

SECOND RECOMMENDATION
from the Implementation Group on Human Biomonitoring
31 March 2006

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Background and Objective

This Second IG Recommendation is to be seen as part of the step-by-step procedure adopted in the development of a coherent approach to Human Biomonitoring in Europe². The Recommendation is based on the discussions at the last meeting of the IG in Brussels on 27th January, on material provided under the BiPRO project³, and on work done within the workpackages of the ESBIO project⁴. Also the considerations from the National Funders (NF) meeting⁵ of 29th November 2005 as well as discussions during the Conference on the 'State of the art of Human Biomonitoring within Europe', Lisbon 20-21 March, have been taken into account.

In its *First Recommendation*, discussed at the Consultative Forum of 19th October 2005, the IG recommended the implementation of at least a so-called 'basic' scenario with biomarkers for one or a few pollutants (such as lead, methylmercury, cadmium, selected persistent organic pollutants, polyaromatic hydrocarbons and cotinine) measured in as many Member States as possible. Such an approach would allow meeting the requirements related to the testing of methodological issues, the establishment of collaboration networks and sharing of methodologies, the promotion of the idea of coordination/harmonisation in biomonitoring and obtaining a first impression of the comparability of biomonitoring data in Europe. The IG also recommended to add to this basic scenario, using the framework established, a more extensive program addressing other compounds of concern according to the priorities of Member States, e.g. pesticides, brominated flame retardants, endocrine disruptors. By choosing biomarkers related to these compounds from a 'shopping list', proposing several options with their pro's and con's, several groups of Member States could take advantage of the efforts within Action 3 of the EU Health and Environment Action Plan in their preparation for a HBM programme

¹ Established in the framework of the European Environment and Health Action Plan 2004-2010 adopted by the Commission on June 9th 2004 (COM(2004)416 final).

² Implementation of Action 3 of the EU Environment and Health Action Plan 2004-2010 (COM(2004)416 final).

³ BiPRO project "Human Biomonitoring: Support to the European Environment & Health Action Plan 2004-2010" funded by the European Commission, Directorate-General Environment.

⁴ ESBIO (Expert team to Support BIOmonitoring) project funded by the European Commission, Directorate-General Research. ESBIO's mandate is to develop a coherent approach to HBM in Europe and in particular the preparation and follow-up of the EU Pilot Project. The final contract was signed on 9 December 2005 and first financial means were available on 19 December.

⁵ See minutes - www.eu-humanbiomonitoring.org

and, in addition to the basic scenario, seek for further coordination. This would lead to a multi-layer model incorporating some flexibility: a core basic level and more advanced levels giving the opportunity to each Member State to include specific interests.

At the *National Funders (NF) meeting of 29th November 2005* strong support was evident for Action 3 of the Action Plan to build a coordinated approach to HBM in Europe and to test out the feasibility of this endeavour in a Pilot Project. However, no consensus could be reached between the Member States on a number of important topics such as the population group to be addressed (children, adults) in this Pilot Project and on the selection-criteria for the pollutants to be monitored. Also the origin and extent of the financial means needed to launch the project remained unclear. An urgent demand was expressed to identify an accurate decision-making structure and process. It became clear that the current systems for consultation do not permit to take the decisions needed to allow a detailed scientific and technical preparation of the launch of the pilot project.

Following the request at the NF meeting (see minutes, conclusions, item e.) for a well-argued, concrete and policy relevant proposal, and in view of the short deadlines with respect to the foreseen launch of the Pilot Project, the *Second Recommendation* presents the further considerations of the IG in the form of a first draft protocol for the Pilot Project. In order to advance the process the group made a selection of compounds to be addressed and proposed a specific study population to be monitored. Arguments for these choices are included. Further aspects of the protocol are included in their current preliminary stage.

The Second Recommendation elaborates the basic scenario, notably the proposal for monitoring in all Member States participating in the Pilot Project, human biomarkers for at least one common substance from the following list: *methylmercury, lead, cadmium, cotinine*. Only markers of exposure are addressed. Critical experience is available in the Member States – as shown by the European HBM inventory - in measuring human biomarkers for these compounds. In this first approach the focus is on harmonisation rather than on implementation of sophisticated technical methods.

The sampling framework to be organized will give optional possibilities to add biomarkers for other compounds of concern, according to the priorities in the different countries, and biomarkers of effects. The IG recommends to measure human biomarkers for additional policy relevant substances, under the condition that at least 5 Member States are interested in the same biomarker. Candidate substances so far are *phthalates and polyaromatic hydrocarbons (PAHs)*. Pending on the decision taken by the Member States and the Commission this extended scenario will be elaborated in the next Recommendation.

The objective of this second IG Recommendation is to receive feedback from the Consultative Forum, and in particular from Member States and Commission, indicating their common vision on the approaches proposed and a rough indication about the financing structures and procedures. This feedback will serve as a basis for the elaboration of a more detailed protocol including the basic and the extended scenario and for next steps such as calculation of budgets, designing of the necessary communication strategy and organisational requirements. The next meeting of the Implementation Group is planned on 18-19 May 2006.

THE EUROPEAN HUMAN BIOMONITORING PILOT PROJECT
A protocol for testing out the feasibility and added value of a
European approach to Human Biomonitoring
WORK IN PROGRESS

1. Background

In Action 3 of the European Environment and Health Action Plan 2004-2010⁶ the Commission announces to develop a coherent approach to Human Biomonitoring (HBM) in Europe based on existing expertise and experiences in the Member States.

The *rationale* for this is that European environment and health policies will be supported by better data comparability and accessibility within and between countries. A coherent approach to HBM in Europe is needed to build a coherent and consistent database covering the whole of the EU. This database will give insight on the presence of chemicals in the human body and their possible health effects for the area covered. Cooperation will lead to a more effective use of resources through shared development of scientific tools and appropriate strategies, and provide a platform for future research on the relation between health and the environment.

It is acknowledged that differences in environmental exposures and national environmental health concerns, different levels of analytical capacities, differences in political and health priorities, cultural differences, and perhaps also different perceptions of ethics may render a common biomonitoring survey carried out simultaneously in several European countries difficult to achieve. Therefore a step-by-step approach is adopted, starting with a pilot project addressing the question whether similar procedures for e.g. recruitment, sampling, data analysis, quality control can be carried out in different Member States, yielding comparable results. Further steps, with more specific questions addressed, will be decided pending the results of the pilot phase.

As indicated in the Baseline Report on “Biomonitoring of Children”⁷, HBM is typically used in survey projects⁸ and in research projects⁹. The pilot project as foreseen by Action 3 of the EU Health and Environment Action Plan clearly addresses the first objective and aims at developing a *framework for surveillance*. Such development is however seen as a research effort in itself. Moreover it cannot be seen separated from research efforts in order to guarantee scientific sound results in a field that is evolving rapidly. Specific research activities are needed to allow e.g.; (i) defining harmonised procedures; (ii) defining best (harmonised) ways of providing information to and obtaining consent from study persons; (iii) the interpretation of measurements with biomarkers; (iv) the integration with other data (environmental and health); (v) the translation into policy. A large scale HBM framework for surveillance will offer a unique opportunity not only as a basis for policy decisions but also as a basis to develop hypothesis driven research programmes¹⁰.

⁶ COM(2004)416 final adopted by the Commission on 9th June 2004.

⁷ http://www.brussels-conference.org/Download/baseline_report/BR_Biomonitoring_final.pdf

⁸ Activities that aim at periodical measurements in order to produce information on the prevalence of exposure to environmental agents and the related public health impact with a view to developing and evaluating policies that protect health.

⁹ Activities that aim at improvement of knowledge on causal links between environmental factors and health by hypothesis generation and testing.

¹⁰ Given the complexity of the link between health and the environment, science may profit from larger population groups with better exposure data to enable testing further hypotheses on e.g. mechanism, expected trends, and combined exposures to low concentrations or cumulative effects.

2. Aim of the Pilot Project

The Pilot Project aims at testing the hypothesis that HBM can be performed in a comparable way throughout Europe and that such a coherent and harmonised approach will provide better information on the relation between health and environment and better support for environment and public health policy, at the national as well as at the international level, whilst also leading to a more effective use of resources through shared development of scientific tools and appropriate strategies.

A surveillance framework and program will be developed and reliable and comparable exposure data on specific pollutants will be collected in European countries. This will allow for :

- Establishing reference values¹¹ and identifying reference ranges¹² for specific biomarkers to which country specific values could be compared;
- Evaluating the effectiveness of policy measures (if biomonitoring is repeated in time).

The framework and program will also provide a sound basis for EU wide research projects on environmental health issues.

3. Choice of the population

Although the SCALE initiative – being the basis for Action 3 of the EU Environment and Health Strategy – focussed on children, several European Member States prefer a wider view and an approach addressing the whole population, although with particular attention to

¹¹ Reference values indicate the upper margin of the current background exposure of the general population to a given environmental toxin at a given time. They can be used to identify subjects with an increased level of exposure (in relation to background exposure) to a given environmental toxin. However, they do not represent health-related criteria for the evaluation of human biological monitoring data (Ewers et al, Int Arch Occup Environ Health (1999) 72: 255-260).

HBM values are derived from toxicological and epidemiological studies and intend to represent health-based biological exposure limits. However, they are currently available for only few well defined environmental toxins and may preferably be used in situations with clearly defined exposure. They refer to only one agent and do not take into account synergistic effects or complex exposure situations. The Commission on Human Biological Monitoring of the German Federal Environmental Agency established in 1993 recommends two different HBM values: HBM I, the concentration of an environmental toxin in a human biological material below which there is no risk for adverse health effects, and HBM II, the concentration above which there is an increased risk for adverse health effects in susceptible individuals of the general population.

¹² Reference ranges: Reference ranges are commonly used for parameters of known physiological relevance (i.e. concentrations of essential trace-elements, cells, enzymes, etc) rather than for the concentration of pollutants of largely unknown dose-response relationships. Reference ranges for particular parameters are derived from a sufficiently large number of individuals, where the measured values ideally show a normal distribution (Gaussian Curve) providing means/medians, min/max-values and standard deviations (s). The curve area within +/- 2s represents the values that 95% of the investigated population fall into and is defined as the reference range, whereas 5% of the population having larger or smaller values. Clearly, reference ranges depend on the analytical methodologies applied and therefore must be stated for comparison purposes by physicians.

However, the US-CDC uses this term also on pollutants/biomarkers of unknown effect levels by stating: "Finding a measurable amount of ... [biomarker/pollutant] in body media does not mean that the level causes an adverse health effect. Whether ... [] levels determined is a cause for health concern is not yet known; more research is needed. The data provide physicians with a reference range so that they can determine whether or not people have been exposed to higher levels of ... [] than are found in the general population. These data will also help scientists plan and conduct research about exposure to ... [] and health effects." (Third National Report on Human Exposure to Environmental Chemicals, July 2005, Department of Health and Human Services, Centers for Disease Control and Prevention, NCEH Pub. No. 05-0570).

vulnerable groups such as children or elderly people. Collecting samples in women of fertile age (20-45 years) and their¹³ children (3-15 years) is in line with these considerations¹⁴. More precise choices will be proposed pending on the agents that will be addressed.

4. Choice of the pollutants and biomarkers

As the major aim of the pilot project is to develop and test a harmonized approach for HBM at the European level, the pollutants and biomarkers addressed should not be too complicated with respect to biological sampling, degradation of compound, analytical methods or interpretation of results. Only biomarkers of exposure will be addressed for the basic scenario. Biomarkers of effect can be added by Member States in the extended approach for research purposes.

For at least one of the following candidates, human biomarkers of exposure should be measured in **all** Member States participating in the Pilot Project: *methylmercury lead, cadmium, cotinine*. In addition to this, human biomarkers for additional substances are recommended, under the condition that at least 5 Member States are interested in the same biomarker. Additional candidate substances so far are *phthalates and PAHs*.

Major criteria to be considered when choosing the pollutants to be addressed in the pilot project were defined as:

- the health significance: the known or suspected role of the pollutant in the aetiology of diseases (serious, frequent, disabling, lethal);
- the known or suspected widespread exposure to the pollutant;
- the number of countries already measuring the biomarker or expressing their interest in doing so;
- the public health concern;
- the availability of a biomarker for which there is already sufficient analytical experience: validated analytical methods with adequate sensitivity, specificity, precision; reference materials and external interlaboratory quality assurance systems available;
- the availability of health-based biological exposure limits¹⁵;
- biological sample needed readily obtainable in the population of concern;
- the practicality of storage and transport of biological samples;
- a small margin of safety between the current known exposure and the exposure level which is expected to cause adverse health effects;
- the knowledge gaps on total exposure;
- the policy relevance and the possibility for policy actions.

¹³ Related mothers and children will be addressed, not independent samples of women in childbearing age..

¹⁴ **France** has a need to measure lead in blood of children of 1-6 years of age. Collecting blood samples in children is however questioned by **Germany** for the following reasons: sampling blood from children may be severely hampered by ethical concerns and might be difficult to solve in the different MS. E.g. the German Ethic-Committee permits the measurement of lead in blood only if a plain health-related benefit is evident. The sampling of blood bears the additional disadvantages that certified medical staff is required and the collection of blood samples from children during a household visit is considered inappropriate.

¹⁵ To date, well-documented health-based recommendations following the results of epidemiologic studies have been formulated for: inorganic lead, inorganic cadmium, inorganic mercury and methylmercury, and fluorides only (see report of WP 6 ESBIO) .

LEAD in whole blood¹⁶

- Target groups: women in childbearing age¹⁷ and children^{18 19}.
- Chronic exposure has effects on the nervous and the haematopoietic system as well as on the kidneys.
- Foetuses and children are particularly vulnerable. The central nervous system (CNS) is the critical organ. A wide range of behavioral tests has been performed on lead-exposed populations to assess the influence of lead on the CNS functions. The meta analysis of the results of epidemiological studies carried out mainly in the USA, Australia and Europe was published by IPCS in 1995²⁰. No NOAEL has yet been established.
- HBM is used for the assessment of total exposure from different sources. In the case of environmental exposure to lead, health effects can be related to the blood lead levels.
- There exists considerable experience in several EU countries although methodologies used are different.
- Very well validated methods for analysis are already available. Lead in blood can be determined by means of flameless AAS or ICP-MS. Reference materials and external quality assurance system at environmental level available.
- Health based values are available: the WHO Air Quality Guidelines for Europe (WHO, 2000) recommend that at least 98 % of the population exposed in the general environment should have Pb-B below 100 µg/l, and the median blood lead level should not exceed 54 µg/l. The Centers for Disease Control (1991) recommended that the Pb-B values in children should be below 100 µg/l. According to recently published results of the studies by Canfield et al.²¹ blood lead concentrations, even those below 100 µg/l, are inversely associated with children's IQ scores at three and five years of age, and associated declines in IQ are greater at these concentrations than at higher levels.
- The geometric mean values in different countries suggest that in women and children the Pb-B levels are approaching the range of 10-30 µg/l considered as a „baseline” of minimal anthropogenic origin.
- The WHO asks to ensure regular biomonitoring of lead (amongst other hazardous chemicals) in at risk children.

TOTAL MERCURY in scalp hair

- Target group: women of childbearing age.
- Total mercury in scalp hair reflects the methyl mercury exposure.
- Methylmercury is a neurotoxin, and the form of mercury that is most easily bioaccumulated in organisms.
- The effects of methylmercury on the adult differ both quantitatively and qualitatively from the effects observed after prenatal or, possibly, postnatal exposure. The critical organ is

¹⁶ Although a declining trend and low blood-lead levels are observed in the last years, this does not necessarily mean that exposure and adverse effects of lead are definitely solved. Some population groups are more vulnerable (children), some population groups are at higher exposure risk (children living in hot spots, minorities, children from families with only basic education level). Also the lowest blood-lead level guarantying no adverse effect is not known exactly, it is a matter of discussion if 100 microgram per liter of blood will not be lowered in the future.

¹⁷ Measuring in pregnant women would reflect exposure of the foetus.

¹⁸ See footnote 15.

¹⁹ Age group to be defined.

²⁰ IPCS Environmental Health Criteria 165 Inorganic Lead. WHO, Geneva, 1995.

²¹ Canfield R.L, et al., Intellectual Impairment in Children with Blood Lead Concentrations below 10µg per Deciliter. The New England Journal of Medicine, 348 (16) 1517-1526. 2003.

the nervous system and the critical effects include developmental neurologic abnormalities in human infants, and paraesthesia in adults. The fetus is at particular risk. Prenatal exposure leads to psychomotor retardation in infants. Developmental neurologic abnormalities in infants are considered the critical effects.

- The analysis of human hair is suitable for evaluating the individual long-term exposure to methylmercury, but not for other heavy metals²². Once mercury is incorporated into hair, it remains unchanged. The level of mercury in hair (Hg-H) is largely dependent on fish consumption.
- Well validated methods and profound experiences to analyse **methylmercury** in hair are available in some MS.
- Reference material and external quality assurance system at environmental level is available (according to WP6 paper). CRM for the round robin tests on hair analyses are commercially available.
- WHO JECFA calculated the safe dose (including fetuses) to be 0.2 µg MeHg kg body weight a day. Benchmark dose calculations have been performed for methylmercury-associated delays on evoked potential latencies in two cohorts of children from the Faroe Islands and from Madeira (Murata et al., 2002). The obtained BMDL 5 % of approximately 10 µg/g maternal hair was similar to that calculated for other neurological variables (Budtz-Jorgensen et al., 2002) in the Faroese children and in the New Zealand population (Crump et al., 1998)²³.
- The EU Parliament in its Resolution on the Community Mercury Strategy called upon the Commission to “ensure that mercury especially in vulnerable populations is included in the biomonitoring programme originally foreseen in the European Environment and Health Action Plan 2004-2010²⁴”.
- The European Food Safety Authority’s (EFSA) Scientific Panel on Contaminants in the Food Chain (CONTAM) published an opinion regarding the possible risks to human health associated with the consumption of foods contaminated with mercury²⁵.

CADMIUM in urine

- Target group: women of childbearing age²⁶.
- Environmental exposure to cadmium can cause kidney dysfunction, osteoporosis,. Some data indicate that cadmium could act as an endocrine disrupter or induce lung cancer.
- Validated methods are available. Cadmium in urine can be determined by means of AAS or ICP-MS. Reference materials and external quality assurance system at environmental level are available.
- Most of the Member States have already essential experiences from previous studies.

²² Wilhelm M & Idel H: Hair analysis in environmental medicine. Zbl Hyg 198: 485-501 (1996); Katz SA & Katz RB: Use of hair analysis for evaluating mercury intoxication of the human body: a review. J Appl Toxicol 12 (2): 79-84 (1992); Harkins DK & Susten AS: Hair analysis: exploring the state of science. Environ. Health Perspect (2003) 111: 576-578.

²³ JECFA 61st meeting – June 2003 (cadmium and methylmercury). Summary and conclusions, 22 p..

²⁴ http://www.europarl.eu.int/omk/sipade3?L=EN&PUBREF=-//EP//TEXT+TA+20060314+ITEMS+DOC+XML+V0//EN&NAV=S&MODE=XML&LSTDOC=N&LEVEL=2&SAME_LEVEL=1#sdocta8

²⁵ http://www.efsa.eu.int/press_room/press_release/258/presrel_contam_01_en_final3.pdf

²⁶ The concentration of cadmium in urine reflects the accumulation of cadmium in the kidney cortex and will therefore be measured in women only.

- Health based values are available: it has been postulated that for the general population the Cd-U levels should be below 2.5 µg/g creatinine. Urinary excretion of early biomarkers of kidney dysfunction can be increased at cadmium levels in urine of about 2.0 µg/g creatinine²⁷.

COTININE in urine

- Target population: women and children.
- Cotinine is a major metabolite of nicotine and its levels are used to track exposure to environmental tobacco smoke (ETS) among non-smokers and to estimate exposure to ETS in every day-life situation. High cotinine levels indicate more exposure to ETS, which has been identified as a human carcinogen. The half life of cotinine in urine is about 70 hours (past few days).
- Active smoking as well as exposure to ETS must be determined in order to correlate potentially elevated cadmium, lead, PAH-metabolites, etc concentrations in urine.
- Validated analytical methods, reference materials and external interlaboratory quality assurance systems are available.

OPTIONAL COMPOUNDS AND RELATED BIOMARKERS

Pending on the decision taken by Member States and the Commission, further details will be elaborated in the next Recommendation.

5. Study Design

[to be further developed]

The pilot study is designed as a cross sectional study²⁸ and will include a number of participants per country in relation to the total number of the population under study in each Member State and in function of the agent to be measured and the variation in the population group of interest (see under 5).

Participants will be recruited to be as representative as possible for the general population or subgroup of interest in the country or region, by random sampling in the population under study.

Each Member state will have a Management Centre coordinating all the activities per country. The chair of this committee will be member of a Central Management body at EU level.

[to be further developed]

6. Selection of participants, number of samples

[to be further developed within WP2 of ESBIO].

²⁷ Järup et al., 1998; Noonan et al., 2002 and Trzcinka-Ochocka et al., 2004.

²⁸ With optional longitudinal components for sub samples - still under discussion for a further developed scenario.

Possible participants will be identified through the following procedure²⁹:

- Sampling locations will be chosen according to the population density;
- Random samples (and equivalent types of sample);
- Sampling of children may go via registration offices, within educational systems (school, vocational schools, day care centres schools etc.) or in the framework of screening tests³⁰.

Inclusion criteria

- Participants preferably should have resided in the sampling area for the previous 5 years;
- Mothers and their related children living in the same household.

Exclusion criteria:

- Serious illnesses (to be defined);
- Accidental overexposures or/and occupational exposure.

Number of samples: the number of samples needed to obtain sufficient statistical background data may vary according to pollutant and population specific characteristics such as the distribution of confounding factors, the distribution of subpopulations with differences in absorption and excretion and the homogeneity of the exposure of interest. Successful biomonitoring programmes have used a wide range of sampling frequencies. On one extreme, the *WHO program on mother milk* collected 50 samples per country. On the other end of the scale, the *Flemish biomonitoring project* collected 5000 samples from a population of 6 million, giving a sampling frequency of one in 1400 inhabitants. The *German biomonitoring program* collected a randomly chosen sub sample of 1800 children from representative samples of 18 000 children taking part in health survey. The population is 80 million if focussing only on the 1800 samples, this gives 1 sample per 45.000 inhabitants. The *CDC program in the US* collected 1500 samples on 280 million inhabitants, a sampling frequency of 1 in 190.000. In the *Czech Republic program* the number of samples is about 400 per year, e.g. 2000 per 5-y period, and about 2 samples per 10 000 inhabitants.

The number of samples could be based on the population (or the number of specific population groups) in each country, with some reduction for countries with a higher number of inhabitants and with a minimum number of samples for countries with low number of inhabitants. Taking for example a sampling frequency of 1 on 50.000, for a country with 400.000 inhabitants this would mean 8 samples needed. For the Netherlands, with 16 million, 320 samples would be required. If all European countries, with 350 million inhabitants, would participate, 7000 samples would be collected.

An objective of the program is to test the recruitment of participants, the collection, storage and transportation of samples and the analysis and communication of results. For these processes 1 sample per 50 000 inhabitants and a minimum of 50 samples per country is suggested. If there is an option to do additional measurements in the collected sample, it should be ascertained that the sample treatment allows for this.

7. Recruitment

[to be further developed]

²⁹ A unique system for all MS may be difficult to achieve, several options may be recommended. SOP (acceptable variations of SOPs) for sampling and logistics will be prepared.

³⁰ Under discussion.

Biomonitoring shall take place on a strictly voluntary basis. Persons who meet the required eligibility criteria will receive a written invitation³¹ to participate. This invitation will also include an information sheet³² explaining background, aim and procedures of the study.

Written informed consent³³ will be asked to each party separately. For the children, parents will be asked for consent. As far as possible given their age (10-15) children will be asked for assent.

Compensation for time and inconvenience will be provided as follows ... [to be prepared within WP 2 and WP5 of ESBIO]

8. Field Work

[to be further developed]

Sampling will be done at the home of the participant or at an examination centre³⁴ by trained interviewers / trained medical personnel in all MS. To avoid seasonal bias, sampling during all seasons is recommended³⁵. The entire procedure of sampling, handling, transport and storage of specimen must follow a protocol and further specified conditions, material and consumables.

9. Questionnaire

[to be further developed]

Participants will be interviewed at the time of the sampling by skilled personnel. The attached questionnaire [to be provided later]³⁶ will serve as a basis for the interview. The Master-questionnaire will be delivered in English first; a precise (one-to-one) translation into the language of participating MS has to be carried out at later stage; lastly a transfer of questionnaire data into the English database is required. The Master questionnaire and its translations will be tested before being made operational.

The first questions will address the criteria for eligibility of the participant (see under study population)

Further questions will e.g. address the following items:

- Personal characteristics (weight, height);
- Socio-biographical data: (nationality, place of birth, educational/ professional/ marital status, language, number of household members, time spent in the current country/sampling area);
- Main exposure routes: (smoking pattern practices and perception incl. cohabiters and coworkers [ETS], dietary habits, consumption frequency/amounts of fish & seafood and time frame prior to sampling, lead water pipes, industrial polluters/heavy traffic in vicinity to home/place of work, previous occupational exposures);
- Professional exposure of parents , practices followed to minimize transfer of work related pollutants to home environment;
- Hobbies.

³¹ A draft invitation letter will be provided.

³² A draft information sheet will be provided.

³³ A model informed consent form will be provided.

³⁴ To be discussed.

³⁵ If only a limited number of samples will be measured, the sampling period may be restricted to a definite time of the year.

³⁶ The questionnaire will be prepared within ESBIO.

[to be further developed]

10. Chemical Analyses

[to be further developed]

Laboratory: for the chemical analyses, either a local facility or a national competence centre of MS can be chosen; either a commercial lab or a governmental institute can be involved. Essential prerequisite is an established and recognized QC/QM-system in regard to the selected target pollutant/biomarker and matrix. In case of tenders, the laboratories require a proven competence in determination of selected biomarkers in matrix. It is recommendable to embed a round robin test into the Pilot Project. For the Pilot Project itself, splitting of specimen material and parallel analyses in a central reference laboratory facility within the EU could greatly improve external quality control.

Sampling, storage and transport: all equipment in direct contact with the specimen needs special attention. In case of organic biomarkers/pollutants/metabolites, proper preservation measures have to be assured, respectively suitable methods of extraction and preservation prior to transport have to be elaborated.

Chemical analyses: Standard Operational procedures (SOPs) for sample preparation and determination of the selected biomarkers/pollutants are required at laboratory level. The chosen methods should be validated and an appropriate instrumentation for necessary analytical parameters ought to be available in MS laboratories.

Specimen: the analyses will be performed on individual samples preferably. Analysis of pooled samples should be only considered in case of budget-, capacity- or time-constraints.

11. Harmonised Data Treatment

[to be further developed]

Data treatment at MS level: the transfer of data to EDP following procedures to be provided by ESBIO will cover the following aspects: preferably promptly data input by skilled staff, English language; unified entry templates to assure that results/entries are expressed in unified values/terms, application of common software and results submitted on unified file format.

Central/regional data management: the submitted data from MS will be subjected to statistical processing by applying common software and strict syntax.

Databases will be made available to all participating countries

12. Evaluation of Data

[to be further developed]

The evaluation of data will take place at different stages of the Pilot Project. Primary steps will be the checking and revising of data obtained from the questionnaires, and the description of the characteristics of the sampled population. Further steps will entail the statistical processing of laboratory results regarding biomarkers in matrix, linked with the results of inter-laboratory comparison (round robin test), and analysis of chemical results in regard to questionnaire data and mother-child correlation.

13. Quality Control

[to be further developed]

Internal Quality Control will be performed at all stages following SOPs. External QC should be added through round robin test and/or splitting of samples and parallel measurements in a reference lab of proven competence. External auditors, consultants will be involved where needed. Exchange of skilled staff within the EU will be encouraged to assist and share expertise.

13. Communication

[to be further developed]

Communication at several steps of the programme is considered key to this project. The goals and limitations of the study, what is expected from the participants, when and how the results will be communicated and interpreted, and the possible follow up to the results will be clearly identified and communicated not only to the participants but also to a wider public.

Communication will be organised at EU level as well as at Member State or regional level at all critical phases: at the launch of the project, during the project and at the end of the project with a view to e.g.:

- Supporting the recruitment;
- Communicate findings to participants, communities, and the general public;
- Support the identification of appropriate policy answers;
- Provide information to policy makers;
- Raise awareness when appropriate to support preventive measures and practices;
- If relevant provide data to support precaution and holistic risk governance.

The communication strategy will address different actors (general population, NGOs, policy makers etc), and will be interactive and dynamically evolving and adjusted.

Study participants will be given the opportunity to receive their individual results³⁷. Reporting of aggregated results at the collective level shall not disclose individual confidential information of participants.

14. Translation into policy answers

[to be further developed]

Data from HBM can provide info to identify trends, evaluate effectiveness of policies or identify areas where policies need to be changed or developed. There fore data will be transformed into concrete information for policy makers.

The results will be interpreted in an environmental health perspective. The obtained biomarker data will be as far as possible presented in a geographical information system, and integrated with environmental and health monitoring data which are available at EU level. Guidance for interpretation and possible follow up of the results will be provided.

15. Ethics

[to be further developed]

Procedures will fully comply with the Helsinki declaration and strictly follow all legislation and regulations at EU and national level. Children will be asked for assent. More details will

³⁷ Procedure to be defined

be provided from WP 4 of ESBIO addressing e.g. ethical issues related to information, consent, data protection and communication.

16. Financial aspects
[to be developed]
