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Guidelines for Population Sampling, Recruitment and Biological Sampling

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Guidelines for Population Sampling, Recruitment and Biological Sampling

Objectives

- Provision of a draft proposal regarding general design, population sampling, recruitment and specimen sampling of the EU Pilot Study on a harmonized HBM Programme
- Scientific and operational input for the implementation of Action 3 of the European Commission's "Environment & Health Action Plan 2004 - 2010"
- Facilitating the establishment of collaboration networks and the sharing of methodologies as adopted in the objectives of the ESBIO Project
- Commitment to Objective 2.3 of the ESBIO contract

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1. Introduction

Within the framework of the ESBIO¹ Project, WP 2 is supposed to elaborate a number of conceptual elements for the intended European Human Biomonitoring (HBM) Programme, starting with a Pilot Study now being part of the Seventh Framework Programme of the European Community for research, technological development and demonstration activities (ENV.2007.1.2.2.1. "European Network on Human Biomonitoring").

The present deliverable will outline strategies for the population sampling and recruitment and propose strategies for the biological sampling.

WP 2 delivered further proposals inter alia for pollutants and appropriate biomarkers and guidelines for collection, analysis and evaluation of HBM data, as well as for the organisation of laboratory work and interlaboratory comparison.

All these working steps are reflected by corresponding deliverables as a result of extensive discussions with various national experts and members of ESBIO and the 'Implementation Group on Human Biomonitoring' (IG).

The reconciliation of opinions, interests and expectations regarding the scope and set-up of the European Pilot Study proved to be a particularly challenging task. Beside the consensus on the key objective(s) of this Pilot Study further controversial issues emerged merely through the nature of a transnational harmonization process in the domain of Human Biomonitoring.

An outline of this decision-making process can be found in Annex 2.

This context accounts for the step-by-step elaboration of consecutive "Recommendations from the Implementation Group on Human Biomonitoring" which served as basis for discussion and fine-tuning with representatives of Member States (MS), European Commission and stakeholders, e.g. the Consultative Forum.

Chapter 3 of the present proposal does reflect the major decisions of the 3rd Recommendations (IG, 2006).

It was recognized that due to the national differences in (infra-) structure, legislation, governance, culture and ethics among the European MS it shall be deemed to be neither purposive nor feasible to specify a rigid scheme of mandatory standardized procedures (*protocols*) for all study elements.

¹ See: <http://www.eu-humanbiomonitoring.org/index.htm> (ESBIO / IG)

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Instead, the individual MS Units must be allowed some flexibility to adopt certain study elements (following provided *guidelines*) according to the national realities, in order to use existing resources but to ensure the quality of adequate procedures and validity of data alike. Therefore, conceptual guidelines for the adaptation on national level will be delivered.

It is of fundamental importance to define objectives and associated hypotheses of an intended HBM study first of all, and to assess required resources and means as precise as possible. Subsequently, all further study elements and operational sequences (*study design*) can be planned out.

It will be exemplified later that alterations of virtually any study element have an impact on the entire process and ultimately on the expected value, quality and credibility of the study outcome.

By integrating all above mentioned factors and keeping the diametrically opposed ideas and concepts of IG/ESBIO in mind (harmonization action/technical feasibility vs. scientific research demand), it becomes apparent that this proposal cannot describe the "golden path" to a European HBM Programme, as much as there is no "gold standard" design fitting all HBM studies in terms of objectives, subjects, biomarkers and related specimen.

Instead, some general requirements of a modern HBM study design will be outlined first and afterwards the commonly elaborated issues and procedures of IG/ESBIO are going to be presented whereby Pro's and Con's of various aspects will be sketched if possible.

2. General Remarks on Human Biomonitoring Studies

Environmental health sciences focus on the relationship between exposures to environmental chemicals of concern and their relationship to health outcomes. As analytic techniques have evolved rapidly, there has been an increasing focus on development and use of HBM for evaluating human exposure.

Originating as a valuable tool from the domain of occupational medicine, HBM gained wider importance in environmental health/medicine and monitoring activities (environmental surveillance) whereby reported data caused considerable public attraction by media coverage. Modern HBM studies can provide data on a broad array of chemicals in blood and urine and in other fluids and tissues such as saliva, breast milk and human hair. These data have often supplemented or even supplanted the previously performed estimates of human exposure based predominantly on environmental measures.

Albeit the applied tools and approaches, human exposure data frequently prompt questions regarding the relationship between levels of environmental chemicals in humans and external exposures, the "baseline" or "background" level against which individual levels should be compared, and whether the measured biomonitoring values allow for conclusions about individual and/or population health.

Within the scope of this proposal lie the characteristics of a scientifically robust HBM study, highlighting the strengths and limitations of biomonitoring programmes in order to propose a suitable study design.

2.1. Sophisticated Human Biomonitoring Study Design

The design of any scientific study incorporates several key components, such as study goal(s) and hypothesis, the properties of the biomarkers to be used, the selection of the population to be sampled and ethical and communication issues. Each of those components depends on the intended utilization of the biomonitoring data.

However, fundamental features that make for scientifically robust and credible studies exist (WHO, 1983; NAS, 2006) and elaborated design proposals are preferentially subject to review, e.g. in the US through EPA's Office of the Science Advisor (HSRB, 2007). The initiators of HBM studies must focus on key research needs and develop recommendations for ensuring that objective, hypothesis and study design parameters are realistic, transparent and scientifically robust alike.

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Since HBM studies involve human beings the entire process has to be conducted in a way that protects the study participants' rights and well-being, and must have the oversight of a Research Ethics Committee (REC) and/or an institutional review board, clearly addressing data protection and ethical standards according to the Declaration of Helsinki^{II} (WMA, 2007; LaKind et al., 2005). The Good Clinical Practice Guidelines of the International Conference on Harmonisation (ICH, 2005) aims at comparable standards regarding human subjects.

2.1.1. Sampling Frame

The extent to which HBM results can be generalized to a wider population will depend almost entirely on the sampling frame and extent of the study. The best sampling frame is a random selection from a clearly defined population (group) featuring a high level of response, participation agreement and compliance, which enables study results being almost entirely generalizable to that population (group).

The extent to which generalization from the sample to the targeted population will be restricted depends on whether

- i) the net sample size is appropriate
- ii) the sampled population matches the target-population parameters
- iii) the population is self-selected (e.g., volunteers)
- iv) there is a high and/or non-uniform degree of non-participation.

2.1.1.1. Population Sampling

HBM researchers in population exposure surveillance studies have no choice but to obtain specimens from a *sample* of the targeted population, from which statistical inferences will later be drawn regarding the (generally much larger) group as a whole. The appropriate strategy is similar in concept to taking a poll before an election in order to compose this sample as representative as possible. This demand of representativeness is a challenging task and polling organizations go to great lengths to achieve such a sophisticated approach.

Ideally, the selection of suitable HBM study participants (sample population) should represent the targeted population in terms of age, sex, geographic location, socioeconomic status (SES)

^{II} The issues of ethical approval and study-related aspects are covered by ESBIO WP 4 (Ethical Issues, internal communication within the Pilot Project)

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and related demographic factors, including race and/or ethnicity, or any other characteristics considered a priori to be important (e.g. diet, personal habits, moral hazards).

These selection criteria are linked to the definition of eligibility, also denoted as "inclusion / exclusion" criteria of the study.

Common exclusion criteria are potential participants' lack of language competence, dwelling in hospitals, mental institutions or detention centres.

For various reasons some researchers tend to choose *samples of convenience*. For example, it is far more convenient to obtain the requested numbers of people in a small geographic area or to take specimens from people who appear at a clinic or hospital for unrelated reasons.

Such groups may have the right mix of age, sex, and even other characteristics specified, but they are just convenient for the researcher, and may not be representative of any larger group.

Although it may be possible to draw some insights from such groups of self-selected volunteers, they cannot be presumed to be representative of the population of interest, nor can any valid comparisons be made with non-sampled members of the population.

The US National Research Council (NAS, 2006) concluded that "convenience sampling tends to be the norm rather than the exception" and that the associated "*selection bias* is the Achilles heel of such samples" and claimed "when such convenience samples are reported, the strategy used for recruitment and selection must be made completely transparent and explicit so that scientists can assess the distortions or biases that may result from analyzing measurements in such groups as though they were true population samples".

It must be understood that "perfect representativeness" of a population sample demands for enormous efforts, and even if those principles of choice and full transparency are rigorously adhered to, there remains in every situation an important degree of uncertainty because of random variation - who was sampled and who was not - so results should ultimately be expressed with respect to that uncertainty. In general, the smaller the group sampled and so lower the concentration of a biomarker and the larger the variation in values because of inter-individual differences and laboratory variation, the more uncertain the results will be.

The confidence interval (CI) of parameters computed represents such estimation of uncertainty.

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Two strategies can be used by researchers to assess precision by approximation.

If a single population is of interest (*intrinsic inference*), means, medians or inter-quartile ranges can be estimated with a required degree of precision which in subsequence determines the sampling effort. If the comparison of two populations is intended (*comparative inference*), the power of the proposed sampling frame^{III} should be assessed in order to detect meaningful differences between the two populations.

2.1.1.2. Sample Size

Statistical calculations on the number of samples required to achieve an expected statistical power at a set significance level^{IV} are commonly conducted in clinical trials, case-control-studies or epidemiological research whenever an endpoint, a bio-medical index (incidence, prevalence, etc) or a relevant difference is known or reasonably defined a priori.

In the context of HBM for comparative exposure assessments such input parameters are rarely available and similar calculation procedures are highly complex and depend on, inter alia the number and selection of biomarkers regarding sensitivity, specificity and practicality, the latter based mainly on the pharmacokinetics of the targeted compound. But also additional features like the variability in exposure pattern of the study population (due to intra-individual and inter-individual variability in pharmacokinetic determinants, such as workload, body build, and metabolic genotype) as well as the distribution and number of confounding factors impair the modelling of valid sample size estimates by increasing uncertainty.

In fact, the estimated number of samples required for a given $(1-\beta) = 0.80$ would depend on the "weakest" biomarker, thus inflating the sample size of the entire HBM study.

This applies in particular to multi-biomarker studies of population-surveys like the US-NHANES (CDC, 2005), the Flemish Biomonitoring Project (TWG/VITO, 2004) and GerES. Accordingly, statements of that kind are scarce or even lacking completely in the reports.

The US National Research Council, Committee on Human Biomonitoring for Environmental Toxicants (NAS, 2006) concluded in its study "that it is critical for biomarker researchers to adhere to appropriate statistical principles when sampling populations to ensure that the biomonitoring results [for all markers investigated] are valid and representative of the sampled population".

^{III} It will be outlined below that such power calculations for population-based multi-biomarker HBM studies without (previous) knowledge of prevalence, incidence, causal relationships or justified relevant differences are quite difficult to impossible to perform.

^{IV} Usual ranges are Power = $(1 - \beta) = 0.80 - 0.99$ and $\alpha = 0.05 - 0.01$.

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2.1.1.3. Randomized Sampling and Recruitment Strategy

A scientifically sound and efficient population sampling procedure will be a multi-step-process. In the following, three major steps will be outlined^V.

It must be stressed that any participation in studies involving humans is strictly voluntary, which implies that the participants' efforts and inconveniences (e.g. travel to the examination centre, blood sampling) should balance with the expected public utility and personal benefit. The use of appropriate incentives or minor monetary compensations proved helpful in maintaining that balance.

A broad communication to all stakeholders and target-group-oriented information campaigns at all stages of the study are essential for the general public acceptance and for an efficient accomplishment of the entire programme.

2.1.1.3.1. Pre-selection

- A. Stratified random **selection of geographical sampling spots**, drawing proportional to frequency of a regional class (classification can be based on community type, population density or environmental and economical indicators)
- B. Contact of accountable registry offices in order to perform **random selections** (of equal number) **of parameter-grouped persons** in order to obtain basic personal data and addresses; over-sampling by 50 - 100 % [*gross sample*] is recommendable to make up for equipollency, non-responders and drop-outs in order to maintain the projected *net sample size*
- C. Transferred registry data make up for a **database using a coding system** to cover sample spot, gender, age (class) and address

2.1.1.3.2. First Contact and Information Campaign

- A. **Mailing of invitations** to participate by providing **all relevant information** about the study in a clear but brief manner (e.g. contracting entity, national institution in charge, contact person/numbers/ address, national web-site/hot-line, scope and intention of study, approval by Ethics Committee/IRB, data protection and participants' consent, methodologies, questionnaires' content and extent, scheduled

^V The sketched sampling strategy offers certain similarities to the German Environmental Surveys (GerES).

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time frame, sequence of activities and locations, conduct of specimen sampling procedures and associated potential risks of invasive sampling, use of data and reporting of results), **including request to return an enclosed form** via prepaid envelope or freepost mode.

Tenor of such reply form: preliminary assessment of interest to participate, correct/current address plus telephone/mobile and favoured time and mode of further contact.

- B. Assisting **information campaign** via broadcast/print media; flyer and poster at public places, referring to websites / telephone hot-lines
- C. **Evaluation of first response**, target-actual-analysis, letter of **thanks for responders** and **notification of preliminary specimen sampling** locations/time frame, **second invitation round to non-responders**, if possible, home visits by trained field workers

2.1.1.3.3. Endorse Contact and Informed Consent^{VI}

- A. Evaluation of overall response, target-actual-analysis, **confirmation letter** for responders with **enclosed informed consent form** in stamp addressed envelope to be signed by participant and returned to National Study Centre. For studies **involving minors**, there is need to **obtain parental consent** prior to the child's participation in the research. The minor may likewise (dependent upon age) be given opportunity to give an assent for participation.
- B. The informed **consent of participants** must address the following **topics**:
 - confirm having received exhaustive information and to decide/act by freewill
 - acknowledge general comprehension of the study, its conduction and ethical/IRB approval
 - confirm understanding the rules of data protection and use of data
 - agree on collection of specified specimen for specified measurements/determinations
 - acknowledge comprehension of potential risks and discomforts in regard to invasive specimen sampling

^{VI} Suitable examples of informational letters, various informed consents and a minor assent can be found at: http://www.clemson.edu/research/orcSite/orcIRB_Consent.htm

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- agree on the [potential] storage and anonymous re-analysis of specimen (caution and full transparency in regard to genetic investigations)
 - confirm understanding the right and mode to withdraw a given consent
 - acknowledge the possibility/ indicate the choice to obtain a summarized or personal report
 - agree on/ indicate choice to be re-contacted in case of follow-up/new programme
- C. Evaluation of returned consents, target-actual-analysis, re-contact and follow-up if needed (visits preferable to mail or telephone contact), generation of **recruited study participants' database**.

2.1.1.3.4. Further Implementation Planning

A detailed planning should focus on efficiency regarding means and resources of the study programme. This embraces the **modus operandi and utilization of questionnaires** (self-administered filling vs. interview-guided by study personal, postal sending/return vs. personal delivery, extent/depth of questions, particularly regarding SES), an optimized **specimen sampling scheme** over a specified period (non-invasive sampling differs from invasive methods, equipment, consumables and logistics, detailed instructions to study subjects and staff, invasive sampling requires defined locations of facilities, periods and persons in charge, assisting measures, etc), and the timely forwarding of all **information and instructions to participants and staff**.

2.1.1.4. Biological Sampling

Biological sampling refers to the collection of suitable specimen(s) from the study participants. The specimen, also denoted as the *matrix* which contains the biomarker of interest, must be well chosen and handled with great care in order to gain meaningful results of the HBM study.

Firstly, by selecting the specimen for a HBM study, the researcher must fully understand the utility and quality of that specimen, as well as its biologic significance and complexity of the matrix. Therefore, specimen selection is often a balance between purely scientific issues and the practicality of obtaining the specimens for a population study in a cost-effective way that is least invasive and risky to the study subjects. However, matrices such as urine or saliva,

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while easy to obtain and non-invasive, are often not suitable for the determination of certain chemicals.

For instance, lipophilic chemicals with long half-lives are usually measured in blood. Since the principal exposure source is usually the diet, they are readily absorbed into the blood supply; blood concentrations then decrease as the blood supply equilibrates with lipid-rich tissues. After this initial equilibration, the concentration measured in a blood sample is related to body burden and should not change substantially in the short term.

The importance of the biologic half-life is illustrated with a hypothetical example in Figure 1. In the example given (☛), the determinant is eliminated with a half-life of ten hours and the biological level mainly reflects the exposure on the day prior to sampling (contribution of 70%); to a relatively small extent, it reflects the exposure during the previous hour and week (contributions of 10% and 20%, respectively).

Such considerations are of importance for example regarding exposure assessments on tobacco smoke: while nicotine has a relatively short half-life of about 2 hours, its primary metabolite cotinine has a half-life of approximately 20 hours. Therefore, cotinine provides a more stable marker of exposure since there is less variability in cotinine throughout the day than that observed for nicotine. Additionally, the renal excretion makes urine a superior specimen compared to blood/serum.

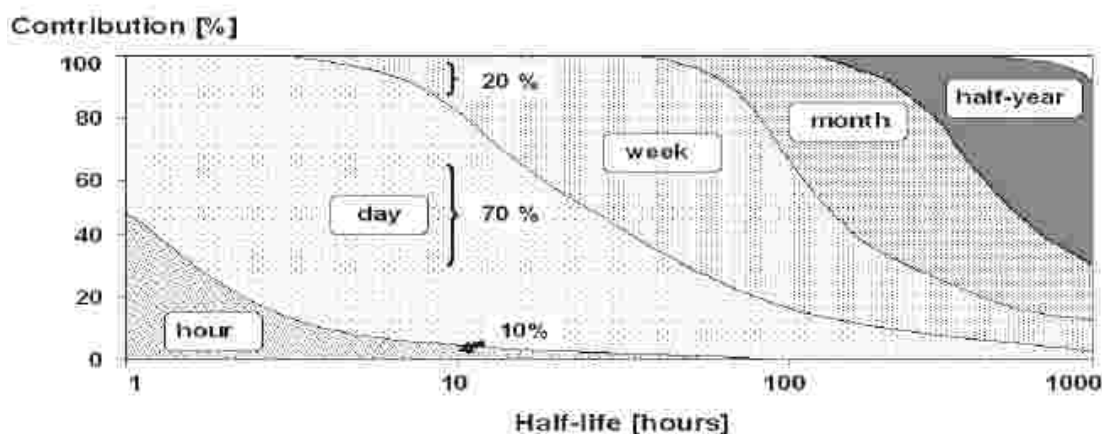


Figure 1. Effect of half-life on contributions of exposures during the last pre-sampling hour, day, week, month, and half-year to biologic levels of determinants. Half-lives were calculated by a one-compartmental model. Source: American Conference of Governmental Industrial Hygienists, 1995

Secondly; an improper collection, transportation, and/or storage of specimens can significantly affect the biomonitoring results (Wax et al., 2000). Biological sampling and further pre-analytical steps must follow validated protocols that are appropriate to the matrix

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and analyte(s) of interest. Most samples require collection into a suitable storage container, clean techniques for handling and storage, chemical or physical stabilization of the sample, storage under appropriate conditions, and control of the number of times a sample is removed from storage and assayed. Failure to follow these steps can produce either degradation or speciation change of -mainly organic- analyte(s) or contamination of the sample from external sources. For example, until the 80ies it was not widely appreciated how easily a sample can become externally contaminated with chromium, principally from stainless steel and other metal components.

Likewise, the collection of blood into a typical *vacutainer* can highly influence the analysis of cadmium, plasticizers, and other compounds that may leach from rubber stoppers, vial seals and walls of containers. Researchers must standardize and report their sample protocols because such exemplified contaminations can be critical in interpreting and comparing results across studies.

For further considerations and recommendations see Deliverable 2.4, *Protocol for harmonised way of collecting and analysing selected pollutants and for data management*.^{VII}

^{VII} Soon available at: http://www.eu-humanbiomonitoring.org/doc/esbio_wp2.4.pdf

2.1.1.4.1. Sampling of Different Types of Specimen

Historically, adipose tissue has been considered the most appropriate matrix for HBM focused on persistent chemicals which tend to bio-accumulate in fatty tissues. Due to their re-mobilization during female lactation, the less-invasive sampling of breast milk gained later acceptance as alternative matrix. In the last 15-20 years, urine and blood have been used more commonly, though nowadays focus lies on non- or minimal-invasive samples such as urine, saliva, sperm, cord blood, exhaled air, hair and fingernails.

In large population-surveillance studies, such as those of the US-NHANES (CDC, 2005), GerES or the Flemish HBM Project (TWG/VITO, 2004) biomonitoring is usually conducted on urine and blood samples.

Urine serves as a route and medium of elimination for many chemicals, especially non-persistent chemicals (chemicals with short biologic half-lives); the persistent chemicals are eliminated primarily in faeces.

For reasons of practicality and study participant convenience, most investigators collect first morning urine samples. It was concluded that mainly for chemicals with short biological half-life, those are generally found in the urine not only as their original "parent" structure but more frequently as metabolites.

On the other hand, utilization of blood permits better comparison of exposed populations with national averages, repeat sampling of persons who have high tissue concentrations, and opportunities to follow chemical clearance with time. In addition, surveys based on blood can also detect important tissue residues of persistent chemicals.

Ultimately, the biochemical and pharmacokinetic properties of a biomarker determine the choice of specimen needed for the study, and since it is not feasible to study a wide array of tissues/body fluids in a general population sample, it is important to identify tissues that most accurately account for the body burden of most of the chemicals of concern. As mentioned above, the choice of matrix is also affected by the invasiveness of collection and whether it is appropriate at a given life stage.

Figure 2 shows the main routes of exposure and the matrices available for analysis of biomarkers based on the metabolism and pattern of bioaccumulation and excretion.

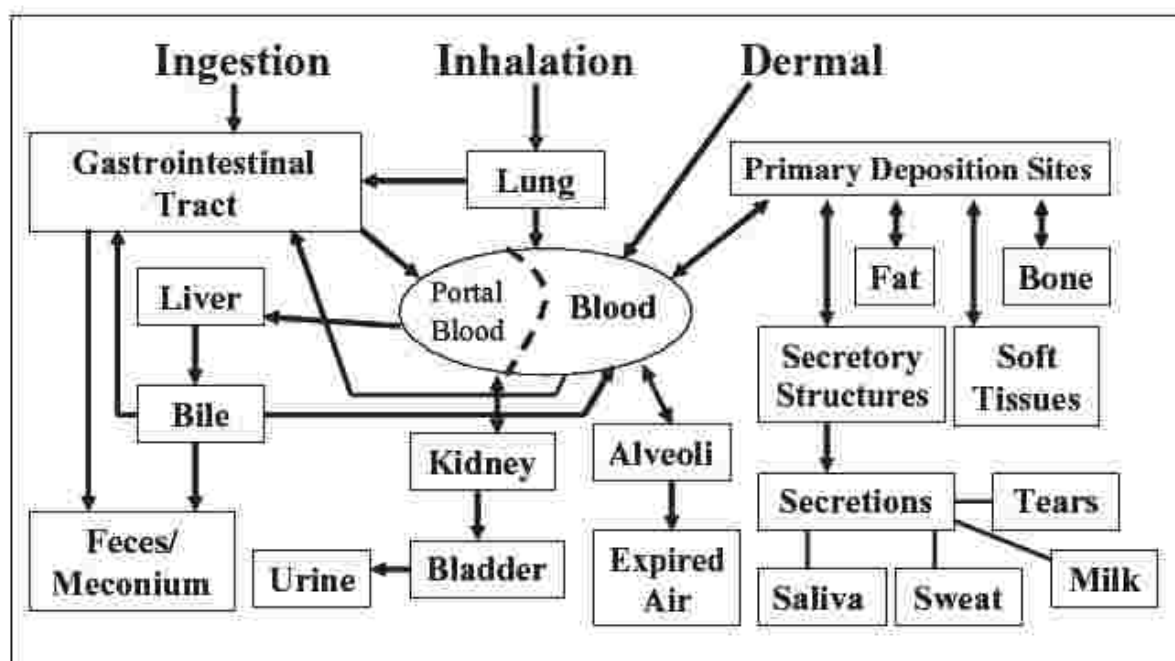


Figure 2 Pharmacokinetics of environmental chemicals in body and what tissues/specimens are available for analyses. (Source: Needham et al., 2005)

Urine is one of the least invasive matrices to collect, but its amount varies widely with age of children and the age-associated difficulties of collection into a vessel. With older children and adults the amount of urine is not a limiting factor, and the main focus in consideration of study design shifts to the timing of collection.

For example, a morning void may be needed to acquire a more concentrated sample for low-level chronic exposures, and it has been concluded that measurements of urinary metabolites (e.g. of organophosphates, pyrethroids, phthalates) in the first morning urine more accurately represent total daily exposure than measurements in spot urine samples collected at other times during the day (Kissel et al., 2005).

Unfortunately, urine is an unregulated body fluid and it varies in volume and concentration of endogenous and exogenous chemicals from void to void.

In order to account for urine dilution, creatinine adjustment of urinary metabolites has been the standard method, although urinary creatinine concentrations vary with age, sex, race and ethnicity and body-mass index, and there are several ways to address this fact at the stage of data processing (Barr et al., 2005).

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Blood offers inherent advantages for HBM studies. Regardless of the route of exposure, a chemical must be absorbed into the bloodstream and circulate to the tissues to have an effect (exceptions are direct inhalation effects on the lung and blistering agents on skin). Blood is also -contrary to urine- a quite regulated matrix, translating to a constant amount of blood for a given body weight, so that measurements can also be normalized to this amount. Blood is routinely collected with *vacutainers* and other suitable containers without anticoagulant or with heparin, EDTA, or citrate, depending on the types of assays expected to be conducted. Serum and plasma are most commonly used for measurements of chemicals in blood. It is important to decide on the type of *vacutainers* to be used and the number and volumes of resulting aliquots that are required for planned analyses without additional freeze-thaw cycles, which may affect sample integrity.

Regarding the required amount of blood samples, for some analyses, such as dioxins, large volumes of blood (70 ml or more) are needed, thus eliminating susceptible subpopulations from the study, such as children and pregnant women. CDC's National Centre for Environmental Health does not permit blood sampling of children less than 6 years old (except to analyze lead, cadmium and mercury in the future) due to the difficulties to collect necessary blood volumes.

Comparably, the Ethics Committee in charge of the GerES considered blood sampling of children between 3 and 6 years of age only justified if a "plain health-related benefit for the sample-donor is evident" and restricted the blood volume to 2 ml.

For persistent organic chemicals, a blood sample can be taken to identify exposures years after such exposures have occurred, but investigators might have no information about the temporal aspects of the exposure.

Sample collection for non-persistent-chemical measurements should reflect the residence time of the chemical in each individual matrix, since the half-lives of non-persistent chemicals are typically much shorter in blood than in urine (Barr et al., 2005).

2.1.1.4.2. Sample Banking

The main goals of a sample bank (or bio bank) are to make future analysis of currently unknown biomarkers possible and to minimize research costs of future studies by using previously collected specimens. As outlined, human specimens -if already fundamentally characterized regarding its anonymous donors and the initial HBM study- might prove too

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valuable to be discharged at the end of a survey. Therefore, systems which can store one or many types of biologic specimens for later analysis from single or multiple studies under conditions which permit efficient retrieval and optimum stability of the sample, sometimes referred as "bio-repositories", gained wider interest and called for international collaboration to ensure standardized procedures.

The International Society for Biological and Environmental Repositories (ISBER, 2005) reflected the collective experience with collection and banking of human samples in a document that contains comprehensive information on the handling of blood, urine, saliva, breast milk, and other tissues. Each sample needs to have a secure chain of custody, processing, location, and temperature-stability records compiled in an accessible location. Intelligent bar-coding and partial to complete automation of a bio-repository will help to address these issues.

The human-specimen repositories vary widely and include commercial banks, national or international project banks, and much smaller bio-repositories associated with scientific laboratories or universities. The latter may have 10,000 samples or fewer; larger bio-repositories, such as CDC, the US National Cancer Institute and the German Human Specimen Bank (HSB, 2007) keep millions of specimen (Goodman et al., 2006).

3. The European Human Biomonitoring Pilot Study

In 2004 the European Commission launched the Environment and Health Action Plan, covering the period 2004-2010 (EHAP^{VIII}), which served also as the Commission's contribution to the Fourth Ministerial Conference^{IX} on Environment and Health, organised by the WHO in Budapest in June 2004.

For the preparation of EHAP several scientific groups had been assembled in order to counsel the Commission, mainly the "Consultative Group on Environment and Health" and three Technical Working Groups (TWG) further divided in several sub-groups.

Out of the four Technical Working Sub-Groups which focussed on *Integrated Monitoring*, the group "Biomonitoring of Children"^X elaborated several recommended options^{XI}.

Under the caption "**Explore Possibilities to Develop an EU Biomonitoring System** with focus on children" it was noted, *inter alia*

- Develop **guidelines** for a harmonised EU approach for biomonitoring of children, starting from existing experiences and expertise
- Conduct a EU wide **pilot study** to test and validate common harmonised approaches
- Develop tools for **translation of results in to a policy response**. This requires integration of biological monitoring data with environmental monitoring, the development of (child specific) reference values and action levels and appropriate communication strategies
- Set up a selection of clear aims for biomonitoring focussed on children
- Collect information to establish European database on biomonitoring activities

This conceptual spadework was widely appreciated and incorporated into the framework of EHAP which outlines three key elements, whereby the first element reads: "*improving the information chain by developing integrated environment and health information*".

In order "*to understand the links between sources of pollution and health effects*", Action 3 of the Plan reads: "*Develop a coherent approach to Biomonitoring in Europe*".

^{VIII} See: <http://ec.europa.eu/environment/health/pdf/com2004416.pdf> ("The European Environment & Health Action Plan 2004-2010", COM(2004)416 final)

^{IX} See: <http://www.euro.who.int/budapest2004> (Fourth Ministerial Conference on Environment and Health "The future for our children")

^X See: http://ec.europa.eu/environment/health/pdf/twg_biomonitoring.pdf (The Group consisted of HBM experts from several Member States and Croatia. This TWG has been expanded to include 18 Member States and is now called the Implementation Group (IG) on Human Biomonitoring)

Find web-link to the TWG baseline report under TWG/VITO (2004) at Chapter 4. References.

^{XI} See: http://ec.europa.eu/environment/health/pdf/merging_options.pdf

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The Commission's EHAP received common acceptance and consequently triggered a broad commitment of participants attending the WHO Conference which culminated in the "Declaration of Budapest"^{XII} and adopting the "Children's Environment and Health Action Plan for Europe" (CEHAPE)^{XIII}.

These developments gave the Commission's plan on Human Biomonitoring a fresh impetus and reinforced the main emphasis on children's health.

Within FP6 of the European Commission (Directorate-General Research, in close cooperation with Directorate-General Environment) the ESBIO project was launched in October 2005. The project team consists of nearly all members of the IG on Human Biomonitoring and is dedicated to work on the conceptual framework and technical preparations of the European HBM Project.

The following chapters will cover the proposed study design, the targeted population, the envisaged number of participants and how they can be recruited.

Annex 2 provides further information on this decision-making process, which started from a broad array of discussed options and by following decision-trees and finally redounded to the topics proposed.

Some examples and alternative options will be presented casually, if appropriate.

Regarding the practical implementation in a Pilot Study - to test out the developed coordinated approaches - a call has been published within the EU 7th Research Framework Programme (ENV.2007.1.2.2.1. "European Network on Human Biomonitoring").

^{XII} See: <http://www.euro.who.int/document/e83335.pdf> (Declaration of Budapest)

^{XIII} See: <http://www.euro.who.int/document/e83338.pdf> (CEHAPE, feat. Regional Priority Goal IV)

3.1. Objectives of the Pilot Study

As presented in chapter 2.1, the definition of objective(s) is the first step in the set-up of any epidemiological study since it has an impact on the study design and its various elements.

The main objective of the Pilot Study was basically preset by the ESBIO-task and reads:

"To develop and to test a coherent and harmonized approach throughout Europe by means of commonly developed strategies, guidelines, protocols and scientific tools ensuring reliable and comparable data, whilst also leading to a more effective use of resources involved."

This implies a focus on the organisational, technical, logistical and infrastructural feasibility.

Further specific objectives derived from the main goal are presented in Deliverable 2.1,

Proposal for Objectives of EU HBM Approach and of the EU Pilot Project^{XIV}.

A secondary - more scientific - objective was defined by the IG with assistance of ESBIO:

"To obtain preliminary^{XV} reference values^{XVI} of selected biomarkers from all participating Member States" (IG, 2006) which shall allow for evaluation and comparison.

Both objectives make an impact on the population sampling scheme.

As an example, the number of samples required for the objective "feasibility" might be small and set at will while the objective "reference values" requires the consideration of statistical demands (Poulsen et al., 1997).

The following Table 1. shall exemplify the impact of different study objectives on the general design and some study-related consequences.

^{XIV} See: http://www.eu-humanbiomonitoring.org/doc/esbio_wp2.1.pdf (Specific objectives, page 10)

^{XV} In view of the scope of the Pilot Study the foreseeable number of samples may lack the required representativeness so that the data obtained might not fulfil the scientific criteria for a "reference" base.

^{XVI} Reference values are statistically derived values of the 95% confidence intervals of estimated 95th population percentiles and indicate the upper margin of background exposure to a given pollutant in a given (sub-) population at a given time. Recommendations on standardized biological reference values have been published by the International Organization for Standardization (ISO) and the International Federation of Clinical Chemistry (IFCC). Reference values can be used to identify subjects with elevated levels of exposure. However, they do not represent health-related criteria for the evaluation of HBM data (Ewers et al, Int Arch Occup Environ Health (1999) 72: 255-260).

D2.3.1 Guidelines for Population Sampling, Recruitment and Biological Sampling

<p>Objective # impact on: hypothesis</p> <p>Effect on:</p>	<p>1. To test the developed guidelines and procedures for field work, questionnaires, chemical analyses, data handling and processing. <i>Includes:</i> To test ethical guidelines and communication strategies, gaining experience on legal and infra-structural aspects of participating MS. (Assessment of operational structuring of harmonized EU HBM-Project)</p>	<p>2. To establish national reference values that can be used for comparisons among MS and to identify people with elevated exposure levels. (Assessment of EU background exposure)</p>	<p>3. To obtain representative data for specific areas with expected different types of environmental loads (urban areas, dense traffic, industry, intensive agric.) (Assessment of EU "hot spot" exposure)</p>
<p>Sampling Scheme</p>	<p>random or convenient</p>	<p>randomized, representative to target population (age, gender, SES, region, etc)</p>	<p>randomized, representative to regional class and target population</p>
<p>N/MS (min.)</p>	<p>fixed number (e.g. 50) or fixed ratio (e.g. 1: 100.000)</p>	<p>fixed ratio according to proportion of target population (sub-groups' weight)</p>	<p>fixed ratio according to proportion of population and regional class (areas' weight)</p>
<p>Remarks to N</p>	<p>fixed ratio results in disproportionate means and efforts for individual MS</p>	<p>See 1.; adjustment to minimum number per MS (e.g. 120 subjects per sub-group)</p>	<p>See 2.; requires prior national classification and larger number of samples as 1. + 2.</p>
<p>Consequences on Study Results</p>	<p>very limited information regarding Environ. & Health, exposure levels, pathways and spatial distribution</p>	<p>net sample size and biomarkers' variances influence validity of reference ranges</p>	<p>basic biomarkers not suitable to evaluate the different types of environments</p>
<p>Consequences on financial and organizational aspects</p>	<p>least expansive / laborious for convenient sampling</p>	<p>substantial efforts for randomized sampling and exclusion of the specifically exposed (e.g. smokers)</p>	<p>elevated initial efforts for EU & MS study management</p>

Table 1. Objectives of HBM-Pilot-Project vs. Study Design and related consequences.
 General Assumptions: Study population = mothers and their biological children
Biomarkers and Specimen = as proposed for Scenario 1

3.2. Proposed Study Design

The EU Pilot Project aims at developing a framework of environmental health surveillance and is therefore designed as a cross sectional study.

As outlined in chapter 2.1. et seq. and summarized in Annex 2, many aspects need to be considered and procedures adhered strictly to, in order to approximate an **ideal** study (design/performance/outcome).

It is obvious that such demand does require enormous means and efforts, bearing in mind the vast differences in size, population, structure, legislation, capacity and -not at last- experience in HBM surveys among MS.

Therefore, regarding the character and objective of the Pilot Project many "best possible" approaches are neither needful nor feasible, and several scientific as political ambitions have to be cut back.

The following chapters will address several issues concerning the IDEAL and the FEASIBILITY of a Pilot Project.

3.2.1 Proposed Study Population

The proposed study population consists of two sub-groups of priority regarding precaution and future assets:

Children aged 6-11 years and their related Mothers, probably aged 22-50 years.

The rationale for suggesting these one-to-one dependent samples links to the EHAP respectively CEHAPE, throwing a focus on the most vulnerable groups of the European population.

Among those children rank first, particularly due to their developmental vulnerability to chemical agents, their dependency on a given environment and their potentially elevated portion of total DALYs^{XVII}.

Nearly equally ranked are women of childbearing age as the developing foetus is potentially exposed to numerous agents by mother-child-transfer. Though not all biomarkers proposed are of concern regarding a pre-natal mother to child transfer, some of the listed pollutants (organic mercury, lead, phthalates, etc) bear health relevance for the future offspring.

^{XVII} DALY: "disability/disease-adjusted life years", a tool for rationalizing the measurement of disease burden, evaluating interventions and comparing public health care programs.

D2.3.1 Guidelines for Population Sampling, Recruitment and Biological Sampling

The study population of the Pilot Project will therefore reflect the EHAP/CEHAPE emphasis; hence children are going to represent the prime target group.

In order to gain more easy access to and acceptability for future surveys on children and to cope with the preference of several European MS for a wider view and an approach addressing the whole population, it is recommendable to include the children's respective biological mothers (regarded as being in reproductive age) into the scope of the Pilot Study.

Sampling women of childbearing age serves additional purposes, too.

Measuring biomarker levels in women enables extrapolations on the respective exposures of their (potential) infants and toddlers which are particularly vulnerable but beyond the scope of the Pilot Project.

Inclusion of mothers provides insight into exposure levels of a population group which is usually more susceptible to preventive measures, and moreover their inclusion facilitates awareness raising in young households.

A rather practical aspect relates to the fact that the accompanying questionnaires on exposure and socio-economic status (SES) have to be filled anyway by the parent/mother.

The recommendation to define the children's age group ranging 6-11 years originates from practical/ethical considerations in regard to sampling of younger children, but also includes scientific aspects such as to limit intra-group variability of values and to enable a direct comparison with data of the US-NHANES Report (CDC, 2005).

The biological mothers within the probable age range of 22-45 years do represent the second study sub-population and will provide values that can be compared with the NHANES's female gender and age group "20 years and older".

3.2.2. Sampling Frame

As outlined in chapter 2.1.1. et sqq., a randomized sampling of the target (sub-) populations - according to SES and other related demographic factors as well as to geographical, economical and environmental variations of each individual MS - would be the ideal manner. In fact, such approach implies vast preparatory works and resources just on this element for the majority of MS.

In consequence, a simpler sampling frame and scheme must be offered as a reasonable alternative for a start of the temporary Pilot Study.

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It is suggested to develop a trifocal sampling and recruitment pattern according to areal classifications^{XVIII} as a minimum.

Such classifications could evolve into

> urban - rural - industrial <

respectively

> industrial - agricultural - natural <

Classifications shall somewhat reflect the dominant characteristics of a country, but requires prior definitions by consultations of participating MS to meet best harmonization.

Surely, this minimum procedure deviates from the *ideal* towards the *convenience sampling*^{XIX} and should hence be extended in representativeness as much as existing structures and capacities of participating Member States allow for.

Within the 3rd IG Recommendations (IG, 2006) it was stressed that sampling and recruitment should be embedded in already existing health care systems, such as medical examinations at schools or children's health monitoring programmes. This will surely reduce costs and efforts, could make HBM part of already existing health care structures and should eventually allow to collect additional health information which might be important for linking the HBM results to health. These desired synergies and operational efficiencies are likewise connected to have an effect on the sampling grade, though a proper pre-selection of recruitment areas on national level helps to curtail an immoderate sampling bias.

On the other hand, similar basic sampling strategies have proved satisfactory for other EU FP5/6 projects on environmental epidemiology^{XX}.

Ultimately, such approaches only call for full transparency at all stages of communication in order to assess the various limitations and consequently the validity of study results.

^{XVIII} It was proposed that the sampling areas should allow geographical comparability with collection of other regional indicators in the EU in the future, and therefore be based preferentially on the NUTS classification which could enable efficient linkage with other environment and health indicators that are already gathered by the EEA, EuroStat, and other relevant bodies. Such matching with NUTS classifications could also be performed a posteriori. A rationale for NUTS can be found in Deliverable 3.1

(http://www.eu-humanbiomonitoring.org/doc/esbio_del_wp3.pdf); more details about NUTS are available at http://ec.europa.eu/comm/eurostat/ramon/nuts/home_regions_en.html

^{XIX} See chapter 2.1.1.1.

^{XX} See: PHIME (<http://www.phime.org/>), PINCHE (<http://www.pinche.hvdgm.nl/>) or CASCADE (<http://www.cascadenet.org/>)

3.2.3. Sample Size

Associated considerations characterized lengthy discussions on the number of samples to be collected at MS level.

The design of a cross sectional study would demand for an equivalent number of participants per country in relation to the total number of the target population in each MS. However, due to the acknowledged vast differences in national population size, it was concluded that a fixed number of samples per MS^{xxi} can serve the purpose for a start.

The number of participants should just be sufficiently large to allow (minimal) statistical evaluations of pollutants' reference values. In this context the International Federation of Clinical Chemistry (IFCC), endorsed by IUPAC/Clinical Chemistry Division, recommends measuring the values of at least 120 individuals per group for the determination of reference values (Poulsen et al, 1997).

This translates to a minimum number of 240 participants (120 mother-child samples) per Member State in order to approximate the scientific objective of establishing reference values for Scenario 1 biomarkers ("*research element*").

Due to this minimal setting and the requested reduction in sample size by some least populated MS, it seems justified to instead refer to *preliminary* reference values.

As outlined in chapter 2.1.1.2. on the matter of variability in exposure and pharmacokinetics within the study population and in relation to analytical variance of individual biomarkers, the required number of samples for statistically significant comparative inference analyses among different national (sub-) populations depends on the differences of the geometric means and their associated standard deviations (SD).

This multi-factorial interdependence can be alternatively used to evaluate the statistical power of comparative inference analyses in relation to the finally obtained number of samples and the analytical performance at the end of the study.

Annex 1 depicts more details by exemplified values for Pb-B of children and Cd-U of women on basis of German Survey data.

^{xxi} Deviation of the "fixed number approach" was proposed by applying a population-stratified sampling scheme for MS based on the NUTS 2 level. Such approach would range from 25 (min) to 250 (max) mother-child samples. See: http://www.eu-humanbiomonitoring.org/doc/esbio_del_wp3.pdf

3.2.4. Population Sampling and Recruitment Strategy

Following the above considerations, the definition of minimal standards/procedures in sampling and recruitment represents the major challenge. Wherever possible, conceptual improvements (e.g. of the trifocal approach, the number of samples) towards a more sophisticated design on national level are encouraged but can't be demanded or expected a priori.

In order to reduce - respectively to enable the identification of - a major selection bias, the pre-selection of the later sampling and recruitment areas (e.g. regions and communities and possible primary schools, health centres or related facilities located therein) must be performed by the National Study Centre. An accurate and comprehensive documentation of planning and progress at all levels is essential, whereupon prompt communication to the Central Study Centre is highly recommended. Only extensive communication can enable the Central Study Centre to advice and efficiently support the National Centres, as well as to evaluate the utilization and comparability of national data delivered finally.

The first step to recruitment is gaining access to pre-selected study participants by appropriate means, depending on the law, registration systems and common practice of MS.

If a sophisticated strategy as outlined in chapter 2.1.1.3. et sqq. is deemed infeasible, it is proposed to access the study population via two possible basic paths.

A.) One option is to approach the mother directly via school authorities, family doctors/ paediatricians, or national health care system in form of verbal or written information (depending on national registration systems and laws on protection of data privacy).

B.) The alternative option is to approach the mother indirectly through the child first; children can be accessed via day care facilities, sports clubs, and the educational system (primary or secondary school), respectively medical examinations at school if existing. The children are supposed to forward the written information about the intended HBM-programme to their mothers/ parents.

The ultimate goal is to gain the targeted number of interested and committed participants which match the pre-set characteristics and do comply with the period and all embedded elements of the study.

3.2.4.1. Communication and Information Campaign

It must be stressed again, that HBM can only take place on a strictly voluntary basis, and therefore it remains the most challenging task to raise peoples' interest and wish to participate in such a study.

Contrary to medical/therapeutic/intervention studies where participation is predominantly driven by personal health/illness-related motives^{XXII} and response rates are usually high, the psychological conditions in HBM studies - targeting largely healthy people - are quite different.

Commonly HBM-Study associated questions of an adult potential participant are:

- A. What do I gain from participation, what are my personal benefits?
- B. What justifies my foreseeable discomfort (blood sampling) and efforts (filling the questionnaires)?
- C. What guarantee on data protection and strict avoidance of misusing my personal data is given? Can I trust such guarantee?
- D. Can I be assured that neither genetic investigations nor drug-screenings^{XXIII} are performed without my knowledge and consent? What about my bio-banked specimen?
- E. Can I expect efficient assistance and professional guidance in case my values cause serious concern? Will I be informed at all?
- F. Do I have the choice NOT to know any results?

Though all these participation-related concerns must be adequately addressed by the information campaign, only C. - F. can be covered and legally regulated by the informed consent, unlike the individuals' major motivational aspects (A. and B.).

The latter might even be negatively amplified when children shall be sampled, which often show profound aversion to (invasive) medical techniques.

Although averseness to surgeries and hospitals might not be overcome entirely, it proved helpful to embed specimen sampling into a basic medical-physical examination with predominant non-invasive methods (e.g. determination of body weight and height, blood pressure, eyesight, orthopaedic check-up, etc).

^{XXII} Monetary motives are most apparently found in pharmaceutical studies of phase II or unethical settings exploiting poverty and dire straits of humans.

^{XXIII} During the ESBIO workshop in Copenhagen, 11-13 March 2007, several researchers reported that private matters such as smoking habits and consumption of alcohol/drugs do effect the compliance of participants as well as the validity of questionnaire data regarding these issues.

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Such medical examinations in varying extent are often part of large-scale population surveys and allow -beside an assessment of the individuals' health status- for an enhanced acceptance and participation rate.

Comparably, minor incentives (for children) and reasonable monetary compensations (for adults) regarding travel expenses and expenditure of time, respectively, can serve the same purpose.

Therefore, these measures are recommended for the Pilot Study and in addition facilitate the approach to include the biological mothers.

This matter must be practically considered within the planning of the HBM Study and requires to be appropriately reflected by the entire information campaign.

4.2.4.2. Informed Consent and Minors Assent

The legal imperative to obtain an informed consent of the participant is presented in chapter 2.1.1.3.3. and practical implications and recommendations concerning parent's consent and/or minors assent for the EU Pilot Study are elaborated by ESBIO WP 4^{XXIV}.

Regarding the content of the consent form which must reflect the associated information campaign of the Pilot Study it is recommended to:

- A. include choice of participant to receive individual or aggregated or no results
- B. include choice to be contacted in case of future studies
- C. include long-term storage to make re-use of sampled -made anonymous- specimen
- D. **explicitly exclude** any genetic determinations and other measurements than intended/listed for the study

4.2.5. Biological Sampling

General aspects regarding specimen sampling are already addressed in chapter 2.1.1.4., while particular ones regarding the Pilot Study are content of Deliverable 2.4 (Protocol for harmonised way of collecting and analysing selected pollutants and for data management, Chapter 3. et sqq. "Sampling") and the rationale for specimens proposed are part of Del. 2.2 (Proposal for pollutants /biomarkers in regard to EU Human Biomonitoring Project).

^{XXIV} ESBIO WPs (<http://www.eu-humanbiomonitoring.org/sub/esbio/wp.htm>); see also footnotes II and VII.

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During the recent years there has been a growing focus on human specimen that can be obtained by least- to none-invasive sampling methods.

Such focus is contributed to minimize the subjective discomfort as the objective medical risk for study participants alike. The work of IG/ESBIO does reflect this emphasis as much as reasonable and scientifically justifiable, respectively.

In order to minimize a seasonal bias, sampling during one and the same season in all Member States is recommended for the Pilot Study.

3.2.5.1. Proposed Specimen

Which kind of specimen to use mainly depends on the nature of the targeted biomarker.

In other words: the specimen must represent a suitable matrix for the determination of a selected biomarker, because -unfortunately- there is no single specimen having such general suitability.

The 3rd IG Recommendation (IG, 2006) differentiated among two groups of biomarkers (and phrased it Scenario 1 and 2), whereby Scenario 1 contains chemicals which are supposed to be measured obligatory in all MS, and Scenario 2 proposed a number of facultative biomarkers which shall be measured additionally according to national preferences.

Basically, a total of three kinds of proposed specimen (urine, blood and hair) will enable to measure all biomarkers (of both scenarios) suggested.

In general with regard to the type of specimen material, urine is regarded superior in ranking to blood (invasive sampling, resulting in elevated ethical concerns regarding children, and associated limitations in sample volume) and to hair (limited suitability to reflect the exposure to chemicals with convincing accuracy/reproducibility).

Urine is the matrix of choice whenever chemicals are subjected to more rapid metabolism ("clearing") to water-soluble compounds and subsequent renal excretion (e.g. phthalates, polar pesticides, low molecular PAHs, nicotine). But also most ions of heavy metals can be detected in urine, being principally a good indicator for long-term, chronic exposures.

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Blood is required for measurements of lipophilic, persisting, bio-accumulating pollutants (e.g. PCBs, highly chlorinated pesticides, poly brominated/fluorinated chemicals) and for a more accurate determination of exposure to lead, which is predominantly bound to the membranes of erythrocytes.

Scalp hair is commonly used in epidemiological studies as a minimal-invasive specimen of choice for assessing the long-term exposure to mercury. It is widely acknowledged that organic species account for more than 80% of the total mercury detectable in hair. Under normal conditions -in the absence of acute exposure- hair mercury concentration reflects the concentration in blood.

3.2.5.2. Specimen Sampling

A. Urine sampling is non-invasive and samples of abundant volume are relatively easy to collect. Ideally, all urine produced during a defined period of time (for example 24h) should be collected. For practical reasons, morning urine shall be sampled in the EU Pilot Study. It is recommended that suitable vessels (usual 1 l wide-neck polyethylene flasks) for urine collection will be provided in advance by the national Study Centre, and that adults and children shall perform the sampling independently and with parental help if necessary, respectively. Therefore the participants will receive a detailed written instruction how to handle the vessels and how to perform the sampling. For the convenience of participants it was suggested to make arrangements for some kind of pick-up-service of urinary samples the same day. If such service proves feasible, the sampling day can be specified according to the pick-up schedule and will be part of the instructions to the participants.

B. Blood sampling is surely invasive but at the same time of some importance for the success of the Pilot Study. Particularly regarding children, it has to be verified prior to sampling that the parental consent (possibly the child's assent, too) has been obtained and is still valid. If consent is missing or revoked, no sample can be taken. The situation during the sampling process has to be monitored likewise. If a participant shows a negative attitude towards the procedure suggesting that he or she disagrees with its conduction, sampling must be cancelled right away.

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Therefore, efforts will be necessary to motivate the participants to consent to blood sampling. In order to facilitate this procedure the application of a topical anaesthetic (e.g. EMLA-plaster) should be offered to children and adults alike.

Such remedy does numb the skin and prevents or eases the pain caused by needle insertion. However, blood sampling requires authorized personnel and an appropriate facility, which means that participants need to visit a designated location.

The recommendation to link blood sampling with some form of basic medical-physical examination at an appropriate health facility has been rationalized in chapter 3.2.4.1..

C. Scalp hair is easy to collect by a minimal-invasive mean and does neither require special equipment nor conditions for transport and storage. Additionally, sample preparation for spectroscopic determination of mercury is simple.

But to prevent exogenous contamination of the hair sample, scissors made of titanium nitride should be used and the sampling itself performed by trained staff.

The procedure can be performed within the arrangements necessary for blood sampling, or be part of a home visit, respectively of the suggested urinary sample pick-up service.

3.2.6 Potential Banking of Specimen

As stressed in chapter 2.1.1.4.2. and bearing in mind the amount of effort required to collect human specimen for environmental exposure assessment, it is obvious that these samples are precious and represent a valuable scientific resource. Regarding the Pilot Study it would be a thoughtless waste to dispose unused and remaining specimen material of expedient amount, which at individual level will likely concern urinary samples only.

In respect of the volume-restricted blood samples it is suggested to pool small aliquots of individual specimen according to groups/classes (e.g. age, gender, geographic/national level). These long-term storage samples will enable further research such as retrospective and sentinel investigations (e.g. screening and target analyses in the context of REACH^{XXV} processes) or re-analysis by means of advanced methodologies in the future.

^{XXV} The operative implementation of REACH demands for an efficient integration of evaluating and monitoring tools of national and European Authorities. See: REACH Regulation at http://reach.jrc.it/legislation_en.htm , RIP 4 - Guidance Documents: Development of guidance documents for authorities (<http://ecb.jrc.it/reach/rip/>) and "Guidance on Dossier and Substance Evaluation" (http://reach.jrc.it/docs/guidance_document/evaluation_en.pdf)

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In order to fathom the scientific prospects it is needed to assess available resources and to initiate a framework to harmonize protocols and standard operational procedures on storage and retrieval of human specimen.

Close linkage of projects and initiatives to integrate bio-banking throughout Europe could enable to identify potential facilities suitable for the scope of environmental exposure assessments.

However, financial constraints might hamper the desirable long-term storage of human specimen collected within the HBM Pilot Study.

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5. Annex 1

Sample Size Estimates and expected effects

(prepared by Margarete Seiwert)

From the questionnaires can be drawn which participants are exposed and their main exposure factors, e. g. smoking status, fish consumption, gender and age.

A significant difference in Cd-U between non-smokers and smokers is expected.

It will be tested whether the GMs for the two groups differ significantly.

For Hg-H differences of the GM between women with frequent and rare fish consumption will be tested.

And a significance test can be applied to discover significant differences of GMs among different MS.

The distributions of all pollutants' concentrations are log-normal. The geometric mean (GM) is the appropriate measure of central tendency and is commonly used instead of the arithmetic mean (AM). Accordingly, t-tests are performed with the log-transformed data.

Sample size calculations are based on the difference of the AMs of the log-transformed data.

Finally, the group AMs of the log-transformed data are re-transformed into GMs.

The standard deviations (SD) of the log-transformed data can be transformed to GSD. These facts have to be taken into account when estimating the necessary minimum sample sizes.

All these comparisons can only yield significant results ($\alpha = 5\%$) with sufficient power ($1-\beta = 80\%$), if the sample size is large enough. A smaller sample is sufficient, if a large effect of the exposure factor is expected, i.e. the difference between the means is large.

In addition, the minimum sample size depends on the variation of the data (here SD of log-transformed data).

In order to depict these complex calculations, two examples for sample size estimates are presented overleaf: Pb-B with a low variation and Cd-U with a larger one.

The following table gives some minimum sample sizes and difference between GMs necessary for significant result with $\alpha = 5\%$ and $1-\beta = 80\%$.

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	Pb-B, children, 6-11y.		Cd-U, women, 20-50 y.	
GM-difference of the two groups	min sample size of one group	min total sample size	min sample size of one group	min total sample size
50%	22	44	65	130
40%	31	62	94	188
30%	51	102	154	308
20%	105	210	319	638
10%	384	768	1167	2334
28%	58	116		
53%			59	118

Assumptions:

log-normal distribution; comparison of GM, t-test for log-transformed values; 2 groups of equal size with equal standard deviation; two-sided test; common alpha = 5%; 1-β = 80%

Estimates based on data from German Environmental Surveys:

Pb-B: GerES VI (2003/06); children 6-11 years (unpublished); GM=16.4 µg/l; GSD=1.6

Cd-U: GerES III (1998); women; 20-50 years (unpublished); GM= 0.212 µg/l; GSD=2.27

Computed with: <http://home.clara.net/sisa/samsize.htm>

If a large effect of the exposure factor is expected, e.g. that GM of Cd-U is on average 50% higher in smoking than in non-smoking women, a sample of 130 females is sufficient to get a significant result.

If the Pb-B levels of MS_x children are 10% different to the German children participating in this study, this GM-difference can only be confirmed by t-test with a sample of at least 384 children from each country.

6. Annex 2

Outline of the ESBIO/IG decision-making process, illustrating the two major steps applied in order to select and propose the study design related issues. The final steps covered reasonable objectives versus feasibility features of a Pilot Study (see: Table 1, page 21).

First Step:

Sampling and classification of issues to be considered

- 1. Study Population**
- 2. Study Design**
- 3. Selection of Participants, Sample**
- 4. Recruitment**
- 5. Field Work**
- 6. Questionnaires**
- 7. Sampling**
- 8. Quality Control**

1. Study Population

- Choice of member states
- Choice of population segment regarding exposure
- Choice of population segment regarding age
- Choice of vulnerable population segment
- Exclusion criteria
- Miscellaneous (families, tissues)

2. Study Design

- Type of study
- Depth of evaluation
- N of participants per MS
- Management

3. Selection of Participants, Sample

- Type of sample
- Choice of sampling locations
- Self-selection of participants?
- Choice within population data bases
- Choice within educational system
- Choice within medical system
- Choice within specific groups
- Miscellaneous

4. Recruitment

- Information / invitation
- Consent
- Compensation for time / money spent

5. Field Work

- Personnel
- Avoiding seasonal bias
- Place of examination and interview
- Miscellaneous (way of contact, incentives, reporting of results)

6. Questionnaires

- Way of questioning
- Necessary questionnaires
- Amount of information on sources of exposure
- Additional topics of questionnaire
- Miscellaneous

7. Sampling

- Individual vs. pooled samples

8. Quality Control

- Internal quality control
- External quality control
- Control of field work
- Control of institutions in MS

Second Step:

Advantages and disadvantages of the alternative ways to perform the study

DESIGN: 1. Study Population				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
Choice of member states	▶ All member states	Full picture of EU	High costs	
	▶ Only member states without established HBM-programme	Less expensive, development of resources and know-how in these countries	Existing programmes have to be evaluated according to objectives and quality criteria, all institutions have to be trained	Quality criteria (different years of study, compliance of the population, analytics, data handling) must be defined
	▶ Only one member state out of each group of member states	Less expensive	Definition of groups	Definitions of groups: regional (East, West, North, South), level of exposure
	▶ Only member states providing financial resources	Highly motivated countries	Not representative for whole EU	
	▶ Only member states experienced in HBM	Less expensive, institutions with expertise	Other states cannot develop resources and know-how, not representative for whole EU	

DESIGN: 1. Study Population				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
Choice of population segment regarding exposure	▶ "hot spots"	Smaller sample size; comparison before vs. after specific measure to reduce exposure	Reference values for population can not be derived	
	▶ General population, but only background exposure	Reference values for population can be derived	People with extraordinary or specific exposure have to be excluded	High exposure groups can be identified in subsequent surveys
	▶ General population	Full picture of exposure in population; reference values for population can be derived	Larger sample size is needed	High exposure groups can be identified
Choice of population segment regarding age	▶ All age groups	Full picture	Higher costs, sample size	
	▶ Children only, restricted to specific age groups	Sample size can be reduced according to age groups, assessment of exposure in vulnerable phases	Blood not easy to sample, urine sampling difficult under the age of three; ethical considerations; consent by parents	
	▶ Children <u>and</u> adolescents	Full picture of childhood and youth	Children's blood not easy to sample, low response rate of adolescents	
	▶ Adults	Blood easy to sample; fewer ethical considerations necessary; consent by participant him-/herself	No focus on "vulnerable groups"	

DESIGN: 1. Study Population				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
Choice of vulnerable population segment	▶ Newborns	vulnerable phases; repeated examination useful	Small sample volume, sample collection difficult; ethical questions; consent of parents	Communication of benefits Breast milk can serve as an alternative specimen to indicate child exposure, interpretation of HBM data
	▶ Babies			
	▶ Toddlers			
	▶ Preschool children	as above	Small sample volume; ethical questions; consent of parents	
	▶ Schoolchildren	easy to sample	Small sample volume; ethical questions; consent of parents	Assent of children
	▶ Mothers, pregnant women	trans-placental exposure; repeated examination useful	Recruitment	Examination of child
	▶ Women in childbearing age	Potential pathway mother/child		Consider recommendations with regard to diet, smoking etc
	▶ Seniors	Accumulation of accumulating and persistent pollutants	Vulnerable?	Influence of morbidity factors

DESIGN: 1. Study Population				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
Exclusion criteria	▶ Healthy participants only	Ability to participate	picture of the general population incomplete	Definition of "healthy"? Potential participants with e.g. HIV and hepatitis must be excluded if body fluids are sampled.
	▶ Exclusion of immigrants	Less expensive, no language barrier	No complete picture of the population of MS. Input for translations of written field material Native speaker as interviewers	Definition of "Immigrant"? Partly problems with accessibility.
	▶ Exclusion of people living in hospitals, homes, homeless	Less expensive	More non-responders, homeless not accessible. No complete picture of the general population	
	▶ People with a minimum time of living at the same place	No imported exposure	No complete picture of the general population	Definition of "minimum time"? Most commonly 5 years
Miscellaneous	▶ Families	Comparison of exposure between the family members, generations	Consent of several family members is needed	
	▶ Tissues from dead bodies		For sampling organs or fat tissue only	

DESIGN: 2. Study Design				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
Type of study	▶ Cross-sectional study, nation-wide, approaching representativeness	Complete picture of one point in time	One point in time and correlations only	Development of exposure can be observed by comparing data of two representative cross-sectional studies; sample size determines validity
	▶ Cross-sectional study with optional longitudinal components for sub-samples	Less expensive than a classical longitudinal study; individual changes; leaves option for future examinations	longitudinal components only for smaller sub-samples, efforts to avoid loss of participants	
	▶ Longitudinal design, cohort study	Observation of exposure trends (also at individual level)	Higher organisational complexity; longer time until all necessary information is collected; efforts to avoid loss of participants; not suitable for the pilot project	efforts to keep participants in study; mobile participants

DESIGN: 2. Study Design				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
Depth of evaluation	▶ Basic programme	Less expensive; suited to round-robin-test	Limited information	
	▶ Extended programme, - array of chemicals increased or - more complex study design.	More information	Higher costs, more complex to organize	Increasing problems with decisions on priorities, effect on level of harmonization
	▶ Information on non-responders	Possibility to tests for selection bias	Only very limited information can be collected.	
N of participants per MS	▶ Fixed number of participants per MS	Equal burden for every MS	Not according to total population	Might be sufficient for a pilot project
	▶ Number of participants according to target population of each MS	Increased validity of data, representative approach	High burden for large populated countries	Up- and down-sizing adjustments to cover disproportionateness
Management	▶ One management centre for all MS	More effective organisation; preconditions for harmonisation and standardisation	Known problems with "centralization", management needs high intercultural competence	Language problems, communication and responsibilities
	▶ Centres in the different MS (+ central management)	Smaller distance to participants; cooperation and shared responsibility	More efforts for organisation and standardisation	Training and acceptance of SOPs

DESIGN: 3. Selection of Participants, Sample				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
Type of sample	▶ Based on persons	Advantageous if registration offices exist	More than one person of one household might be chosen, relevant for environmental sampling	Similar advantages and disadvantages for groups such as school classes or day care centres.
	▶ Based on households	Advantageous if no registration offices exist; examine several household members	Unwilling household members	
Choice of sampling locations	▶ Sampling locations according to community size (village - small town - city)			
	▶ Sampling locations according to exposure level (hot spot - area with low exposure)	Relevant for known hot-spots, provides picture on upper & lower margins	Provides no reference values, incomplete information on national population	Exposure level depending on biomarker, assumptions vs. knowledge,
	▶ Sampling locations according to region (administrative units)	Similar intra-national structures & capacities, might facilitate merging E & H databases	Vast intra- and inter-national differences of administrative units	
	▶ According to population density			

DESIGN: 3. Selection of Participants, Sample				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
Selection of participants	▶ Sample of volunteers, self-selecting sample	Motivated participants	Selection bias; possibly people with higher exposure	Convenient sampling needs to be made transparent
	▶ Random Sample (and equivalent types of sample)	Less selection bias	Convincing information campaign, non-responders	Consider GROSS to NET sample size
Choice within population data bases	▶ Registration offices	Easy to access, almost complete database of all citizens	Cooperation of the offices is needed; data protection; confidentiality	Age of available data
	▶ Social security data bases	Almost complete database of all working citizens?	Relevant in all MS? Adults and working people only, cooperation of the offices is needed	
Choice within educational system	▶ Universities	Many unexposed, probably highly motivated participants at on place	Social and educational bias; consent of administration	
	▶ Schools, vocational schools	Generally good response as pupils like unusual experiences	Support of the head master and teachers needed	Type of school must be considered: social and educational bias
	▶ Kindergarten, day care centres	Many participants at one place	Support of the staff needed	Social bias must be considered

DESIGN: 3. Selection of Participants, Sample				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
Choice within medical system	▶ Physicians, paediatricians	Additional information on health status might be gathered, doctors can be good motivators	Support of the physician is needed, only "patients" visiting a doctor	Selection bias, sensitive doctor-patient relation and data privacy
	▶ Gynaecologists	Pregnant women	Social bias, support of the physician is needed	Cooperation of personnel/doctors is needed
	▶ Screening test when entering school	Many participants at one place, additional information on health might be gathered, boosted motivation?	support of the health authorities is needed, only small age group (5-7 years of age)	might not be possible in all MS
	▶ Preventive medical check-up	Additional information on health status might be gathered, doctors can be a good motivators	Not used by all to which offered, only for children in special age and for adults social bias, disease bias	Data protection? In which MS are those check-up examinations offered? Cooperation of personnel/doctors is needed

DESIGN: 3. Selection of Participants, Sample				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
Choice within specific groups	▶ Public announcement in media	Volunteers, highly motivated	Social, educational bias	
	▶ Blood donors	Easy to organize; easy access to samples	Adults only, social bias support of the institutions is needed, strict exclusion criteria	
	▶ Churches		Not a relevant institution in every member state	
	▶ Members of a research institute	Easy to sample, motivation, interest in results	No representative picture, not even suitable for a pilot study	
Miscellaneous	▶ Over-sampling of certain populations	Groups with low response rates representatively included		
	▶ Use of census data	To test bias		

DESIGN: 4. Recruitment				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
Information / invitation	▶ Public relations	Essential		The study and advantages of participation should be explained to participants, institutions and media
	▶ Public announcement		Social, educational bias	
	▶ Written invitation /information to recruited person	Essential		The study goal, programme and means MUST be explained in plain language, includes data protection and potential risks of invasive sampling.
Consent	▶ To each part of the study separately	Essential		Informed consent
	▶ By participant (adult, adolescents)	Essential		
	▶ By parents for child	Essential		
	▶ Consent to be contacted in case of follow-up study	Essential		
Compensation for time / money spent	▶ Participants (adults, children, parents)	Higher participation rate	Higher costs	Controversial ethical discussion
	▶ Cooperating personnel (doctors, nurses ...)	Better motivation	Higher costs	Controversial ethical discussion

DESIGN: 5. Field work				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
personnel	▶ Trained interviewers	Improves quality of data and communication with participants	Higher costs and training required	
	▶ Trained medical personnel	Essential for blood sampling and can improve communication with participants		Requires appropriate facilities alike
avoiding seasonal bias	▶ Sampling in the same season in all MS	Short duration of study	Over- or underestimation of exposure if season is relevant for exposure	For pilot study acceptable
	▶ Sampling during all seasons in all MS	Average exposure over year; test significance of seasonal effects	Duration of study: one year	
Place of examination and interview	▶ Visit at an examination centre	Neutral environment, all equipment at one place, questionnaires could be checked, no transport of study personnel	Inconvenience for the participants, time & travel	Travel-compensations
	▶ Visit by an interviewer at home	Interviewer can validate answers of participants, more convenient for participants, questions can be answered by the trained interviewer	Interviewer need initial training, transport required, repeated attempts to succeed possible	Some people do not like to be visited, cultural misunderstandings have to be considered

DESIGN: 5. Field work				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
Miscellaneous	▶ Way of contact (personal - by phone - by letter)	Various means and reruns increases response rate	Higher costs	Number of trials to contact potential participant
	▶ Commercial sub-contractors for field work	Might be experienced in field work	Higher costs, QC by the study management is needed	
	▶ Financial incentives or gifts	Higher response rate	Higher costs, ethical considerations (suitable amount, social bias)	Special incentives for special age groups
	▶ Reporting results to participants	Essential to achieve a high response rate	Higher costs	level of interpretation, in case of high exposure values essential because of ethical reasons
	▶ Duration of interviews and sampling			Maximum 2 h

DESIGN: 6. Questionnaires				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
Way of questioning	▶ Questionnaire filled in by participants and/or parents	Less expensive	Only easily understandable questions possible, people must have language competence and must not be illiterate.	Translated questionnaires in several languages, must be validated.
	▶ Interview, based on questionnaire	Better data, interviewer can give additional explanations	Higher costs, duration of interview	Extent of training of interviewers
Necessary questionnaires	▶ Questionnaire containing questions about criteria needed to decide, if potential participant belongs to study population	Essential		
	▶ Non-responders questionnaire	Check bias, some crucial information on exposure	Higher costs	
	▶ Questionnaire for participants	Essential		

DESIGN: 6. Questionnaires				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
Amount of Information on sources of exposure	▶ Including no information on exposure routes	shortness	No correlation between sources and exposure assessable	
	▶ Including all possible exposure routes	Quantification of exposure pathways by multivariate evaluation	Extended time frame to fill in, higher costs	Time-activity patterns and food frequencies are time consuming
	▶ Including only main exposure routes	Quantification of exposure pathways by multivariate evaluation, sufficient for the definition of reference values		Pb: only gender and age needed for the definition of reference values
Additional topics of questionnaire	▶ Including sociodemographics	Essential	Sensitive issue of privacy for many people, biased or incomplete data	Age, gender, social status, education, income (of the parents), professional status (of the parents), type of school might not easily be compared in MS.
	▶ Including time of living at the sampling location	Might elucidate some spatial exposure sources	Correlation with age	Might be left out
	▶ Including nationality	Essential		In combination with place of birth, time spent in the current country, language spoken at home
	▶ Including food frequencies	Important exposure source	Time consuming, no good ratio between effort and output	

DESIGN: 7. Sampling				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
Individual vs. pooled samples	▶ Individual samples only	individual results, range of exposure, enables spatial and temporal statistical comparison	No expensive chemical analyses	
	▶ Pooled samples	Less expensive, more volume, more substances can be analysed and detected	No individual results, no range of exposure, no reliable statistical comparison	Summarization of questionnaires is needed, minor motivation for participants to take part
	▶ Individual <u>and</u> pooled samples	Special (expensive) analyses with the pooled sample possible		relevance in regard to bio-banking of specimen
DESIGN:8. Quality Control				
Topic	Alternatives	Advantages	Disadvantages	Issues to consider
▶ Internal quality control		Essential, less expensive	Routine-blindness	
▶ External quality control		Desirable	Extra costs	It is not always easy to find adequate cooperation partners
▶ Field work		Recommended	Expertise needed, higher costs	Knowledge of different languages needed, if performed by a central unit
▶ Institutions in MS		Essential	Expertise needed, higher costs	