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**Deliverable D3.3: Guidelines for integration scenarios and implementation strategies
for biomonitoring results to be tested in the pilot study**

R. Smolders*, G. Schoeters

VITO
Environmental Toxicology Department
Boeretang 200
2500 Mol
Belgium
Tel: 0032 14 336216
E-Mail: roel.smolders@vito.be

* Corresponding author

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1 INTRODUCTION

The current document contains a proposal for an integration and implementation strategy of HBM data with additional environment and health data for the European Pilot Project on Human Biomonitoring. The main goal of the document is to provide readers with a brief overview of which policy and research activities are occurring at present, and how they contribute to an improved interpretation of HBM data.

A first part of the document deals with the identification of a number of recent policy developments such as the REACH and INSPIRE Directives and related Fifth and Sixth Framework Programme research projects on Environment and Health that may be of immediate relevance for the Pilot Project on HBM in Europe. These developments, like for example the FEHES initiative, may provide a future framework for the European Pilot Project, new tools to improve the links between exposure metrics, HBM data, and resulting health effects, a better insight in the dose-response relationships of hazardous substances and the applicability of novel techniques such as the “omics” for a further development and strengthening of HBM applicability and research in Europe.

A second part of the document redirects its focus towards the possibilities for integrating all different sources of environment and health information with HBM data, through the application of geographical information systems technology (GIS) and the related Policy support under the recent INSPIRE Directive. Although there are currently still many uncertainties regarding the feasibility of a geographically representative HBM network in Europe, GIS offers excellent integration opportunities for a future European HBM network.

A third and final part of the document includes a proposal for a statistical analysis framework for HBM data, both at a European level and for individual Member States. Although this final part needs to be seen as a first attempt to attend to some of the basic analysis needs of the Pilot project, it is only an example and a discussion document highlighting what this analysis framework could look like, and should be treated as such. At this point, there are too many unknown variables regarding the effective realization of the Pilot Project and it would be premature to warrant a concrete analysis plan. However, the current proposal could provide a platform on which to build such a statistical analysis plan once the details of the Pilot Project become known.

2 CREATING SYNERGIES WITH NEW AND ONGOING ENVIRONMENT AND HEALTH RESEARCH

2.1 HBM and REACH

On December 18th 2006, the European Parliament and Council introduced Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorization and Restriction of Chemicals, more commonly known as REACH. The philosophy behind this REACH Regulation is to ensure a high level of protection of human health and the environment from the potential hazardous effects of chemicals, to promote the development of alternative methods for the assessment of hazards of substances, and to generate more and better toxicity data for a large number of chemicals which toxicological potency until now remains largely uncharacterized, without jeopardizing the competitiveness and innovation potential of the European business market.

An important objective of the Regulation is to identify substances that are of potential concern for both human and environmental health because of their persistent, bioaccumulating or toxic properties, and to encourage their replacement by less dangerous alternatives. In order to achieve this, manufacturers and importers need to generate data on the substances under consideration, use these data to assess the risks related to these substances and develop and recommend appropriate risk management measures.

Risk assessment of chemicals essentially is a comparison of the predicted environmental concentration (PEC) with the predicted no-effect concentration (PNEC). If the expected concentration of a substance in the environment or in humans is higher than the concentration at which this substance is assumed to have an effect, taking into account long-term exposure and chronic effects, risk is assumed to be present and action needs to be taken. In the context of REACH, the main application of Human biomonitoring (HBM) is in the estimation of expected concentrations (PEC) in human matrices, by directly measuring the internally accumulated dose, rather than by estimation from often poorly understood processes such as bioaccumulation, degradation or excretion.

In the following, we would briefly like to provide the reader with an overview of possible pathways by which human biomonitoring (HBM) can provide added value to the REACH process, and vice versa. HBM as a tool to measure internal dose of a substance can be of use for manufacturers and importers, but also regulating agencies such as the European Chemicals Agency can benefit from the added value of HBM.

2.1.1 Predicted environmental concentrations

REACH introduced a radical change in the way the risk assessment of chemicals was perceived by both industry and policy makers, by laying the burden of proof that a substance is not harmful for humans or environment by industry, manufacturers, importers and downstream users. This way, the responsibility for the identification of hazardous properties of substances, the potential adverse effects on human health and the environment and the management of the risks lies with the natural or legal persons that manufacture, import, place on the market or use these substances.

During this process, where the manufacturers or importers need to illustrate that substances are not hazardous, or risks can be sufficiently restricted to allow for marketing, HBM may provide an interesting source of information to define testing priorities, limit the amount of additional testing requirements, improve extrapolations or include detailed end-user information in the risk management proposals:

- **Define priority chemicals:** It is estimated that around 100.000 chemicals are in need of testing in order to comply with the REACH authorization procedures. Since it is not technically, financially nor practically feasible to test all these chemicals at once, prioritization for the most urgent chemicals to be tested is foreseen in REACH. Although the REACH regulation has included some criteria for priority setting, based on either production volume or evidence of carcinogenicity, mutagenicity, or reproductive toxicity, HBM can provide additional information on prioritizing of chemicals to be tested. Documented presence in human matrices is as such not a criteria that is currently introduced in the REACH Regulation, but can be of major help to rank chemicals according to the need of testing. For example, a temporal increase in body burden in the general population, as is well documented for e.g. brominated flame retardants, may be a stimulus for the prioritizing of substances, even if no or only ample data on toxicity or health effects is available;
- **Limit testing requirements:** It is clearly mentioned that within REACH resources should be focused on substances that meet the criteria for classification as carcinogenic, mutagenic or toxic for reproduction categories 1, 2 or 3, as a respiratory sensitizer, or in respect of other effects on a case-by-case basis. Also, in Annex XIII of the REACH Regulation, the criteria for

the identification of persistent, bioaccumulative and toxic substances, and very persistent and very bioaccumulative substances are outlined. However, these criteria are generally based on tests in terrestrial or aquatic environment, or tests with marine or freshwater organisms, and are not always an adequate proxy for human exposure or dose. Particularly, if it can be adequately illustrated that bioaccumulation in humans is not comparable to bioaccumulation in (eco)toxicological tests, this information may be used to limit further testing requirements to assess human exposure and risk. Data from occupational human biomonitoring schemes may be an extremely relevant source of information in this context;

- **Improved dose extrapolations:** frequently, toxicity tests are expressed in terms of exposure concentration added to the relevant test medium instead of absorbed dose. Although it is acknowledged that the use of dose requires at least basic knowledge of the main target organs, it is a much more relevant metric for risk assessment than mere exposure concentration. Moreover, using internal dose as a metric in toxicity testing may make toxicity testing much more relevant for extrapolation to human risk assessment, since also human dose can be estimated using HBM. Using dose as a metric, both in (eco)toxicological and human biomonitoring, may greatly improve the relevance of risk assessment, and may identify discrepancies between proxy organisms such as rodents or fish, and humans. One example to highlight this is the important difference in bioaccumulation factors of dioxins between rodents (expressed in terms of days) and humans (expressed in terms of years). If regulatory animal toxicity testing would be described in terms of (internal) dose-response relationships rather than (external) exposure-response relationships, a more sensitive and relevant extrapolation from test organism to human and hence an improved risk appraisal would be possible;

Besides these issues where manufacturers and importers can benefit from the information provided by HBM programs, also the regulators, particularly in person of the European Chemicals Agency (ECA), can benefit from HBM.

- **Registration screening:** Given the vast amount of chemicals that is up for evaluation, it would not be practically feasible to perform a thorough

screening of all submitted REACH dossiers. Hence, it is foreseen that only a certain percentage of dossiers are identified for compliance checking, no lower than 5%. HBM can assist in selecting which dossiers should preferentially be screened, by providing information on the distribution of background concentrations in the general population. It is obvious that substances that are present in human matrices in measurable concentrations should be monitored more closely than substances that have not been detected in humans;

- **Follow-up of risk reduction strategies:** Generally, risk reduction strategies will focus on reducing exposure to hazardous substances. Hence, it would be useful to monitor the efficiency of the risk reduction strategies that are proposed in the different dossiers in order to be able to evaluate whether the proposed strategies are effective in reducing the risk for users. HBM is a useful tool to track temporal and spatial evolution of the distribution of substances among the general population, and hence may provide relevant background information on the efficiency of risk reduction strategies.

2.1.2 Predicted no-effect concentrations

As was illustrated above, HBM can provide assistance for the REACH process, mainly through providing information on the presence or change of substances in the general population. However, REACH will also generate a large amount of data on the toxicological potency of substances, many of which are until now largely uncharacterized. This influx of data may highlight the presence of substances which pose currently unknown threats for human and environmental health. Based on this laboratory information, new and currently unexpected environmental health threats may be identified, based on the improved toxicological profiling of substances.

2.1.3 Contributions of the European Pilot Project on HBM

Evidently, HBM has a significant contribution to make to the entire REACH process, both in providing information on the relevance of PEC estimates, and in identifying substances which may require more or less detailed attention of risk assessors, based on their tendency to be detected in human tissues.

A European Pilot Project will allow for the development of harmonized data throughout all Member States, where currently, different Member States may gather their own HBM data,

using non-comparable methods, with different analytical sensitivities and hence different REACH implications. The development of a network to gather comparable and representative data for the whole of Europe will stimulate the applicability of HBM data within the REACH process, by developing harmonized methods, comparable throughout Europe, limiting the presence of conflicting information, hampering the decision making process.

2.2 HBM and health examination surveys

As previously mentioned, HBM is not an island of information, and should preferably be linked with environment and health data in order to improve interpretability and relevance of HBM data. Because the ultimate goal of HBM survey programs is the protection of the general public well-being, HBM survey projects have often gone hand-in-hand with much broader projects on the general health assessment of the general public.

2.2.1 HBM and NHANES

Probably the most extensive survey projects on human health running worldwide at this moment is the US National Health and Nutrition Examination Survey (NHANES). Since 1999, NHANES has become a continuous program that examines a nationally representative sample of about 5000 persons each year, with a changing focus on a variety of health and nutrition measurements. Oversampling of special emphasis groups such as adolescents, persons 60 and older or specific ethnic groups is conducted to enable accurate estimates for these groups. The NHANES detailed interview includes demographic, socioeconomic, dietary and health-related questions and the examination component consists of medical and dental examinations, physiological measurements and laboratory tests. The findings from NHANES are used among others to determine the prevalence of a number of diseases, medical conditions and health indicators in the general population (CDC 2007):

Anemia	Mental health and cognitive functioning	Reproductive history/behaviour
Cardiovascular diseases	Nutrition	Respiratory diseases
Diabetes	Obesity	Sexually transmitted diseases
Hearing loss	Oral health	Vision
Infectious diseases	Osteoporosis	
Kidney disease	Physical fitness/functioning	

From a random sample of participants from the NHANES study, blood and urine samples are collected to measure more than 100 chemicals or their metabolites. Although it is acknowledged that for many of the substances measured, knowledge on the health effects is lacking, specific public health uses of the exposure data are identified (CDC 2005):

- To determine which chemicals are present and at what concentrations;
- To determine the proportion of the populations with levels above those associated with adverse health effects if toxicity levels are known;
- To establish reference ranges that can be used to identify persons with unusually high exposure;
- To assess efforts to reduce exposure;
- To determine whether exposure levels are higher among vulnerable groups such as minorities, children, or women of childbearing age;
- To track evolutions over time;
- To set priorities for research on human health effects of exposure.

For most of the chemicals included in the HBM pillar of NHANES, this last use category is essential to determine whether exposure at levels reported is a cause for health concern. Hence, HBM may not only be a source of information to identify exposure and link this to potential health effects, but HBM data may also be used to identify future research needs on the toxicology and health effects of currently undercharacterised substances.

2.2.2 HBM and KIGGS

The German Health Interview and Examination Survey for Children and Adolescents (KIGGS) is an equally extensive project designed as a representative nationwide health survey of children and adolescents, where data are collected regarding objective measures of physical and mental health as well as self-reported information regarding among others subjective health status, living conditions and environmental determinants of health (KIGGS 2005). Between May 2003 and May 2006, more than 17000 participants were enrolled nationwide, and also here, a subsample of 1800 participants was selected for analysis of chemicals or their metabolites in the German Environmental Survey 2003-06 (GerES IV) for Children. Children were selected as the target group because of their specific exposure behaviour patterns and specific physiology. In association with the collection of blood and

urine samples for HBM of a wide variety of metals and organic contaminants, information was also gathered on the presence of contaminants in the domestic environment (e.g. dust, drinking water, chemical and biological pollutants in indoor air) (Umweltbundesamt 2005).

2.2.3 HBM and FEHES

Similar to the two examples NHANES and KIGGS mentioned above, the European Union has funded a project, Feasibility of a European Health Examination Survey (FEHES), through the Programme of Community Action in the Field of Public Health (2003-2008). The objective of FEHES is to contribute to the development of a European Health Examination Survey System in EU Member States. Specific objectives of the FEHES project are (FEHES 2006):

1. To create a network of experts and institutes for implementing HES in all EU MSs.
2. To describe and analyse the feasibility of models of HES with different intensity and cost.
3. To collect and assess in all EU-countries information on factors affecting feasibility of HES
 - Legal, data confidentiality and ethical issues
 - Availability of sampling frames
 - Previous HESs, experience and expertise
 - Perceived importance and interest in HES on the national/regional level in each of the MSs
 - Perceived interest in a European HES in each MS
4. To make proposals and recommendations for the future of HES in EU and all MSs
 - European HES combined with current national HIS or HIS/HES or separate European HES
 - Models of HES of different comprehensiveness and complexity
 - Topics for HES core modules and their measurement methodology
 - Resource needs
5. To prepare a proposal for a European HES pilot to be carried out both in MSs
 - with previous national HES experience and
 - without such national surveys

The FEHES project is expected to finish in March 2008, and may open the door for a EU-wide, standardised Health Examination Survey (HES). Needless to say, the establishment of such a HES could also be an appropriate framework within which to allocate a Network on Human Biomonitoring in Europe, much like what was illustrated above for the USA and Germany.

2.3 HBM and FP5 and FP6 projects

In addition, there has been considerable research funding from the European Commission for Environment and Health-related topics, mostly through the Fifth and Sixth Framework Programs. A recent publication by DG Research provides an overview of all EU research on Environment and Health funded by the Fifth Framework (EC 2007). This document among others provides summaries of projects on air pollution-related health impacts, chemicals and health impacts and multiple stressors and factors and health impacts. Also electromagnetic fields, noise, UV and ionizing radiation, waterborne stressors, climate change and nanoparticle related health impacts are covered, and contacts and links for further information are provided. Information on (ongoing) projects within the Sixth Framework programme can be found at <http://cordis.europa.eu/fp6/projects.htm>. Moreover, most of these ongoing projects have their own websites, where additional information and contact addresses can be found.

Several of these projects, such as ECNIS, NEWGENERIS, GA²LEN,... have a biomonitoring angle included in their project development. However, since these projects generally are research driven, and focus more on the development and validation of new biomarkers for faster, direct and more sensitive identification of health effects, their outcomes may not be immediately available for routine application. However, it has to be kept in mind that these hypothesis-driven research projects will in the future provide the tools for a better and more relevant interpretation of human biomonitoring data. Hence, keeping close contact with these projects may be desirable from a HBM point of view.

3 PROVIDING LINKAGE AMONG DATA SOURCES

3.1 Exposure-dose-response Triad

Within the Environment and Health Action Plan 2004-2010, the need for integration of human biomonitoring (HBM) with environment and health data is specifically foreseen:

“Biomonitoring is not an automatic instrument, which can be considered in isolation, but has to be integrated with environmental monitoring, toxicological and eco-toxicological data and especially with considerations related to analytical epidemiology. (EC, 2004b)”

From this, it must be clear that HBM should be considered a stepping stone between environmental and health data. The final aim of this process should be an integrated and holistic system where HBM can on the one hand lean on environmental data to provide detailed information on the sources and pathways of pollutants that enter the human body, and can on the other hand clarify new and existing hypotheses on the relationship between environmental pollutants and the prevalence of diseases or the occurrence and identification of disease clusters (Thacker et al 1996, Hoppin et al 2000).

An integration of information on environment, HBM and health should preferably be obtained within an Exposure-Dose-Response Triad approach (EDR-Triad). *Exposure* aims at quantifying the amount of a pollutant present in the environment through different routes or compartments, *dose* focuses on the internal concentration of this pollutant bioaccumulated over time and pathways, and *response* incorporates the physiological and/or epidemiological consequences of the observed internal dose (Committee on Biological Markers of the National Research Council, 1987; Andersen et al, 1992; Dietert et al 2000, Smolders and Schoeters 2007). Within this EDR-Triad, dose is the central pivotal point, as a midway marker with an optimal trade-off between interpretability and toxicological relevance. The advantage of using the internal dose as a proxy for pollutant exposure assessment is clear if you keep in mind that dose refers to the amount of a pollutant that has actually entered a person’s body, and that the link between exposure and effect is a continuum rather than a series of distinct events (Decaprio 1997, Nuckols et al 2004). This makes dose a more or less person-specific, integrative parameter, as bioaccumulation and elimination characteristics such as age, gender, physiological condition, as well as factors related to behavior and activity are taken into account in a dose. Furthermore, dose can also serve as an integration of all previous exposure conditions through different pathways. This is especially important for bioaccumulating substances, since current conditions may not necessarily be representative for historical exposure. Also, since most health responses are the result of long-term, chronic exposure to environmental pollutants, linking exposure to response without looking at the dose will overlook the exposure history of individuals.

Finally, the use of dose together with exposure monitoring has been shown to be an important tool to gain insight into the specific toxicity mechanisms (Hoppin et al 2000, Mortensen et al 2003, Bushnell et al 2005) and will in the future remain an essential intermediate to assess cause-effect relationships and a more relevant risk assessment (Stokstad 2004).

3.2 GIS and mapping information

Although it is recognized that there is a wide variety of data available in the context of the EDR-triad among different European Union Member States (MS), it is often difficult to integrate these different data layers because of differences in database structures, geographical detail and spatial distribution, or most importantly because the data simply were not meant to be gathered and interpreted in the context of integrated human risk assessment. Based on the “European Environmental Health Action Plan 2004-2010”, a wide variety of research and consultancy activities have originated, aiming at the establishment of an “Integrated Health and Environment Information System”. This system will evaluate the myriad of environmental monitoring programs, and will highlight research and development needs for making the information accessible in a more harmonized way.

Geographical Information Systems (GIS) are “automated systems for the capture, storage, retrieval, analysis, and display of spatially referenced data” (Clarke et al 1996; Higgs and Gould 2001). A unifying feature in GIS is that all objects are unique since they are spatially explicit and can be linked to a geographical framework, usually in the form of mapable objects. This feature implies that different types of data, in our specific interest environmental, HBM, and health data can be presented under a common denominator, being their spatial location or distribution. This is achieved by depicting the different data types as layers, and superposing these layers in the same geographical reference grid. Apart from for example simply plotting environmental monitoring data or morbidity/mortality information on a map, GIS also offers important opportunities for inter- or extrapolation of data, for a geographical representation of monitoring or modelling data and for the visualization of overlaps between different layers of information.

Since it is the aim of the European Commission to establish a Europe-wide HBM network, this geographical component of a GIS system will prove to be indispensable for an efficient representation of Europe-wide HBM data, and furthermore to create links with environmental and health data (EC 2004a, 2004b). Additional advantages of using a GIS

environment are the opportunity to represent data at different geographical levels, thus incorporating local, regional, national and Europe-wide information within the same platform, and an efficient dissemination of results through the production of readily interpretable maps. It is obvious that, in order to develop an EDR-triad that is as sensitive and relevant as possible, data will be required at a fine geographical scale throughout Europe. Apart from creating a link with environmental and health data, a number of issues in HBM could easily and routinely be resolved:

- ☑ Highlighting ‘hot-spots’, areas with significantly elevated biomarker values;
- ☑ Spatially and temporally illustrating the effects of remediation actions;
- ☑ Target resources for disease prevention;
- ☑ Identification of information gaps;
- ☑ Integration of different geographical levels in one platform.

3.3 The INSPIRE Directive

With regard to spatial information in Europe, fragmentation of datasets and sources, gaps in data availability and lack of harmonization between datasets at different geographical scales makes it difficult to access and use available spatial data throughout Europe. At the same time however, the need for high-quality geo-referenced information to support and better document for example environment and health policy making is increasing rapidly. On the 14th of March 2007, the European Parliament and the Council agreed on Directive 2007/2/EC on “Establishing an infrastructure for spatial information in the European Community (INSPIRE)” (EC 2007). The Directive is aimed at providing more, better, and easily accessible spatial information in Europe for the formulation and implementation of community policy on the environment by triggering the creation of a European spatial information infrastructure that delivers integrated spatial information services to potential users. Member States will need to ensure that all newly collected and restructured datasets and the corresponding spatial data services will become available according to general implementation rules and a Geoportal network will be constructed where all these data are aggregated. Included in the spatial data themes that will need to be made available are among others themes such as land use, environmental monitoring facilities and human health and safety data. Particularly this last item is of importance within the context of the current publication, since it addresses the “*geographical distribution of dominance of pathologies (allergies, cancers, respiratory diseases, etc.), information indicating the effect*

on health (biomarkers, decline of fertility, epidemics) or well-being of humans (fatigue, stress, etc.) linked directly (air pollution, chemicals, depletion of the ozone layer, noise, etc.) or indirectly (food, genetically modified organisms, etc.) to the quality of the environment”. It is clear that the incorporation of a European Network on Human Biomonitoring may be highly plausible with regard to this latest data theme.

3.4 Confounders of spatial analysis

However, there are a number of factors implicit to the spatial characteristics of HBM data that may have a profound effect on the appropriateness and applicability of this type of data:

- Micro-mobility: People are generally moving study objects, which makes it difficult to exactly quantify environmental exposure and uptake of chemicals. Although people spend most of their time indoors, most environmental monitoring and modelling is generally done using data on outdoor concentrations of pollutants. Also, mobility in itself may be a major source of exposure, due to increased uptake of contaminants in e.g. traffic. To a certain extent, information on micro-mobility can be gathered using time-activity patterns, either through simple fill-in diaries or more complex techniques such as GPS tracking (Hanssen et al 2006, Elgethun et al 2003, 2007);
- Macro-mobility: Additional to these every day spatial patterns of micro-mobility, there is also the issue of macro-mobility, where people move around to go live, study or work in other parts of the country or the world throughout a lifetime. While this macro-mobility may not have important effects on biomarkers which are rapidly metabolized and excreted from the body, effects on biomarkers for bioaccumulating substances or substances with long half-lives such as dioxins in body fat stores (5-15 years) or cadmium in kidney (30 years) may be considerable. This effect can be partially taken into account by including the requirement in the participant selection that constituents in HBM studies must have lived in the study area for a number of years, but this might be problematic for young adults that move out of their parents' houses or go to university to study;
- Non-spatial variability: Even when micro-and macro-mobility are accounted for, there remain many non-spatial variables that can have a profound effect on the biomarker values in individual constituents. Through individual food preferences

such as consuming home-grown vegetables or eggs (Pruvot et al 2006, Schoeters and Hoogeboom 2006), lifestyle factors such as smoking or drinking behavior (Heinrich-Ramm et al 2000, Sorensen et al 2007), or even genetic susceptibility (Norrpa 2001, Abdel-Rahman et al 2005); two people with comparable mobility patterns and residence may have considerably different biomarker concentrations due to differences in behaviour, lifestyle, genetics ;

- Privacy and confidentiality issues: HBM data give information on the internal pollutant concentration in constituents, which has considerably different ethical and legal requirements than e.g. air pollution monitoring sites. Due to basic privacy requirements, it would not be advisable to report HBM data at a precise geographical scale. While for example air pollution monitoring sites can be represented by an X- and Y-coordinate, this is much less desirable for HBM data. So there is a need for at least some form of data aggregation if HBM data are to be compared among different geographical areas or scales (NAACCR 2002, Elliot and Wartenberg 2004, Olson et al 2006).

From the above, it should be clear that there are certain issues related to the spatial relevance of HBM data in the context of environment and health research which advocate the use of some kind of aggregation of HBM data at a currently undefined geographical scale. Many of the confounders mentioned above are in themselves scale dependant. When for example the local or regional impact of a waste incinerator is assessed (Nawrot et al 2002, Reis et al 2007) micro- and macro-mobility may have an important impact on the outcome of the study. It can be assumed that this source of variability is much less relevant when a Europe-wide study is under consideration, such as is currently proposed in the European Network on Human Biomonitoring.

4 DEFINING GEOGRAPHICALLY RELEVANT AREAS

4.1 A common currency

If HBM is to be linked to environmental or health data, both in the context of INSPIRE but also more generically in a wide variety of research and survey projects, a common “currency” for these three sources on information needs to be developed. As discussed above, a GIS environment offers a platform where this common currency can be applied.

However, due to privacy reasons (see earlier), there is a need for at least some level of aggregation of HBM data. Different types of aggregation are potentially useful and several options are addressed more in detail below.

4.2 The NUTS classification

A possible and highly attractive candidate currency for the whole of Europe has already been established in the form of the NUTS classification. The Nomenclature of Territorial Units for Statistics (NUTS) was established by Eurostat as a uniform breakdown of territorial units for the production of harmonized regional statistics for the European Union. In 2003, a Regulation of the European Parliament and of the Council on the NUTS was adopted and from 1 May 2004, the regions in the 10 new Member States have been added to the NUTS as a cornerstone of the European Statistical System (EU, 2003). In order to improve the comparability of regional statistics, it was required that these regions were of a comparable size in terms of population.

The NUTS classification was established and serves as a reference for three specific target goals:

- For the collection, development and harmonisation of Community regional statistics;
- For the socio-economic analyses of the regions;
- For the framing of Community regional policies.

This NUTS classification results in a subdivision of Europe into 89 (NUTS1), 254 (NUTS2), or 1214 (NUTS3) separate statistical sectors according to a hierarchic system. Depending on the variable concerned, regional statistical data at one or more of the 3 NUTS levels is available in a wide variety of publications and databases. Many variables and time series for regional data in different domains such as environment, health, are available on the Eurostat website (<http://epp.eurostat.ec.europa.eu/>). At an EU-level, a wide variety of data is gathered at the relevant NUTS levels. This data is generally gathered by the relevant National Statistical Offices and is later stored in a unified database structure named REGIO, a part of Eurostat's New Cronos. This regional database domain REGIO has 12 distinct collections, including demographic, health, and environmental statistics. The standard level of data availability is NUTS2 level, while certain data are also available at the NUTS3 level (European Communities 2005). It should be stressed that this collection is not exhaustive, and certainly future development of the REGIO database may even further increase the

applicability of these regionally available data. Figure 4.1 provides an example of the usefulness of the NUTS classification with regard to the depiction of population density data at a NUTS 3 level throughout Europe.

4.3 Land use/cover

Land use or land cover may be a useful proxy for estimating the overall exposure of humans. Based on their natural surroundings, people can be classified living in for example rural, semi-urban, or urban environment, which may reflect their general exposure status ((Nielsen et al 1996, Van den Heuvel 2002). Certainly, people living in highly industrialised or densely populated urban environments have a higher probability of getting into contact with certain contaminants, such as those closely related to traffic or intense industrial activity. Also hotspots, areas of above-average concentrations of pollution, are more likely to be found near areas of higher industrial and economic activity. However, this relationship is not always as straightforward as it may seem, as can be illustrated by the generally higher exposure to pesticides in agricultural areas, or the observation that home-grown foods, which generally are more often consumed in rural areas, may have significantly higher levels of contaminants than foods from supermarket purchase (Schoeters and Hoogeboom 2006). Hence, caution needs to be used when using land use/cover data as a proxy to identify rural, semi-urban or urban living conditions.

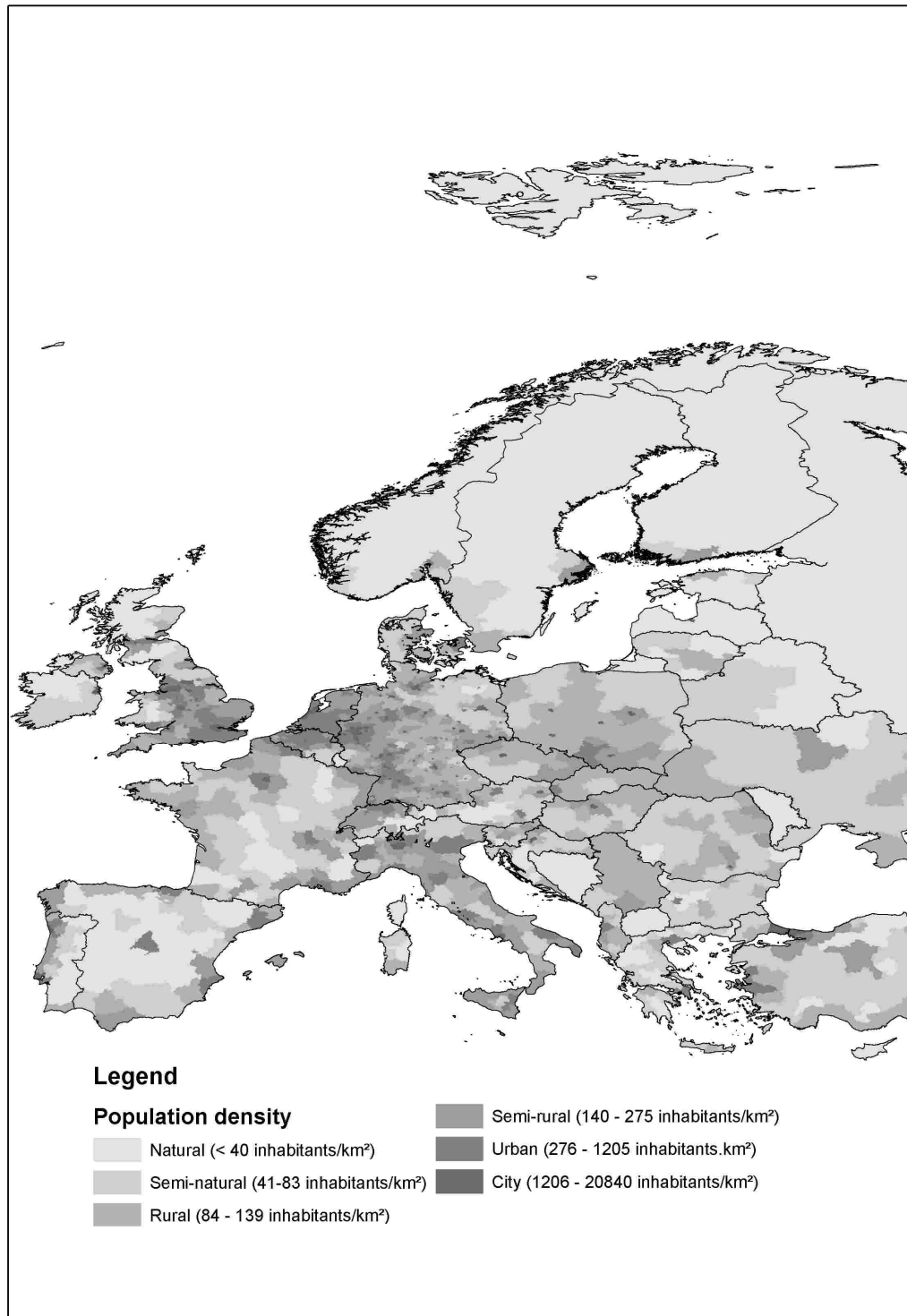


Figure 4.1: Overview of population density in Europe, based on NUTS3 level data aggregation.

The CORINE programme (EEA 2000) aimed at gathering a comparable and consistent computerised inventory on land cover data across Europe. Roughly, CORINE identifies 5 main land cover themes (artificial surfaces, agricultural areas, forest and semi natural areas, wetlands, and water bodies), which are further subdivided into as much as 44 different land cover categories. Although it is only a proxy for generic exposure, and should hence be interpreted as such, it may be a good approach to use basic CORINE land cover for estimating overall exposure (Figure 4.2).

Alternatively, it can be left to each partner or Member State in a project to define for themselves whether constituents are living in an urban, semi-urban, rural or natural environment. While this approach offers optimal flexibility, it does not guarantee a harmonised appraisal of exposure and less-than-optimal comparability of results, since a natural area in a densely populated area such as Belgium may be defined quite differently than a natural area in Sweden or Finland.

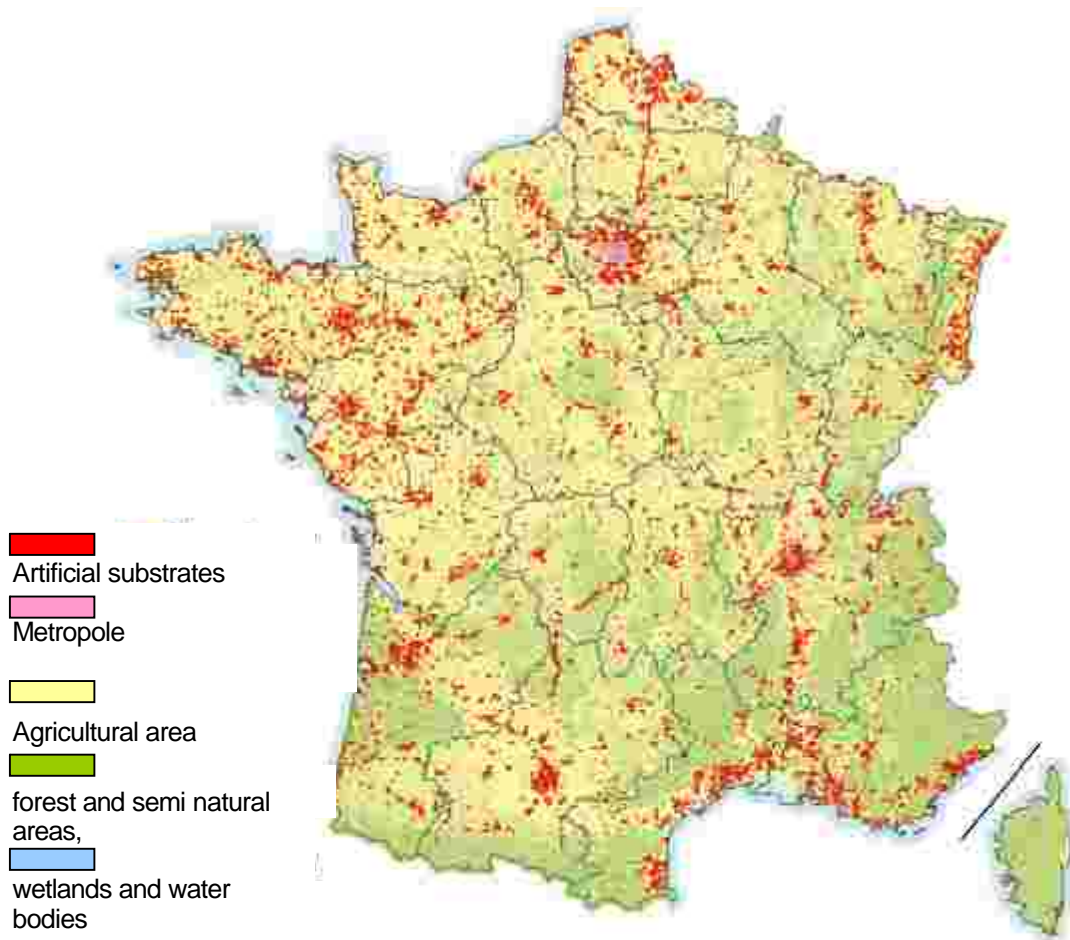


Figure 4.2: CORINE Land cover data for France

4.4 Predefined exposure/health clusters

A third approach which may prove useful to define geographically relevant areas is based on the predefinition of potential exposure or health clusters. This approach may be considered as one where the burden-of-proof is reversed, and constituents are geographically classified along their predicted or assumed exposure (low, medium or high exposure), e.g. based on existing pollution distribution models. Figure 4.3a gives an example based on the EMEP-modelling of Pb deposition through air across Europe. The advantage of this approach is that research hypotheses can be tested more efficiently because exposure conditions are a priori implemented in the study design.

However, the approach also has a number of limiting features as well:

- Areas of low, medium, or high exposure have to be designated for each pollutant under consideration individually, which limits the comparison of biomarker levels among different areas;
- Using Europe-wide modelling or sampling data at a macro-scale may underestimate the presence of local differences in pollution exposure at a micro-scale, thus not representing a full picture of the actual background exposure of constituents;
- Areas are generally defined on the basis of only one exposure route, such as deposition from air (Figure 4.3a). This may lead to an underestimation of true exposure from all possible sources. However, when it is possible to identify one main source of environmental contamination, it may very well be possible to show the added value of this approach. For example, it may be possible to predefine areas of high exposure to Arsenic based on geological data or FOREGS data on As in soils and river sediments (Meliker et al 2006, Singh et al 2007).

A comparable approach may be possible through the use of health data. Figure 4.3b gives an overview of standardized death rates per 100.000 inhabitants due to malign neoplasms in Europe (Data from Eurostat).

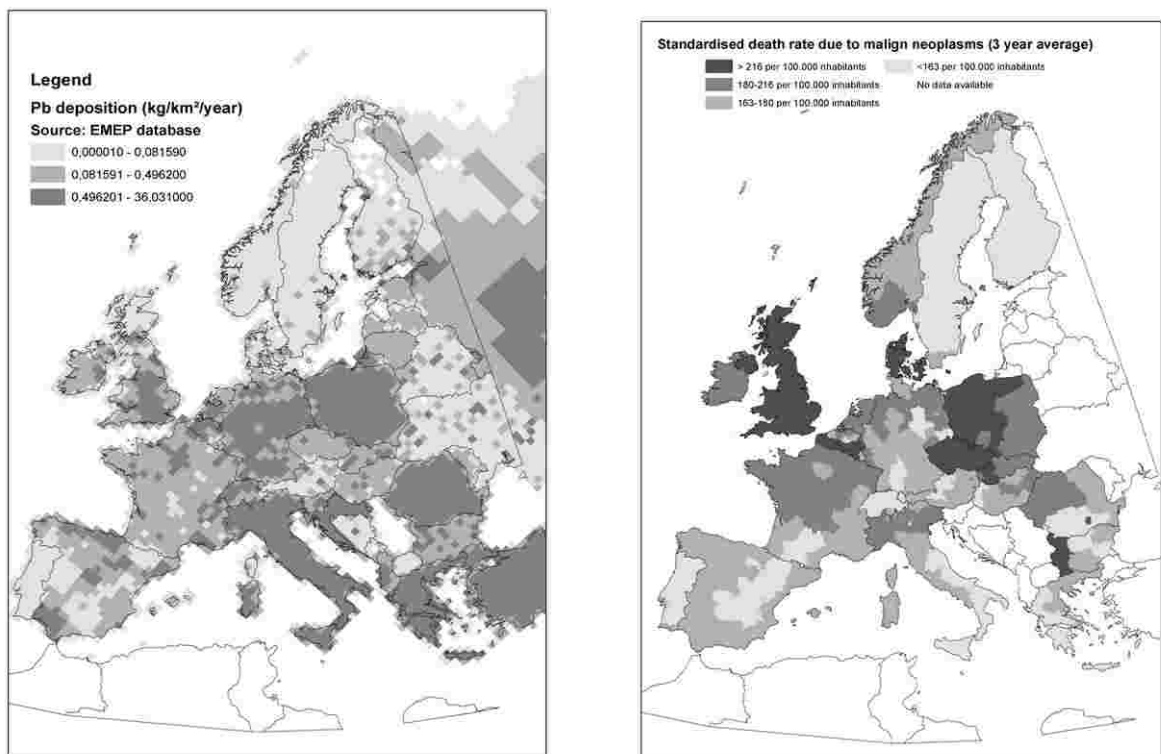


Figure 4.3: Possibilities to identify predefined exposure or health clusters, based on a) Pb deposition data or b) mortality rates due to malign neoplasms

4.5 Grid data

Potentially the most objective way of defining geographical areas in Europe is based on a grid. This approach is to some extent already illustrated in figure 4.3a, where air quality is modelled in the EMEP program, based on a 50x50 km grid overlaying Europe. The advantage of this approach is that it is an objective way to define geographical areas, and that such a grid is a flexible tool to include different levels of spatial detail in one harmonized layer, e.g. by breaking down a single 50x50 km into four 25x25 km grids, 25 10x10 km grids or any other level of detail. Using this hierarchical approach, different levels of geographical information can be included in one single data layer, providing the possibility of simultaneously representing large-scale survey studies and localized hot-spot identification programs.

Drawbacks of this approach are the observation that data gathering and handling may be difficult for grids that include two or more Member States, and that it is difficult to use this type of data for policy advice. Additionally, when one harmonized sampling grid is used, there is a threat of overrepresentation of areas with low populations, and underrepresenting

regions with a high population density, however this drawback may be alleviated by taking into account an appropriate breakdown of sampling grids to smaller or larger areas, as discussed above or use of weighing factors.

4.6 Opportunities for HBM and inspire

The INSPIRE directive calls for a more representative and harmonized use of geographically relevant data on environmental quality in Europe. Although HBM certainly has a contribution to make in this context, issues like mobility, interpersonal variability and especially privacy and ethical considerations, have an effect on the geographical relevance of HBM data. However, different aggregation options are available, which do not overly restrict the applicability of HBM data as a layer of data on environment and health, and which can be included in the INSPIRE spatial data infrastructure. Which type of aggregation scheme is preferable, mainly depends on the spatial scale of the HBM survey, the specific aim of the study, the contaminants under consideration and the availability of high-quality environment and health data.

5 A PROPOSAL FOR (STATISTICAL) ANALYSIS AND LINKAGE OF HUMAN BIOMONITORING DATA

5.1 Introduction

The rationale behind this proposal for (statistical) analysis and linkage plan is the rationalization of the main statistical methods used for the Pilot Project on Human Biomonitoring in Europe and a priori specification of some of the potential research questions which may be addressed by the Pilot Project on Human Biomonitoring. The proposal is primarily based on the statistical analysis plan that was developed for the Flemish Human Biomonitoring Program (2002-2006), with modifications to better suit the purposes of the European Pilot Project on Human Biomonitoring and we have taken over some of the statistical procedures that were outlined in “Protocol for harmonized way of collecting and analyzing selected pollutants and for data management” (Becker et al 2007).

It needs to be stressed that the statistical tests or research questions proposed here, or the (sometimes arguably artificial) break-up of HBM data, are based on common sense, but do by no means intend to be final. The use of alternative tests or other items is open for

discussion and comment by all. The current document only intends to outline the analysis and linkage options and provide a workable document.

5.2 Aim of the Pilot project on human biomonitoring

The main aim of the Pilot Project on Human Biomonitoring is to develop and to test a coherent and harmonized approach throughout Europe by means of commonly developed protocols, strategies and scientific tools ensuring reliable and comparable data, whilst also leading to a more effective use of resources involved. This implies a focus on organizational, technical, logistical and infrastructural feasibility.

Hence, the first step of the Pilot Project is the gathering of comparable data, not only on biomarker measurements, but also on supporting information such as questionnaire data on lifestyle, individual exposure patterns or socio-economic conditions. The development of these procedures has been elaborated on by other Work Packages within ESBIO, and is hence not the intent of this report. However, based on the findings of this report, contributions to the questionnaire development will be made.

This report aims at proposing procedures for a harmonized data analysis, not only to compare biomarker values among different Member States, but also to link as much as possible biomarker values with environment and health data. By defining the planned comparisons before the start of the Pilot Project, there is no immediate need to correct for multiple comparisons (e.g. Bonferroni corrections).

So this document is intended to provide guidelines that come of use once all the samples have been taken, the relevant biomarkers have been analyzed, and the information available from the questionnaires has been stored in the relevant databases. At that point, the laboratory data and questionnaires will be processed in an integrated way, with the aim of addressing the following research questions:

1. Defining provisional reference values for the measured biomarkers in Europe;
2. Comparison of biomarkers of exposure with a European average value;
3. Comparison of biomarkers of exposure among different Member States;
4. Linkage of biomarker values with environment and health data in both Member States and Europe.

5.3 Data analysis at member state level

5.3.1 Exploratory and preparatory statistics

Description of the populations in the whole of the EU and in the different Member States

The sampling population in the EU and in the different member states should be described in terms of age of the mother, age and sex of the child, smoking behaviour of the mother, socio-economic status, nutritional habits and traffic exposure of both mother and child. It is important to gain a detailed insight in the composition of the sampling population in the EU and in the Member States since possible social, behavioural and professional differences among constituents for the different participating Member States may cause considerable differences in exposure and effects. This data is harmoniously gathered through the appropriate questionnaires, which are an integral part of the Pilot Project.

Descriptive statistics for the biomarkers

Descriptive statistics and graphical representation will be used to initially describe the biomarkers and associated data. The chosen statistical and graphical methods are determined by the type of data :

	Categorical value	Continuous value
Descriptive statistics	Proportion, table	Geometric mean, standard deviation, median, percentiles
Graphical representation	Bar or pie diagram	Boxplots, cumulative frequency distributions

At first instance, the descriptive statistics are presented for each of the participating Member States separately. The procedures for doing this have been developed in detail by Becker et al (2007) and will not be fully repeated here. Only a brief overview of their findings will be presented. For more information, we would like to refer to the original document (WP2, Deliverable D2.4). The initial representation of the data per Member State is given in a basic table. Table 5.1 depicts the distribution of pollutant concentrations for mothers and children.

Table 5.1: Distribution of biomarker values (taken from Becker et al 2007)

		LoQ	N	% < LOQ	P ₁₀	P ₂₅	P ₅₀	P ₇₅	P ₉₀	Max	GM	95% CI
Biomarker	Mother Child											

The following parameters are included in the table and are essential for the reporting of the biomarker data:

- LoQ (Level of Quantification): The LoQ represents the chemical-analytical performance of the measurement used. Although chemical-analytical techniques have rapidly evolved and improved in the last decades, there is a limit at which any chemical can be reliably quantified. If a biomarker value is below the LoQ, it will be set to LoQ/2;
- N (number of samples): Generally, the number of samples per Member State is set at 120 mothers and 120 children. However, due to practical, financial or technical reasons, it may be that not the full 240 samples are available. This needs to be indicated;
- %<LoQ: this percentage represents the number of samples that fall below the LoQ, and thus also represents how many values are set at LoQ/2;
- P_x (xth percentile): the percentile is a way of providing estimation of the proportions of the data that fall above and below a given value. The xth percentile is a value such that x % of the observations are below this value, and hence that (100-x) % is greater. The 50th percentile (P₅₀) is the median, the number that divides the higher half of a sample from the lower half;
- Max (Maximum)
- GM (geometric mean): 2 measures of central tendency should be reported, the median (P₅₀, see higher) and the geometric mean (GM). The GM is often applied since the distribution of pollutant concentrations is often lognormal;
- 95% CI (95% Confidence Interval): the 95% CI of the GM is computed to provide information on the precision of the GM.

5.3.2 Confounder correction of raw data - stratification

The interpretation of raw data (i.e. uncorrected data, see further) has to be done with care and proper reservation, since significant differences in biomarker values among different

Member States could be due to differences in the composition of the population (eg. differences in average age of the mothers, gender of the children,...). Hence, alongside these raw data, there is a clear need to use “adjusted” data. An efficient way of dealing with this is stratification, where the dataset is broken up into different strata.

As Becker et al (2007) have indicated, stratification of the data can be based on a number of different parameters (Table 5.2):

Table 5.2: Examples of stratification of data by pollutants

	Pb	Hg	Cd	Cotinine
Women	Age, area	Age, area, fish consumption	Age, area, smoking, ETS exposure	Age, smoking ETS exposure
Children	Age, area, gender	Age, area, fish consumption, gender	Age, area, gender, ETS exposure	Age, gender, ETS exposure

Since the number of samples per Member State is limited, stratification per individual Member State may not be advisable due to the low statistical power of these subdivided datasets. It could be proposed to aggregate Member States’ datasets based on predefined parameters, such as aggregating MS with high, average, and low national fish consumption, geographical area (e.g. comparing Scandinavia, Eastern, Southern, and Western Europe), population density (high, average, low average population density) or a number of other methods. Examples on how data can be geographically aggregated are given in Deliverable 3.3, Chapter 4. Nevertheless, aggregation of Member States should be performed based on the specific research questions for each individual pollutant under consideration.

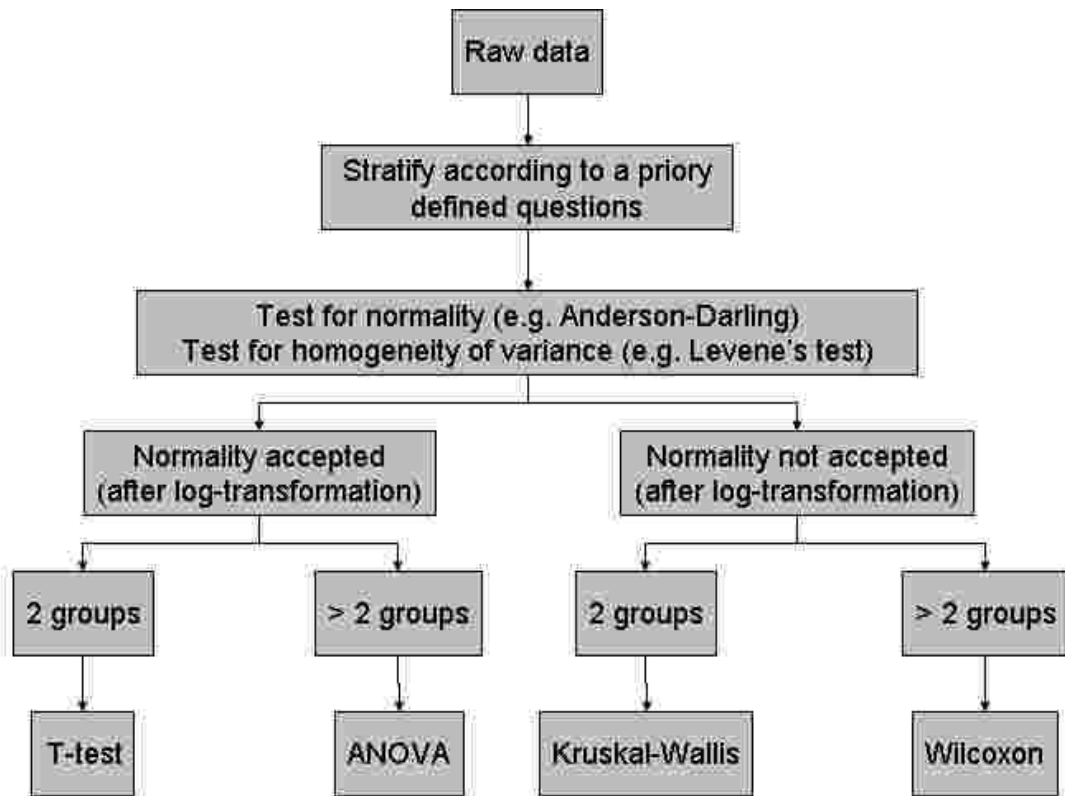
The statistical analysis procedure outlined below is based on Becker et al (2007), and only defines suggested statistical tests (Figure 5.1). Although the proposed tests are often used to perform the statistical operations outlined, and it is preferable to use a harmonised set of tests for all Member States, the use of alternative tests for whatever reason remains open for discussion.

As mentioned already earlier, biomarker data are only seldom normally distributed, so comparison of geometric means should be carried out with the logarithmically transformed concentrations. The assumption of normality should be checked with the appropriate test (e.g. Anderson-Darling or Kolmogorov-Smirnov tests), as must the homogeneity of variance be assessed (e.g. Levene’s test). If normality (of transformed data) is assumed, the differences between the geometric means of the subgroups are preferably tested by a t-test if

2 groups are compared (e.g. “How do HBM values differ with the gender of children”) or one-way analysis of variance (ANOVA) if more than 2 groups are compared (e.g. “How do HBM values differ with the age of mothers”). If the assumptions for normality and homogeneity of variance are not met, the corresponding non-parametric tests (resp. Wilcoxon test for comparing 2 groups, Kruskal-Wallis for comparing more 3 or more groups) shall be used.

For the analysis of paired samples (e.g. “How do HBM values correlate among women and their related children?”), the appropriate test (t-test when normality assumptions are met, Wilcoxon test if assumptions are not met) can be used to evaluate differences.

Figure 5.1: Flow-chart proposing SAPs for comparison of HBM data within MSs.



However, due to the relatively small sample size (120 mother-child pairs per Member State), the break-up of data in too many strata could have a profound effect on the power of the statistical analysis. Hence, caution in the interpretation of the data is required at all times.

5.3.3 Confounder correction of raw data – multiple regression

An additional method to correct for confounding variables is the use of multiple regression equations to simultaneously correct for different parameters. With multiple regression, there is more than one independent variable in the model, which allows for the possibility to study the effect of multiple independent variables at once, when all other variables remain constant. The general equation for multiple regression is:

$$E(Y_x) = a + b_1X_1 + b_2X_2 + b_3X_3 + \dots \text{ for linear regression}$$

$$\text{Log}(p/1 - p) = a + b_1X_1 + b_2X_2 + b_3X_3 \dots \text{ for logistic regression}$$

In these equations, b_1 depicts the effect of X_1 on Y (or p in case of logistic regression), after correction for X_2 and X_3 . The theoretical example presented here is based on continuous parameters, but the multiple regression model can easily be adapted to the use of nominal parameters (such as Member State, Area,...) using dummy variables. These dummy variables take the value of 1 to represent the presence of some quality, and the value of zero to indicate the absence of that quality (for example, smoker=1, non-smoker=0). Ordinal coefficients may indicate age groups (for example, 20-30=1, 31-40=2, 41-55=3).

Of course, it is not possible to study the relationship between the biomarkers and all measured or questioned parameters. Hence, there needs to be an a priori determination which independent variables will be included in the model. This choice needs to be based on literature research and discussions among scientists. The list in Table 3.2 is a good example of confounders that can be included, however this list can be altered by any means following discussion. Hence, the following multiple regression equations can be proposed based on the a priori identification of confounding factors listed in Table 3.2:

$$\begin{aligned} Pb_{\text{corr, w}} &= a + b_{\text{age}} * X_{\text{age}} + b_{\text{area}} * X_{\text{area}} \\ Pb_{\text{corr, ch}} &= a + b_{\text{age}} * X_{\text{age}} + b_{\text{area}} * X_{\text{area}} + b_{\text{gender}} * X_{\text{gender}} \\ Hg_{\text{corr, w}} &= a + b_{\text{age}} * X_{\text{age}} + b_{\text{area}} * X_{\text{area}} + b_{\text{fish}} * X_{\text{fish}} \\ Hg_{\text{corr, ch}} &= a + b_{\text{age}} * X_{\text{age}} + b_{\text{area}} * X_{\text{area}} + b_{\text{fish}} * X_{\text{fish}} + b_{\text{gender}} * X_{\text{gender}} \\ Cd_{\text{corr, w}} &= a + b_{\text{age}} * X_{\text{age}} + b_{\text{area}} * X_{\text{area}} + b_{\text{ETS}} * X_{\text{ETS}} + b_{\text{smoking}} * X_{\text{smoking}} \\ Cd_{\text{corr, ch}} &= a + b_{\text{age}} * X_{\text{age}} + b_{\text{area}} * X_{\text{area}} + b_{\text{ETS}} * X_{\text{ETS}} + b_{\text{gender}} * X_{\text{gender}} \\ Cot_{\text{corr, w}} &= a + b_{\text{age}} * X_{\text{age}} + b_{\text{ETS}} * X_{\text{ETS}} + b_{\text{smoking}} * X_{\text{smoking}} \\ Cot_{\text{corr, ch}} &= a + b_{\text{age}} * X_{\text{age}} + b_{\text{ETS}} * X_{\text{ETS}} + b_{\text{gender}} * X_{\text{gender}} \end{aligned}$$

These multiple regression models can be used to determine average values and probabilities. Based on the collected data, the different parameters in the model (a , b_1 , b_2 , b_3, \dots) are estimated and a prediction for the average values of Y for specific values of X_1 , X_2 , X_3, \dots is made. For example, if X_{age} represents the age of the mother, X_{ETS} the estimated exposure to environmental tobacco smoke and X_{smoking} the number of cigarettes currently smoked, the average expected value of Y can be estimated for every combination of X_{age} , X_{ETS} , and X_{smoking} . Average values for the biomarkers measured that are the result of a multiple regression model are called *adjusted averages*. (hence $\text{Cot}_{\text{corr, w}}$ where w or ch stand for respectively women or child).

The statistical procedures that will be used to analyse differences in these corrected averages among Member States are similar as the ones identified in Figure 3.1.

5.4 Defining provisional reference values or ranges

As a result of the Pilot Project, all participating Member States will be able to generate comparable biomarker values for their country. Bringing all these values together will allow researchers to calculate a “Provisional European Reference Value” (PERV). Since this PERV is made up out of only 120 samples from both mothers and children per Member State, it will be referred to as being provisional. Nonetheless, the PERV and its confidence limits will be a number that reflects the average exposure of the constituents of all participating Member States (i.e. P_{50}), and will preferably be a weighted average. The weight of every Member State will be proportionate to the total population number of each Member State (see Table 5.3). An PERV based on weighted averages per Member State has the advantage of providing a better approximation of the average exposure of all Europeans, since countries with larger populations have a larger influence in this number. On the other hand however, it reduces the contribution of smaller countries, where specific exposure conditions may occur, and where the limited number of 240 samples may provide a better estimate of true exposure for the entire population. It is probably advisable to postpone the discussion on whether weighed averages should be used or not until after an appraisal of sampling representativeness becomes available.

Since special interest in the Pilot Project goes to the identification of regions with high exposure, also a second provisional reference value will be calculated, the High Exposure Provisional European Reference Value (HEPERV). This value identifies the 90th percentile (or 95th as proposed by Becker et al 2007, still under consideration) with its appropriate 95%

confidence intervals and identifies the upper margin of background exposure to any given pollutant. This HEPERV is comparable to the reference value proposed by Becker et al (2007) or other publications (e.g. Ewers et al 1999; Wilhelm et al 2006). These authors define reference values as characterizing the upper margin of the current exposure of the general population at a given time, and usually is the 90th or 95th percentile of the concentration in a representative part of the general population that has no particular exposure profile. We will also refer to this baseline exposure limit, but will address it as High Exposure Provisional European Reference Values (HEPERV). Practically, this means that non-environmental exposure routes need to be taken into account in the calculation of the HEPERV, and a number of exclusion criteria need to be considered. Which these exclusion criteria are, will depend on the chemicals tested in the Pilot Project. For the Scenario 1 chemicals, criteria may include:

- No obvious occupational exposure;
- Only non-smokers (Cd and Cotinin)
- No extensive dental amalgam fillings (MeHg)
- Less than 3 fish consumptions per month (MeHg)
- ...

Other exclusion criteria may be added later, and do not necessarily apply for all different Scenario 1 chemicals. The main advantage of using these exclusion criteria is that the true contribution of the environment on the biomarker values in constituents is measured, but on the other hand it may not be easy to find a sufficiently large study population that fulfils all exclusion criteria. Again, it may be best to postpone the discussion on the application of exclusion criteria until more information on the sampled population becomes available.

Having both PERV and HEPERV values will be informative about the average exposure and about the prevalence of high exposures of the general European population. This may be considered as a first step towards identifying high exposure populations. Member states will be able to compare their own biomarker distributions to the overall European PERV and HEPERV reference values.

Finally, it needs to be stressed that this PERV is only a statistical reference value and does not represent toxicologically derived biological exposure limits. Nothing in itself can be concluded from this PERV, and Member States with biomarker levels above the PERV are not necessarily classified as being at risk, nor do levels below the PERV automatically indicate that there are no health effects possible. The main purpose of these values is to have

benchmarks to identify constituent with increased internal exposure to a defined toxicant (Ewers et al 1999).

Table 5.3: Population statistics for 2005 of all 27 EU Member States (Data source: Eurostat)

	Children (6-11)		Mothers (20-55)		Total population	
Austria	547.133	1,74	2.052.426	1,69	8.206.524	1,67
Belgium	716.286	2,27	2.519.316	2,08	10.445.852	2,13
Bulgaria	404.384	1,28	1.914.996	1,58	7.761.049	1,58
Cyprus	60.726	0,19	194.231	0,16	749.175	0,15
Czech Republic	592.900	1,88	2.597.960	2,14	10.220.577	2,09
Denmark	418.698	1,33	1.269.835	1,05	5.411.405	1,10
Estonia ^a	81.799	0,26	340.050	0,28	1.351.069	0,28
Finland	372.500	1,18	1.062.598	0,88	5.236.611	1,07
France ^a	4.517.116	14,33	15.093.777	12,45	62.130.243	12,68
Germany	4.806.290	15,25	20.014.073	16,51	82.500.849	16,83
Greece	636.736	2,02	2.771.253	2,29	11.082.751	2,26
Hungary	637.575	2,02	2.558.766	2,11	10.097.549	2,06
Ireland	328.065	1,04	1.051.672	0,87	4.109.173	0,84
Italy	3.243.390	10,29	14.366.659	11,85	58.462.375	11,93
Latvia	125.174	0,40	581.330	0,48	2.306.434	0,47
Lithuania	236.868	0,75	866.816	0,72	3.425.324	0,70
Luxembourg	34.765	0,11	114.572	0,09	455.000	0,09
Malta	29.629	0,09	98.243	0,08	402.668	0,08
Netherlands	1.183.971	3,76	4.005.731	3,30	16.305.526	3,33
Poland	2.612.194	8,29	9.993.197	8,24	38.173.835	7,79
Portugal	640.209	2,03	2.678.974	2,21	10.529.255	2,15
Romania	1.350.579	4,28	5.533.103	4,56	21.658.528	4,42
Slovakia	373.365	1,18	1.435.092	1,18	5.384.822	1,10
Slovenia	114.920	0,36	515.989	0,43	1.997.590	0,41
Spain	2.384.995	7,57	11.221.887	9,26	43.038.035	8,78
Sweden	623.070	1,98	2.023.155	1,67	9.011.392	1,84
United Kingdom ^a	4.446.270	14,11	14.354.323	11,84	59.694.353	12,18
Total	31519607	100,00	121.230.024	1,00	490.147.964	100,00

^a Data from 2004

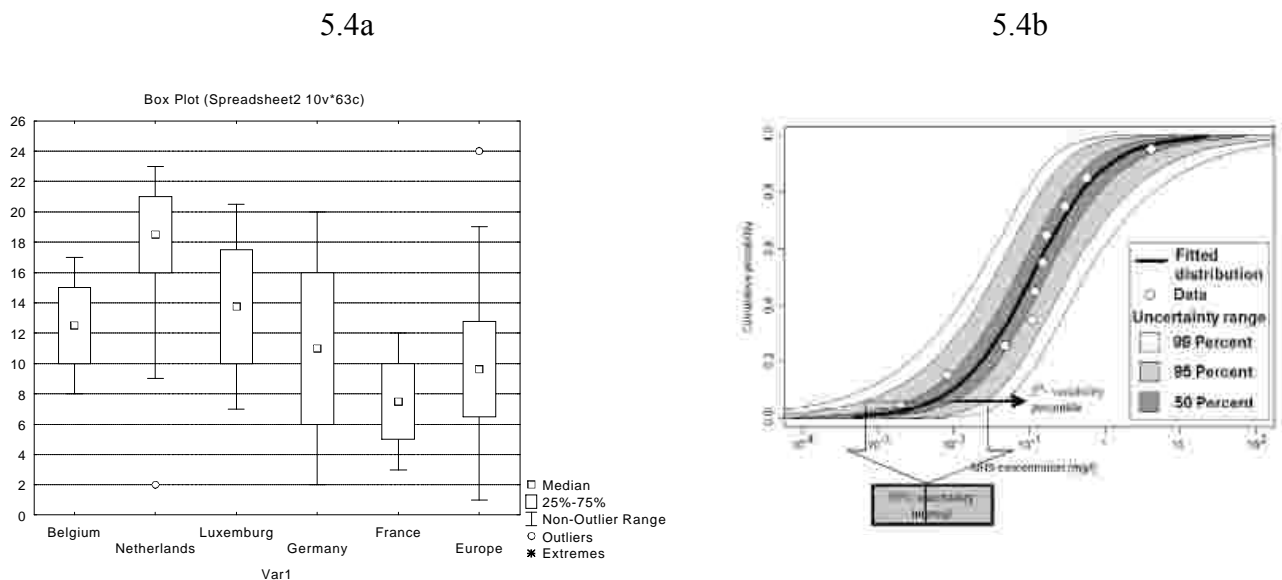
5.5 Comparing values from Member States

5.5.1 Comparing continuous values

Graphical presentation of the data per Member State

A rapid overview of biomarker values per Member State can be given using box plots of raw data or data adjusted for confounders (see chapter 3.3). The advantage of using box plots is that it includes a large amount of information in one graph, and also allows the rapid identification of outliers. A negative aspect might be that when all 27 Member States are included, and also the PERV and/or HEPERV, the interpretation of the box plot may be hampered because of information overload. A possible solution to this is breaking up the data set into different box plots, where every box plot contains the data for one Member State, the European average and the Member State's immediate neighbours. An imaginary example of this type of graph is given in figure 5.4 for Belgium.

Figure 5.4: Example of a) how data per Member State may be represented compared to Neighbours and European averages and b) how the distribution of biomarker values may be represented (imaginary data, for graphical purposes only)



Additionally, the distribution of the biomarker values may be represented using cumulative distribution graphs, for which confidence intervals may be calculated using bootstrapping or Monte-Carlo simulation, assuming for example a log-normal distribution (Figure 5.4b).

Response variables

Because the original biomarker data generally are not normally distributed, the markers of exposure are transformed using the natural logarithmic function. These transformed values are used in the regression models as response variables.

Adjusted averages

Adjusted averages are the averages or proportions per Member State that were calculated, taking into account confounding factors. The confounding factors which have to be taken into account may differ per biomarker, the selection of confounders is based on information in the peer reviewed literature (see further chapter 3.2).

Differences among Member States

To test whether there are significant differences among Member States (after having taken into account the effects of confounding factors), an F-test will be used. If this F-test is statistically significant at the 5% significance level, the following further comparisons will be made:

- The average value for each Member State is compared among the different Member States using ANOVA, and if differences are found, the appropriate post-hoc test to identify differences among Member States;
- The average value for each Member State is compared with the PERV;
- The P_{90} value for each member State is compared with the HEPERV

5.5.2 Graphical representation of differences among Member States

For use in policy support, it is essential that data are readily and easily interpretable, preferably within one graph or table. Hence, significant differences for biomarkers among Member States will be represented with a simple colour code, green implying significantly lower than the PERV, red implying significantly higher than the PERV, and yellow indicating no significant differences. If the HEPERV comparisons indicate significant differences, this will be illustrated by adding an exclamation mark to the relevant table cells. In doing this, it needs to be made absolutely clear that using colours such as green or red do not provide any information on the health effects of biomarker values. It is only a comparison on Member State values with European values. In this context, using green, yellow, and red may not be the most appropriate colours, and others may be suggested.

Not only can data be presented in a tabular form like this, but maps reflecting the same information can easily be developed using this approach.

An example on how this table will graphically look like, is presented below:

Member State	Pb	Hg	Cd	Cotinine
MS1		!		
MS2			!	
MS3	!			
...				

5.6 Bayesian and multivariate statistics - linkage of aggregated data

There is a significant amount of literature available on the link between environmental contamination and epidemiological effects (e.g. Jarup et al 2004; Nuckols et al 2004; . However, the incorporation of dose as the central pivotal point has not often been performed. This is tragically illustrated by the finding of Nuckols et al (2004), who indicated that “we could not find an example of the use of GIS to estimate personal exposure for an epidemiologic study”. However, insights from projects that link environmental exposure, health outcome and risk through integrated geographical information systems should allow us to generate similar procedures for the link between dose and response. At this point, it is very difficult to estimate how, and to what extent, complicated multivariate and spatially explicit models can be applied for the Pilot Project, since this mainly depends on the results of the sampling and analysis of HBM data.

If adequate data of sufficient quality are available for analysis using these sophisticated statistical techniques, projects such as EUROHEIS (A European health and environment information system for exposure and disease mapping and risk assessment, EUROHEIS 2001, 2003) aimed at providing these links among environmental exposure, health and risk assessment will be contacted and hopefully involved in the spatial analysis of HBM data. Furthermore, also within the FP7, there is a call for a Coordinated Action on GIS in Health and Environment (Call ENV2007.1.2.3.2), which might provide room for collaboration with the Pilot Project.

5.7 Exposure-dose-response linkage

5.7.1 Some preliminary notes

It is outside of the scope of the Pilot Project to perform a full-scale epidemiological study on the exposure patterns and health effects of the studied populations. However, in combination with biomarker data, information on environment and health effects can be gathered that may prove very useful in detecting particular exposure patterns, or explicit health effects. Deliverable D3.1 provides a broad overview of available data sources available on environment and health, and highlights some of the gaps and opportunities for exposure-dose-response linkage.

In the following, we will distinguish among two broad groups of linkage opportunities, on the one hand linkage based on geographical entities, and on the other hand linkage based on individual information. The first group mainly uses geographically explicit environment and health data and links this with biomarker data based on geographical overlap. Typical examples of this kind of information is the modelling data for air quality in Europe (e.g. see figure 4.3 or 4.8 in Deliverable D3.1). This type of linkage can be performed by the overlaying of maps and correlating exposure, dose and response data for the relevant geographical entities. Examples of what these geographical entities may look like (NUTS, urban-rural-natural, based on exposure patterns,...) are given in Chapter 4 of Deliverable D3.3.

A second type of linkage is based on individual information. Although this approach does not use individualized exposure, dose or response information, it uses the information gathered in questionnaires to aggregate constituents into distinct groups, such as low, average, or high exposure populations. Linking methylmercury content in hair with the average monthly fish consumption or linking cotinine levels to self-assessed ETS exposure may be examples of this linkage based on individual information.

From the examples given below, it may become obvious that several linkage questions can be situated in both types of linkage (e.g. cotinine in urine may be linked to individual smoking exposure, but may also be linked to National anti-smoking legislation). The difference among both linkage approaches is mainly in scale, where linkage based on geographical entities usually includes Member State or regional data, while individual information obviously is unrelated to geographical scale.

It is important that the research questions that are investigated through these linkage strategies are defined before the actual analysis of the data. This facilitates the analysis of exposure-dose-response linkages and increases the statistical relevance of potential successful correlations (e.g. limits the need for Bonferroni corrections). In the following, we list a number of linkage opportunities which may be tested in the Pilot Project. They are generally selected based on scientific evidence in literature that such links exist. This list has no intention of being complete, and merely offers an indication of the potential of the European Pilot Project on Human Biomonitoring to provide additional information on environment and health issues. It needs to be stressed that the links that may potentially be observed are the result of an investigation with minimal control, and should be interpreted carefully. They should not be used as proof of a causal connection between exposure, dose, and response. However, findings may highlight biomarkers, health effects, or exposure patterns for specific Member States (or regions therein) that may provide guidance for further, more focused, research efforts.

5.7.2 Linkage based on geographical entities

- **Does air or soil quality influence Cd in urine or Pb in blood ?** Through several Europe-wide programs such as EMEP and FOREGS monitoring network on respectively air and soil quality, Europe-wide maps are available describing the distribution of cadmium and lead in the air and soil (See Deliverable D3.1 for more information). Overlaying these maps with HBM data may provide insight to which extend these environmental compartments contribute to the measurement of cadmium in urine or lead in blood;
- **Is seafood quality/quantity related to MeHg in hair ?** The link between the weekly or monthly seafood consumption and MeHg in hair has extensively been illustrated in literature. However, while seafood consumption may be comparable in e.g. Baltic and Mediterranean Member States in terms of quantities, there may be substantial differences in terms of quality of seafood among Northern and Southern European Member States, not only due to differences in fish species but also because of differences in contaminant load of the same species (see e.g. Figure 4.6 in Deliverable D3.1). While the link between quantitative seafood consumption and MeHg may be detected using personal information, the link between seafood quality and MeHg may better be tested for broader geographical areas;

- **Does anti-smoking legislation reduce cotinin levels in urine?** Different European Member States have more or less stringent restrictions with respect to anti-smoking legislation. While some countries are extremely strict in banning smoking from all public buildings, others have not yet included such a radical ban of ETS from everyday life. Linking cotinin in urine to Member State specific anti-smoking legislation may provide insight in the success of this legislation in reducing exposure to ETS.
- **Can a link be detected between metals and asthma/respiratory diseases?** Metals like cadmium and lead have been documented to cause a depression of the immune system and an increased production of immunoglobulines. This may lead to an increased prevalence of asthmatic symptoms or respiratory diseases. While this link obviously can be tested by gathering individual information on asthma prevalence (see further), programs like ISAAC or ECRHS I & II, which gather asthma and respiratory disease incidence at a regional basis throughout Europe, offer the possibility to test this link between HBM and health effects for broader geographical areas.

5.7.3 Linkage of individual information

- **Does quality of housing contribute to increased Cd or Pb levels?** Age and quality of housing often is considered a relevant source of contaminants, for example due to the use of lead-based paint or lead water pipes in older houses. Also house dust may be an important source of environmental exposure, not only for lead but also for other metals such as Cd or As;
- **Does parents' smoking behaviour have an influence on children's cotinin levels?** Cotinine is a biomarker for exposure to environmental tobacco smoke (ETS). It has widely been shown in literature that ETS has a profound effect on several of the health parameters described above, including asthma and allergies, reproductive outcomes and fertility, and neurological development of children (in case of smoking mothers). Hence, also for cotinine measurements, the inclusion of a number of health-related questions in the questionnaire may provide extremely useful information on the link between the measured biomarkers and health effects.
- **Do people suffering from respiratory illnesses have higher Cd or Pb biomarker values?** With some relatively simple questions on the prevalence of asthma in

mother, child, and the rest of the family (husband, other children,...) background information can be gathered which may reflect biomarker measurements. A set of standardized questions that was developed and standardized is available from the European Community Respiratory Health Survey II (screening questionnaire, see www.ecrhs.org for more information). Cadmium has been described in literature as being an immunotoxic chemical (suppression as well as activation of the immune system have been reported). The mode of action of cadmium possibly occurs through the secretion of immunoglobulines and interleukins¹.

- **Are lead and cadmium related to potential fertility problems ?** A reduced male fertility was observed in men that were professionally exposed to lead. This was confirmed by in-vitro studies, where higher lead concentrations in sperm were associated with lowered sperm motility and fertility. An additional mechanism is endocrine disruption, since rats had lower blood androgen levels when exposed to lead. Also for cadmium, in-vitro studies with human cell lines have detected androgen-like effects. Cadmium has also been linked with increased male infertility, yet this may have been due to co-linearity with lead. Cadmium and lead have also been associated with an increased chance of premature birth and/or lower birth weigh.

¹ In the Flemish Biomonitoring Program 2002-2006, significant links between cadmium and lead, and allergic responses such as asthma and hay fever have been described in both mothers of newborns and adolescents (asthma adjusted for smoking and family history, hay fever adjusted for smoking).

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