. Develop a communication package about the survey

Information about the project is prepared for the target audience. The leaflet provides information on the survey on a plain language and explain: the rational for the survey and its objectives; who will be involved; how the survey will be implemented; what risks it could pose to the community and its members, if any; what the benefits for the community are; how the survey results will be communicated; what the follow-up is, in particular, if high levels of exposure to mercury are detected.

3. Ensure support from influential people

The Director General of the Ghana Health Service will be addressed for permission to collect data in the identified health facilities. He will in turn provide letters informing the Greater Accra Regional

Director of Health Services, and medical directors of proposed study facilities of the impending study, seeking their collaboration. Gynecologists /doctors in charge / senior nurses of the maternity services in the facilities will subsequently be informed by the respective medical directors of the facilities.
On community level, religious leaders serving the target population will be contacted to support the survey.

4. Communicate information about the survey to community members

Information about the survey will be communicated to community members, through:

- developing and disseminating an information leaflet about the planned survey; this allows outreach to a wider audience but does not allow immediate answering of questions and providing clarifications;
- reaching out directly to potential survey participants (pregnant women) and their families; that can be done during antenatal visit;
- agreeing joint antenatal visits with gynaecologists and obstetricians serving the community;
- agreement wuth religion leaders to explain the survey benefits in church mass.

5. Keep contact open during the survey implementation

Dirung the survey contact will be kept through field staff and involved medical personal.***6. Communicate the survey results***

The survey results should be communicated irrespective of the measured concentrations of mercury.

There will be a national dissemination workshop which will involve all stakeholders including representatives of sub-metros involved in study, health staff from the study facilities, representatives of Association of Pediatricians, relevant government MDAs (e.g. Fisheries, MOFA,), community & religious representatives. The findings will be shared with all these practitioners, researchers and the MOH, GHS and partners, to determine the way forward. The dissemination should culminate in an action plan on how to move the findings into policy review and implementable actions to address baseline situation.

Individual dialog will be organized with all people with high-level concentrations of mercury.

In case of high-level concentrations will be observed, longer-term actions will be recommended for a follow-up survey in 3–5 years.

7. Follow up with community members who need specific attention and support in implementation of risk-reduction measures, if necessary

In cases of high level concentrations of mercury in biological samples, the participants will receive additional information on how to interpret the results and recommendations on individual preventive measures to reduce exposure. In the unlikely case of very high mercury concentrations, recommendations for individual medical consultations with health-care workers will be communicated directly to the affected participants. Further to providing information at individual level, risk-reduction measures need to be implemented at the community level. This requires active interaction and full engagement of the local authorities in the development and implementation those measures.

Annex 6. Sample Referral Form from Study on Human Biomonitoring for Mercury in Accra

Date:……..

The Medical Officer in Charge

Dear Colleague,

The above named is an on-going study to determine pre-natal exposure to mercury. The study is evaluating levels of mercury in women in the last month of pregnancy using urine, hair, dried bloodspot, and cord blood.

The study is being jointly organized by the WHO, GHS, WRI and the School of Public Health.

We would be grateful if you would kindly attend to the study participant named below who has levels of mercury beyond the WHO acceptable limit

We would appreciate your feedback on the management of the patient and treatment received.

Name:……

Age:……..

Thank you in anticipation.

Yours sincerely

Dr. Edith Clarke

Study Co-ordinator

Annex 7 Budget

N	Budget line/expenses	Cost per unit	Total cost for the budget line, USD
1	***Staff costs***		
1.1	National coordinator	30.0 USD per day x 50 days	1,500.00
1.2	National laboratory coordinator	20.0 USD per day x 50 days	1000.00
1.3	Field staff - Midwives:	15.0 USD per day x 40 days x 12 midwives	7,200.00
1.4	2 Lab Technicians x 2 Hospitals	15 USD per day x 20days x 2 technicians x 2 facilities	1,200.00
1.5	Research assistants	20.0 per day x 30days x 4 persons	2,400.00
1.5	Assistant for biweekly verification & sample collection	50.0 per day x 8 weeks x2 persons	800.00
		Subtotal for staff costs	***14,100.00***
3	Travel	20.0 USD per trip x 10 trips	200.00
4	Copying (questionnaires, SOPs, and other relevant survey documents)	Check rims of paper , toner, printer toner	800.00
5	In-land samples transportation (supervisory visits)	combined with sites visits	0.00
6	Consumables (hair sampling)		300.00
7	Consumables for cord blood collection & storage (gloves, cotton wool, disinfectants Syringes & needles, test tube racks)		500.00
8	Health drink for subjects		500.00
9	Ethical Approval		300.00
10	Data entry into Excel & analysis		1,400.00

11	Report writing		1,500.00
		SUB-TOTAL	19,800.00
		15% to receiving institution	2,970.00
		SUB-TOTAL	**22,770.00**
I.		***TRAINING COSTS***	**3,240.00**
			26,010.00