Institutet för miljömedicin - IMM

IMM-Rapport nr 1/2016

Exposure Assessment

- Challenges and Research Needs

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Exposure Assessment

Challenges and Research Needs

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Institutet för miljömedicin (IMM)

Karolinska Institutet, 2016

Preface

The Institute of Environmental Medicine (IMM; http://ki.se/imm), a department at Karolinska Institutet, is a national center for environmental medicine, with a special assignment to support national authorities in the area of chemical and physical health risk assessment, including the general as well as the work environment. At IMM, interdisciplinary research is performed in the fields of epidemiology, toxicology, physiology and occupational and environmental medicine.

This report is the collaborative effort of several researchers at the IMM who have discussed the chapters and conclusions together. Associate Professor Marika Berglund has been in charge of the project and Dr Ilona Silins has coordinated it. The authors are (in alphabetical order): Tom Bellander, Marika Berglund, Karin Broberg, Ulrika Carlander, Antonis Georgellis, Per Gerde, Olena Gruzieva, Sara Gunnare, Per Gustavsson, Annika Hanberg, Gunnar Johanson, Anneli Julander, Stephanie Juran, Hanna Karlsson, Jolinde Kettelarij, Maria Kippler, Karin Leander, Marie Lewné, Carola Lidén, Petter Ljungman, Johnny Lorentzen, Mare Löhmus Sundström, Klara Midander, Lena Palmberg, Göran Pershagen, Nils Plato, Tove Sandberg-Liljendahl, Ilona Silins, Mattias Sjöström, Marie Vahter, Pernilla Wiebert, Kerem Yazar, Johanna Zilliacus and Agneta Åkesson.

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1.Introduction

1.1 Background

Exposure assessment is an important component of health risk assessment, risk management, status and trend studies, and epidemiological investigations. In risk assessment exposure data is required in order to estimate the level of risk associated with a certain level of exposure while risk management involves deciding on the acceptable level of risk. In status and trend evaluations exposure data allow follow-up of measures taken to reduce exposure and risk. In epidemiology exposure data is evaluated in relation to the occurrence of a disease, or a precursor thereof, in the general population and/or among specific groups in attempt to establish causal relationships.

Exposure can be defined as the contact between a chemical, physical or biological agent and a target, and exposure takes place at the point of contact, while the resulting *dose* is the amount of the agent that enters the target by crossing an exposure surface or absorption barrier (primarily the skin, lining of the respiratory tract, and wall of the gastrointestinal tract) of an organism. Exposure is sometimes referred to as *external exposure* and dose as *internal exposure*.

Exposure assessment has been defined as the process of estimating or measuring the magnitude, frequency, and duration of exposure to an agent, together with the number and characteristics of the population exposed (WHO/IPCS 2004). Ideally, describing the sources, pathways, routes, and uncertainties in the assessment (WHO/IPCS 2004). This definition is also used by the US Environmental Protection Agency (https://www3.epa.gov/).

Major sources of exposure include industrial and agricultural activities, transport and energy production, and waste management, but there are also natural sources, e.g. volcanic eruptions and elements that occur naturally in groundwater used for drinking. Other sources of exposure are related to occupation and/or workplaces.

The relationship between these sources of exposure, dose and effects, is illustrated in Figure 1.1 Individuals may be exposed through contaminated ambient air, food, drinking water, sediments, soil, dust and particles, consumer products and building materials. The major routes of exposure are inhalation, ingestion, dermal exposure and placental transport. The dissemination, transformation and fate of environmental contaminants, as well as physiological (e.g., age and body weight) and other individual factors (exposure factors), related to habits and life-style, will influence both exposure and dose.

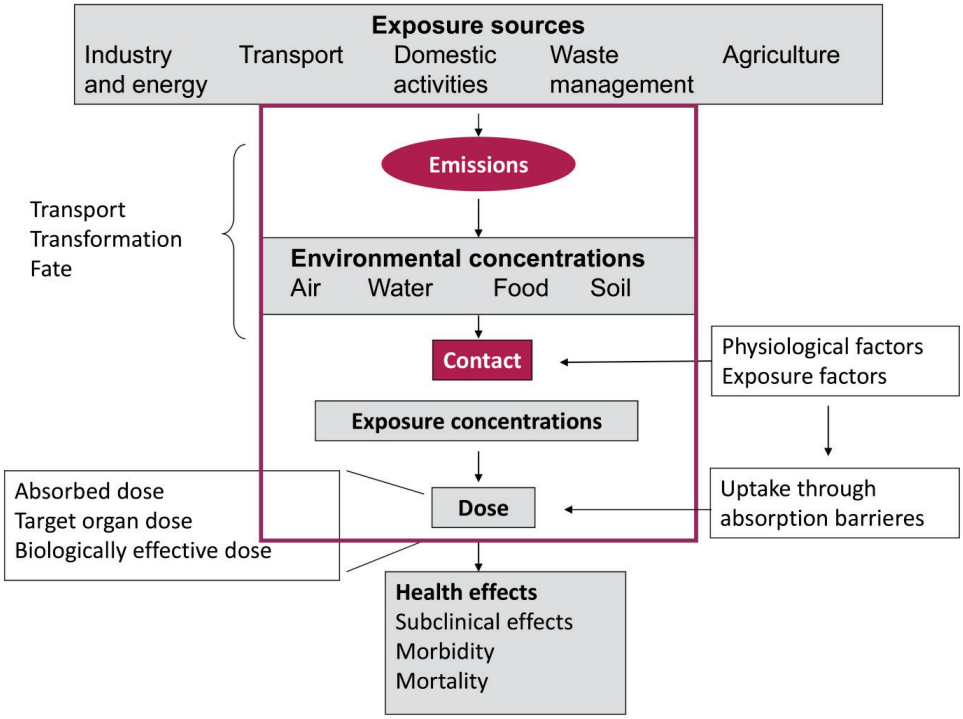

Figure 1.1. The framework for exposure assessment (indicated by the red square).

Exposure assessment has a long tradition. One historical example involves the professional tasters at the Roman court, who consumed some of the food to be served to the emperor to see if it was poisoned. Another example is the use of canary songbirds to monitor carbon monoxide in British mines during the 18th century. As early as in the 17th century, the Italian physician B Ramazzini recognized associations between certain types of occupational exposures and particular diseases, for example that potters became anemic and suffered from palsies due to exposure to the lead salts they used for glazing. An early example concerning the general population was the observation by John Snow in the 1850's that the risk contracting cholera was associated with a disease-causing agent in a certain source of drinking water. He indicated the cholera cases on a map of London and realized that the cases lived in houses served by one of the drinking water sources.

Obviously, the ultimate aim of exposure assessment is prevention. Systematic analysis of occupational exposure and health effects began in the 1950's. At this same time, a series of disease outbreaks drew attention to the fact that environmental pollution can cause severe health effects, including death in the general population as well. For instace, in Minamata the population of fish-eaters developed severe neurological symptoms and

children exposed during fetal development were born with severe brain damage, called the Minamata disease. It was later realized that these effects were due to industrial emissons into the bay next to the village of high levels of mercury. During London fog when air pollution reached extremely high levels due to cold weather, it was noticed that the number of deaths was closely correlated to the level of the air pollution. Thereafter, environmental exposures of the general population and potential associated health effects have been of considerable concern.

1.2 Key considerations in exposure assessment

Temporal and spatial factors are important aspects of human exposure, as are habits, lifestyle and socioeconomic status. Individuals are exposed continuously or intermittently to various agents of various intensities throughout life, over time periods of various lengths, depending on activities and habits, and where they spend their time. Exposure takes place for a specific time period which can last for minutes/hours (acute exposure), days, years or life-long (chronic exposure).

The extent of exposure varies among population and, moreover, certain individuals who are more susceptible, as well as others who are more heavily exposed run a higher risk for detrimental effects. In general, periods of rapid development, i.e., in utero and during childhood and puberty are time windows of increased susceptibility and exposure during such a period may have long-lasting effects. Thus, the timing of the exposure assessment is important. Other important temporal aspects include the period that elapses between exposure and effect, as well as between measurement of exposure and the expected or monitored effect on health.

1.3 Various approaches to exposure assessment

Approaches to the exposure assessment generally involve indirect or direct measurements (US EPA 2016). Indirect methods include the use of questionnaires, mathematical estimations based on environmental concentrations of substances in combination with information on rates of contact, and modelling. Direct approaches include individual measurements (personal monitoring) and biomonitoring. These methods range from simple qualitative to more quantitative approaches. The detail and quality required for the intended use of the exposure data obtained should guide the choice of study design.The aim should be to reduce uncertainty while increasing or taking the actual variability into account.

Groups or individuals in a population can be classified as exposed and non-exposed (e.g. smokers and non-smokers). The classification can be based on for example geographical location, type of work or questionnaire information. Exposure from different exposure scenarios or situations can be quantified using mathematical models, which in their simplest form can be based on environmental measurements (e.g., concentrations of air

pollutants) in combination with assumptions or default values concerning rates of contact (e.g., volume of air inhaled/time unit). Refinement of such models requires more specific information on environmental concentrations and individual rates of contact, as well as consideration of patterns of activity and exposure distributions for different groups of the population. An extensive database on the patterns of human activity (behavior and time use) and exposure factors (i.e., contact rates for air, water, soil, etc.) that can be utilized to quantify exposure is provided in the Exposure Factors Handbook (US EPA 2011). Probabilistic or mixed exposure models include distributional data and thus, take into account individual variation, resulting in probability distributions of exposure.

Methods for the assessment of previous exposure (reconstructive exposure assessment) include the use of questionnaires asking for past exposures, but also human biological monitoring (HBM) and measurement of biomarkers of exposure (e.g., a chemical or metabolite). By measurement of biomarkers in biobanked human samples the exposure at the time of sampling can be determined. It is nessesary to know the half-time of the chemical of interest in the biological media, and have some basic knowledge of its metabolism, in order to be able to translate a concentration into exposure. Knowledge of a chemical's fate in the body can be translated into physiologically based pharmacokinetic (PBPK) models, and such models can help predict the absorption, distribution, metabolism and excretion (ADME) of chemicals in humans, and vice-versa to calculate the resulting dose from a given exposure.

Development and use of accurate and rapid systems for testing chemicals and for risk assessment are sorely needed. The major challenge is to extrapolate actual human exposure to experimental settings (in vitro and in vivo) and vice-versa.

1.4 Challenges associated with exposure assessment

Traditionally, exposure assessment is performed for one agent at a time, but the need for combined exposure assessment has become more obvious considering the omnipresence of chemicals, environmental factors and pollutants. People are exposed simultaneously to numerous environmental factors, multiple chemicals and pollutants from multiple sources, including consumer products (such as electronics, toys, clothes, and cosmetics,) and building material, as well as food, air, water, soil and dust. In general, little is known about how often and to what extent humans and their environment are exposed to various chemicals and/or mixtures thereof or how such exposure may change over time.

More effective methodology for assessing the risk associated with combined exposure to multiple chemicals has been proposed by the WHO, EU scientific committees and the U.S. EPA (WHO/IPCS 2009; EU 2012; U.S. EPA 2003). *Combined exposure* refers to exposure to multiple chemicals by a single route and exposure to multiple chemicals by multiple routes (Meek et al 2011). WHO/IPCS suggested a tiered approach based on a hierarchical (phased) procedure involving integrated and iterative consideration of

exposure and hazard at all phases, with each tier being more refined (i.e., less cautious and more certain) than the preceding one, but requiring more labor and data (WHO/IPCS 2009).

The scientific committees SCHER, SCCS, SCENIHR established by the EU have concluded that in light of the virtually infinite number of possible combinations of chemicals to which humans and other species are exposed, some form of initial screening that allow focus to be placed on mixtures of most potential concern is necessary. These experts also concluded that at present regarding risk assessment of chemical mixtures, a major gap in knowledge is the lack of information concerning exposure (EU 2012). The US EPA has gone further, including both chemical and non-chemical stressors; considering how to group chemicals with similar toxicity and co-exposure as well as taking uncertainties in the assessment into account (US EPA 2007).

The development of biomarkers is one avenue to improving assessment of exposure, susceptibility and occurrence of disease in connection with both environmental and occupational epidemiology (Wild 2005). The omics techniques, including transcriptomics, proteomics, and metabolomics, may prove valuable in this context, although this remains to be seen. The major challenge is to identify biomarkers that are sufficiently sensitive and specific indicators of low-level exposure in human populations.

The *exposome* concept, analogous to the genome, was launched some years ago to draw attention to the critical need for more complete environmental exposure assessment in epidemiological studies (Wild 2005). This concept encompasses total exposure to both environmental and life-style factors, from conception to death, (Figure 1.2), reflecting the need to develop methods with the same precision for an individual's environmental exposure as there are for the individual's genome (Wild 2005). Although sometimes limited to exposures that can be assessed with biomarkers, the exposome should include all types of exposure regardless of their most relevant mode of assessment, including lifestyle factors and factors assessed employing questionnaires, environmental measurements and models.

In light of the considerable variability in datasets, concerning e.g., exposure levels, biomarker analyses, the half-lives of chemicals in the body, the time frame of exposures, etc., prospective studies of the exposome and repeated within-subject assessment of time-varying exposures are warranted. One of the first attempts along these lines is the Human Early-Life Exposome (HELIX 2015) project, a European collaboration that aims to characterize the exposomes of children as they develop through early life (Vrijheid et al. 2014).

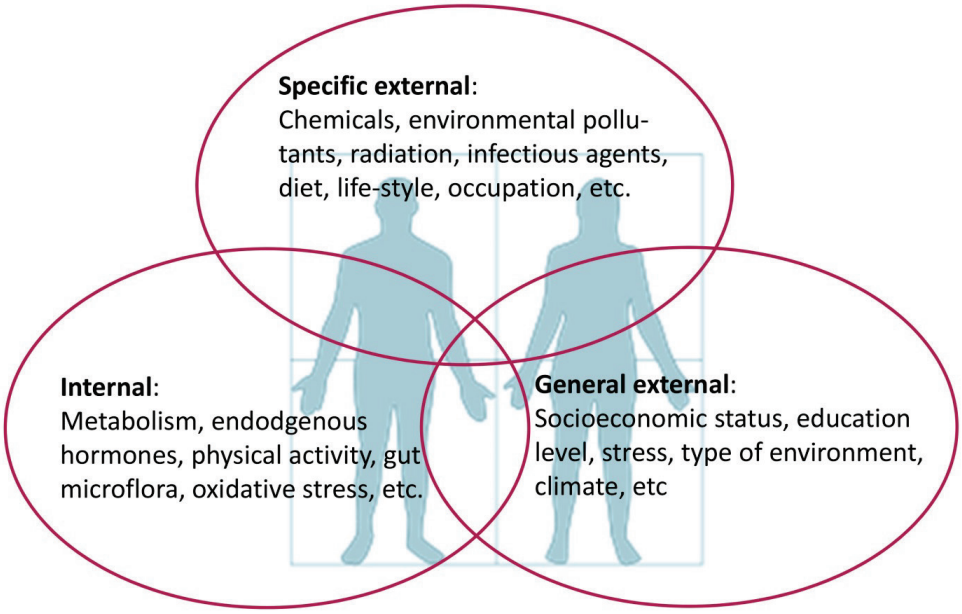

Figure 1.2. The three different domains of exposure involved in the exposome (adopted from Wild 2012).

1.5 Scope and focus of the current report

There is a growing need for accurate data on exposure in the regulatory area for future informed policy decisions as well as in health risk assessment and human health research, and accordingly, development of harmonized and standardized approaches for human exposure which are based on innovative, and state-of-the-art monitoring and modelling of environmental stressors are in high demand.

The present report is by no means comprehensive, but points out certain important areas of exposure science where research, development and innovation are needed. We describe the state-of-the-art and future trends for some urgent areas of exposure research. The main focus is on human exposure to environmental chemicals and physical agents in the general as well as the occupational environment. The target readers are administrators at national authorities that deal with risk assessment and risk management and associated regulations in their daily work, as well as members of research councils that support research in environmental medicine, risk assessment and public health.

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2. Human Biomonitoring

Authors: Marika Berglund, Agneta Åkesson

2.1 Introduction

Human biomonitoring (HBM), i.e., measurements of chemicals and biomarkers (metabolites, products, adducts, etc.), in human material (blood, urine, saliva, etc.), is one approach to estimating human exposure and dose. Biomonitoring integrates all routes and sources of exposure, while also taking into account individual variations in life-style and other factors, such as age, gender, body weight and individual susceptibility. The challenge is to link measurements of biomarkers both to exposure (sources, pathways and routes) and adverse or early health effects (key events along the pathway from exposure to disease). This report is concerned primarily with the use of HBM in connection with exposure assessment.

The value of HBM as a tool for assessing overall exposure of humans to single or multiple chemicals in population monitoring at national level, risk assessment, public health surveys and epidemiological research is becoming increasingly apparent. During the past decade numerous long-term trends in the concentrations of contaminants in populations have been published indicating the likely environmental consequences (Dong et al, 2015). As new and improved methods for analyzing low internal doses of chemicals with high precision have been developed, along with growing laboratory capacity and reductions in cost, the numbers of research studies and investigations that include HBM have accelerated. For example, biomonitoring is increasingly applied in large national prospective cohorts coordinated by the Institute of Environmental medicine, such as SMC, COSM, 60yo and BAMSE. Furthermore, physiologically-based pharmacokinetic (PBPK) modeling, describing absorption, distribution, metabolism and excretion (ADME) of chemical substances in biological systems, can be used for exposure reconstruction, and biomonitoring can help develop PBPK models that understand the ADME process.

2.2 Methodological considerations

Blood and urine have been widely used for HBM measurements of a large number of chemicals and their metabolites. For ethical and practical reasons, non-invasive procedures are sometimes preferred, and matrices such as saliva, hair, nails, breast milk, and feces can sometimes be used. In certain cases the dose absorbed is closely correlated to the level of exposure; however, some chemical-specific, physiological, or pathway-specific factors may result in significant differences between intake and absorbed dose. For example, certain chemicals are rapidly metabolized and excreted, while others bioaccumulate in adipose tissue. Therefore, the application of HBM in exposure

assessment requires basic knowledge of the metabolism of the specific chemical of interest to relate concentration in a biological medium to the level of exposure (Figure 2.1).

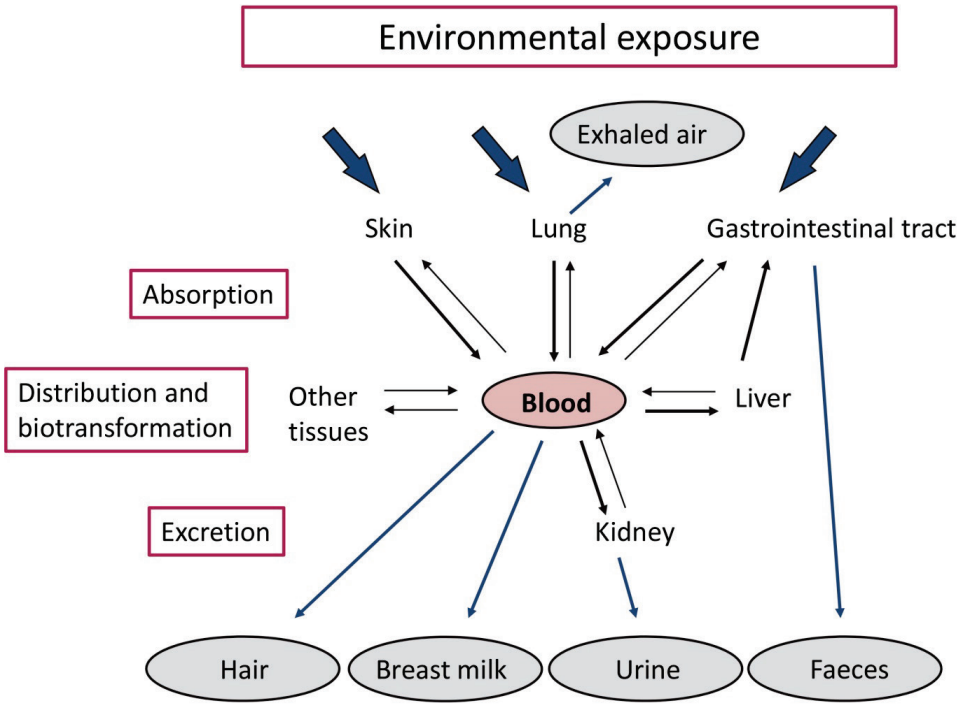

Figure 2.1. Chemicals and their metabolites can be detected in a variety of biological media such as blood, urine, hair, breast milk, or exhaled air.

A crucial factor in conncetion with HBM is the availability of high-quality, validated, high throughput analytical methods. To ensure that the biomarker data obtained are correct, validation and careful control of all all steps, from the collection of samples to evaluation of the analyses are required. Quality control measures should include the collection (time-point, collection equipment, transport), and handling of the samples (transport, treatment, sub-sampling, and storage), as well as the entire chain of analytical procedures. Unfortunately, it is still more the exception than the rule that appropriate tests of quality are performed and/or reported along with biomonitoring data.

There is a continuous need for methods to analyse “novel” or emerging chemicals. Screening for new substances and establishment and optimization of analytical procedure for non-targeted analysis to trace the occurrence of non-identified substances is a new area of exposure science.

As with any exposure assessment technique, biomonitoring offers advantages and disadvantages. The advantages include reducing the number of assumptions made regarding contact with and consumption rates with an exposure media. Furthermore, HBM integrates all routes and sources of exposure, and may thus reveal high end exposures not anticipated or neglected by indirect aggregate exposure assessments or models. HBM also allows identification of emerging chemicals and in particular vulnerable populations.

Although certain biomarkers reflect long-term, sometimes even life-long exposure, many reflect only a snapshot at a given time, which is a disavantage. However, the same is true for other procedures for assessment of exposures based on the concentration of a chemical(s) in an environmental sample or product at a given time, e.g., measurements in a certain type of food at a specific point in time combined with data on consumption rates.

2.3 Variations in human biomonitoring data

The accurate use of HBM for characterization of exposure requires an understanding of factors other than the magnitude and frequency of exposure that may influence biomarker concentrations, such as variations in fundamental physiological parameters (e.g., age and body weight). Another important source of variability involves timing of sample collection relative to the exposure event. For any repeated constant exposure, variability is greatest when the half-life of the biomarker is short and exposure infrequent (Aylward et al 2014).

One substance with a short elimination half-life is BPA, which is excreted rapidly and completely in the urine with a half-life of approximately 4 - 6 hours (Volkel et al., 2005, 2008). Thus, the relationship between the elimination half-life and the intervals between exposure events may markedly affect intraindividual variation in biomarker concentrations. For chemicals with extremely long half-lives, such as persistent organochlorine compounds, interindividual variation in biomarker concentrations is influenced strongly by historical patterns of exposure (Ritter et al., 2009, 2011).

In most investigations, the level of biomarkers in a single biological sample, typically blood or urine, collected at a specific time-point is measured. With biomonitoring involving the occupational environment, some information about the timing, duration, and perhaps magnitude of exposure is often available. However, in the case of general populations, there is usually little or no information concerning when the previous exposure(s) to the chemical(s) of interest occurred. The interplay between chemical-specific toxicokinetics (bioavailability, accumulation, metabolism, excretion) and the time that elapsed between previous exposure(s) to a given dose and sampling determines the concentration present in a single sample. Repeated sampling can help ensure that the

levels of biomarkers reflect actual exposure rather than errors in measurement and/or temporal or metabolic variations.

Dilution of the urine, excretion of creatinine and the content of blood lipids all contribute to variations in biomarker concentrations, as do differences in metabolic rate and other factors that influence the absorption and/or excretion of a compound (Aylward et al 2014). Disease, such as kidney dysfunction may also alter the relationships between exposure and urinary concentrations of biomarkers, thereby, contributing to variation.

2.4 Human biomonitoring in connection with risk assessment and management

Human biomonitoring surveys can be interpreted for purposes of risk assessment and management on the basis of HBM guidance values, which are currently developed by Germany and the United States. The German Human Biomonitoring Commission, established in 1992, defines statistically based reference values; e.g., the 95-percentile of an exposure distribution (RV95), as well as health based HBM values, based on toxicological and epidemiological data (Schultz et al., 2007). Reference values should ideally be based on representative samples of the general population (Schulz et al 2012) and are used for comparison with the exposure levels of individuals or specific population groups.

In the United States, Biomonitoring Equivalents (BE) are developed. These values extrapolate established health-based guidline values (e.g., tolerable daily intake values; TDIs, and reference doses; RfDs) into biomarker concentrations (Hays et al, 2009; Angerer et al., 2011). HBM surveys in combination with HBM values or BEs allow initial screening of population exposure to chemicals, thereby allowing risk assessment under real-world conditions, assisting in priority setting and ultimately improving decision making and risk management.

2.5 Human biomonitoring in connection with environmental and nutritional epidemiology

HBM is being used to characterize exposure in epidemiological studies. High-throughput analytical techniques along with biomarker research, increased laboratory capacity and reductions in analytical cost have accelerated the numbers of research studies and investigations that include HBM. The many biomarkers currently utilized in population studies to estimate exposure or adverse health effects are highly heterogeneous in nature. Some are stable over time, while others have very short half-lives. Some markers can be measured easily with standard laboratory techniques while others require specialized techniques, equipment, etc.

2.5.1 Biomarkers

Biomarkers are typically divided into three categories (Schmidt 2006):

- Exposure biomarkers reflect the actual internal dose resulting from the exposure.
- Effect biomarkers reflect health impairment or recognized disease, early precursors of disease, or peripheral events that predict health impairment.
- Susceptibility biomarkers include intrinsic genetic or other characteristics, such as single-nucleotide polymorphisms, or preexisting disease that result in an increase in the internal dose, the biologically effective dose, or the responses of target tissue(s).

In addition, biomarkers of the biologically effective dose assess the interaction of toxicants with molecular targets such as protein receptors, while others may help predict the risk of future disease.

For purposes of environmental health, biomarkers include a range of indices related to exposure, such as levels of pollutants in tissues and bodily fluids; changes induced in cells or proteins, DNA, and/or other molecules; and inherited genetic variations that influence individual responses (Schmidt 2006). Epigenetic changes caused by environmental factors serve as historical biomarkers of exposure; and while singlenucleotide polymorphisms (SNPs), simple inherited genetic variations, can elevate or lower susceptibility to disease from environmental exposures. In a broad sense, biomarkers also include physical parameters that are clearly associated with a disease or class of diseases. In medicine, these include elevated heart rate and high serum levels of cholesterol, both of which correlate directly with the risk for cardiac disease.

Biomarkers not only allow monitoring of exposure, but also detection of subclinical early effects on health. Ideally, a biomarker should be sensitive, specific and biologically relevant, as well as feasible, practical, and inexpensive to measure. However, these criteria are seldom all met and most biomarkers involve compromise.

The present chapter focuses on biomarkers of exposure, but since the ultimate aim of exposure assessment is prevention, the information on exposure must be coupled to a (known) level of risk or an associated effect. In other words, measurements of biomarkers should not be considered by themselves, but, ideally in combination with both the exposure(s) and an effect.

Identification of biomarkers of exposure is often difficult, since most chemicals are metabolized in the human body. For many compounds, the dominant metabolic pathways have not yet been unraveled, but research on important metabolites as potential biomarkers is growing.

2.5.2 Estimating exposure to food contaminants

The use of biomarkers in epidemiological studies aiming to assess associations between deleterious health effects such diseases and exposure is in general quite costly. Limited availability of stored human samples, expensive analytical procedures and the large

number of samples required for sufficient statistical power all contribute to these high costs.

As a complementary approach, since food is the major source of exposure to a wide range of environmental pollutants (e.g., several toxic metals and persistent organic pollutants), extensive effort has been focused on estimating such exposures via the diet instead. The idea is to link available information on the detailed food consumption obtained in large prospective cohorts to the concentrations of the contaminant in question in all commercially available food products. Such databases are required for each individual cohort and are dependent on that food monitoring programs are available and can provide large quantities of high quality data. They can then be linked to information on food consumption collected with validated instruments such as food frequency questionnaires, dietary records or repeated 24-h recalls. Altogether, this approach allows highly cost-effective assessment of exposure – disease associations in large populations.

A prerequisite for this type of study is that the diet is the predominant source of exposure, with contributions from other sources, such as inhalation, food packaging materials or cooking activities preferably being low. If present, potential trends in the concentration of the contaminants over time need to be taken into account. The crucial question, of course, whether the level of the dietary exposure estimated in this manner provides a valid assessment of exposure.

In contrast to essential nutrients, food contaminants are not an intrinsic part of the food, resulting in larger within food variation in the concentration. Thus, to be able to use the dietary exposure, the estimated levels of the exposure to the specific contaminant under study needs to be validated against relevant human biomarkers of exposure (Bergkvist et al., 2012). Nevertheless, measurement errors in dietary assessment and subsequent misclassification of exposures are inevitable, and this may be especially true for food contaminant exposures due to the larger within food variation. A strength here, however, is that the estimated dietary exposure is not influenced by the disease being monitored, which the levels of exposure biomarkers may very well be. One well-known example of the latter is that blood lipids, which are linked to cardiovascular risk, may also influence the distribution and partition of persistent organic pollutants such as PCBs in plasma, representing reverse causality.

Associations between components of the diet and disease cannot be considered primary if these simply reflect differences in total energy intake resulting from differences in body size, physical activity and metabolism. Such individual differences result in variations in the intake of specific dietary components unrelated to dietary composition since the consumption of most nutrients, as well as contaminants is positively correlated to total energy intake. Accordingly, adjustment for energy intake is standard practice in nutritional epidemiology (Willett and Stampfer, 1986). Such adjustment also reduces the artificial interindividual variation introduced by under and over-reporting of food intake.

For these same reasons, energy-adjustments of dietary exposure to contaminants need to be considered.

2.6 Human biomonitoring programs

Human biomonitoring, which has become a primary tool for characterization of chemical exposure in a wide variety of contexts, including population monitoring at national levels, poses a number of challenges concerning, for example, study design and ethical considerations. Understanding public perceptions and developing effective methods for communicating and interpreting results also remain areas of interest and active research.

Once every two years the National Biomonitoring Program (NBP) of the CDC (Centers for Disease Control and Prevention) in the United States monitors exposure of the population to an increasing number of factors, to date, more than 450 chemicals and nutritional indicators (CDC 2009; 2015). Since 1999, the National Health and Nutrition Examination Survey (NHANES), an annual assessment ofthe health and nutritional status of 5,000 representative adults and children in the United States, conducts interviews and physical examinations, and collects specimens for the NBP. The ranges of exposure, which can be used as references by physicians and scientists to determine whether an individual or group is subjected to unusually high exposure, are based on these measurements. These data also help monitor the impact of public health interventions.

In Europe national HBM-programs have been initiated, in e.g., Germany, Belgium, the Czech Republic and Austria (Angerer et al. 2011). The Health-related Environmental Monitoring program (HÄMI), coordinated by the Swedish Environmental Protection Agency includes both population-based and personal exposure measurements as well as HBM studies, focused on vulnerable groups. The program has been in place since the 1990's and covers a wide range of environmental pollutants and chemicals.

2.6.1 The initiative for harmonization of HBM on the European level

The EC has recognized the need to harmonize the procedures and programs of HBM to enable more coordinated design and development, more effective use of limited resources, attainment of higher quality data and improvements in general. Such harmonization would allow better utilization of biomonitoring data to answer questions concerning chemical exposures and potential health effects, respond to community concerns, improve environmental and public health, and to inform policy.

Therefore, the European Human Biomonitoring Initiative (EHBMI) was launched, on the national and European level, by the Eueopean Commission, and is planned to be carried out in collaboration with various European agencies and directorates, national ministries and experts in HBM. This initiative for the development of a sustainable and rational approach to human biomonitoring is designed to establish a joint European programme for monitoring and scientific assessment of EU citizens, including occupational exposure

and potential impact on health. Based on experience gained in connection with previous activities undertaken by the EU (e.g., the COPHES/DEMOCOPHES projects), as well as at national levels, this programme will allow harmonized and comparable HBM information on chemical exposure in Europe, to be linked to sources of exposure and health effects.

Through IMM and the Swedish EPA, Sweden is participating in the EHBMI and currently preparing an application to be submitted in April of 2016. If approved, this initiative will be linked to the Swedish health-related environmental monitoring programme, thereby strengthening the links between this programme and environmental health research.

2.7 Research and developmental needs

Human biomonitoring has become a primary tool for chemical exposure characterization in a wide variety of contexts. The value of HBM as a tool for assessing overall exposure of humans to single or multiple chemicals in population monitoring at the national level, in risk assessment, public health surveys and epidemiological research, as well as in PBPK modelling, has become increasingly apparent. A harmonized HBM will better utilize the biomonitoring data to answer questions about chemical exposures and potential health effects, respond to community concerns, improve environmental and public health, and to inform policy. A crucial factor in HBM is the availability of high-quality, validated, high through put analytical methods, and analytical methods for "new" or emerging chemicals.

There is a need for

- harmonization of HBM on both the national and European levels.
- development of reference values and health-based guidance values for purposes of risk assessment and management.
- development of biomarkers suitable for quantifying combined exposures.
- improved methodologies for high-through-put analyses, including non-target analysis.

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3. Epidemiology and Exposure Assessment – some general aspects

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3.1 Introduction

Epidemiology, commonly defined as the study of the occurrence and determinants of health-related states or events, can either be descriptive or analytical. Assessments of exposure are central to most research of this kind. The term exposure is usually applied to any factor that may be associated with an outcome of interest. The theoretical definition of an exposure may, however, be different from the operational definition.

Analytical studies should not only assess the exposure of interest but also potential co-varying exposures, which may constitute confounding factors (factors that disturb correct estimation of the association if not taken into account). Exposures that could potentially modify the effect of the factor of primary concern may also be important to assess.

This chapter discusses general aspects of exposure assessment in epidemiological studies, with particular focus on the accuracy of these assessments. Assessment of multiple exposures, with or without hypotheses concerning interactions is also discussed briefly.

3.2 Response rates in epidemiological studies

For population surveys designed to describe the prevalence of exposures, obtaining a high response rate is crucial to the validity of the results, the obvious risk being that exposure to certain factors under investigation may differ between non-respondents and respondents. Epidemiological research that attempts to elucidate causal relations between exposures and illness also requires high response rates, especially when generalization to an external population is desired. Such generalizability is usually referred to as external validity. A study characterized by low external validity may nonetheless be capable of providing information about associations between exposure and illness, if its internal validity (i.e., the validity of conclusions pertaining to the specific study population) is high and all potentially relevant levels of the exposure being examined are well represented in this study population. The definition of a high response rate is somewhat arbitrary, but traditionally, a participation of at least 70% in epidemiological studies has been considered satisfactory (Rothman 2012).

Participation in population surveys appears to be declining worldwide (Mindell et al. 2015), regardless of whether postal questionnaires, telephone contact via public directories, or random digit calling is employed (Galea and Tracy 2007). This problematic situation may lead to increasing selection bias, meaning that they under- or

overestimate the exposure distribution in the population. Furthermore, the decreasing participation rates may lower the accuracy of analyses of time-trends regarding exposure distribution. In turn, this situation may reduce the quality of information on which health care planning, including efforts at prevention, are based.

Some examples of recent Swedish surveys associated with declining response rates are listed below.

- Nationella folkhälsoenkäten, an annual national questionnaire-based population survey among inhabitants 16-84 years of age aimed at describing factors associated with health and lifestyle. In 2014 the response rate was 52%, versus 61% in 2004.
- Miljöhälsoenkäten, a questionnaire-based population survey performed regularly among children and adults (the focus alternates) and designed to describe exposures and health. The response rate in the most recent survey, performed in 2015 among adults 19-81 years of age, was 42% (personal communication by Niklas Andersson) versus 72% for an analogous survey in 1999 (Miljöhälsorapport 2001). The response rate of a survey performed among children in 2011 was 51% (Miljöhälsorapport 2013) versus 71% in 2003-2004 (Miljöhälsorapport 2005). These response rates rose with the age of the parent/legal guardian and fell with increasing age of the child (Miljöhälsorapport 2005, Miljöhälsorapport 2013).
- Riksmaten, a national population survey of dietary habits in adults 18-80 years of age carried out in 2011. The participation rate was 36%, with the highest non-participation among young men (Amcoff et al. 2012). Participation involved documenting individual consumption of foods and beverages over a 4-day period in a web-based log book, as well as filling in a questionnaire. A similar survey performed in 1997-1998 among individuals 18-74 years of age, using a regular rather than a web-based logbook, and documenting the intake of food and beverages over a 2-week period, had a response rate of 60% (Wulf and Pearson 2002).

Among the various reasons for these declining rates that have been discussed, an increasing number of competing interests, including competing surveys, e.g. for marketing, may be the most likely. Changing attitudes, beliefs and norms in the general population concerning the importance of contributing to the community, as well as increasing privacy concerns may also be involved.

Although a low response rate in a descriptive population survey is problematic, different approaches can be used to take non-response into account before drawing conclusions. One common procedure, referred to as calibration estimation, is based on the assumption that non-responders within a specific stratum of the population would, on the average, have responded in a manner similar to the responders in this same stratum (i.e., random non-response within a stratum). These strata can be defined by age, sex, income, education, marital status, country of birth or any other variable or combinations thereof for which data are available, factors sometimes referred to as help factors. When assessing the distribution of exposure in a sample of responders, specific weights are then assigned to the various strata of responders. In this way the relationship between the

number of respondents in each help factor stratum and the size of the corresponding stratum in the target population will be taken into account. Although this approach is assumed to correct appropriately for non-response, the assumption of random non-response within strata requires further evaluation.

Another way to compensate for non-response is to compare simple information on key parameters provided by responders and non-responders. Usually, only a minor sample of the non-responders is approached and among these individuals, the response rate may again be low, so that in general only large differences between responders and non-responers can be identified in this manner.

It is worrisome that the rates of participation in analytical epidemiological investigations are also declining. Approximately 15 years ago, these rates were often around 70-80%, whereas today researchers must be prepared to accept rates of 25-50%. The response rates in recent epidemiological studies where participation involved both filling out a questionnaire and undergoing a physical examination are listed below.

- Swedish CardioPulmonary BioImage Study (SCAPIS): This pilot study involving 1111 individuals was performed in Gothenburg in 2012. The questionnaire was web-based and answered in connection with the physical examination. The overall participation rate was 49.5% (39.9% and 67.8% in areas of low and high socio-economic status, respectively). The reasons for non-participation were as follows: inability to make contact with the subject (37.4%), too busy (15.7%), too sick (6.6%), language difficulties (7.8%), miscellaneous (6.4%) and none given (26.1%). Inability to make contact and language difficulties dominated in areas of low socio-economic status (Bergström et al. 2015).
- LifeGene, pilot phase: Data from index individuals 18-45 years of age were collected from 2009-2010 in Stockholm, Umeå and Alingsås. The questionnaire was web-based and jparticipation rate approximately 25% (Almqvist et al. 2011).
- EpiHealth: By January 2013, 7000 subjects from the Uppsala region had been included in this study, and the participation rate was around 25% (Lind et al. 2013).

Participation rates vary by age, with more pronounced participation by older than younger individuals (Abrahamsen et al. 2016, Amcoff et al. 2012). This might be expected in connection with studies where the participants receive individual feedback on physical examination parameters such as levels of various blood markers along with recommendations from physicians, since, in general, younger individuals may worry less about their health.

Different approaches to obtaining higher response rates in epidemiological surveys and studies have been tested. Multiple contacts have been repeatedly observed to increase the rate of response (Cho et al. 2013). Telephone interviews may provide clearer answers to complex questions, but participation rates in questionnaire surveys are substantially higher (Rocheleau et al. 2012, Sinclair et al. 2012). Mixed-mode surveys (with the option

filling in either a traditional or internet-based questionnaire), especially when younger and more highly educated populations were involved, resulted in response rates similar to those attained with traditional questionnaires, but at a lower cost (Zuidgeest et al. 2011).

The use of incentives, including cost-free participation in lotteries (Olsen et al. 2012), increases response rates in all kinds of surveys (Singer 2002). Whether monetary or non-monetary incentives are most advantageous may depend on the context. Small monetary incentives were found to improve the response rate among nurses (VanGeest and Johnson 2011) and other healthcare professionals (Cho et al. 2013). Reimbursement for travel costs may also help enhance the response rate.

In light of the increasing challenge to recruit participants for population-based studies, as well as the dynamic nature of the general population in terms of attitudes and context, continued examination of the factors that influence response rates is required. Furthermore, novel approaches accounting for the possible bias introduced by non-response must be developed.

3.3 Misclassification of exposure

Exposure can be assessed using sophisticated technical instruments, questionnaires and/or interviews, or inferred from databases. Each source of data has its own typical drawbacks that may compromise accuracy. The exposure assessments may be individual or at an aggregated level. In certain contexts individual data on exposure are collected indirectly, e.g., from a close relative of a participant who is too ill to provide the data herself or may have died prior to data collection. For a number of diseases, the accuracy of such proxy data has not yet been assessed.

The accuracy of both self-reported data and data obtained indirectly may depend, for example, on whether the exposure is difficult to recall, related to stigma, or of interest to the subject so that she is willing to put effort into reporting accurately. Certain types of exposures, such as alcohol intake and consumption of food regarded as unhealthy, are systematically under-reported (Meiklejohn et al. 2012), whereas other types are reported inaccurately in a more random fashion. In addition, specific groups may be less prone to report accurately than others, a well-known example being the underreporting of energy/food intake by overweight and obese individuals.

Moreover, survey methods/instruments vary in their ability to measure exposure accurately. The accuracy of measurements is usually expressed in terms of sensitivity (defined as the probability of classifying a subject who is truly exposed as exposed) and specificity (the probability of classifying an unexposed subject as unexposed). Ideally, instruments employed for exposure assessments should be validated, preferably for the specific population under study (Bergkvist et al. 2012).

Even when highly validated instruments are utilized for exposure assessment, potential misclassifications should be considered when designing epidemiological studies and

interpreting their results. In the case of analytical studies, severe bias may be introduced if the sensitivity or specificity of measurements differs between individuals diagnosed with the disease of concern (cases) compared to those free from this disease (non-cases), so-called differential misclassification. Even if identical exposure measurements are made on cases and non-cases, the cases may, for various reasons, be more likely to under- or over-report certain types of exposure.

Potential "reversed causation" must therefore be considered particularly when interpreting the results of epidemiological studies involving a cross-sectional or case-control design. Even in cohort studies, where exposure assessments usually take place prior to the development of disease, differential misclassification may occur. With a cross-sectional or case-control design, exclusion of fatal cases and cases too ill to participate introduces misclassification of exposure, if the exposure is related to the severity or mortality of the disease.

When deciding on a procedure for exposure assessment, the researcher is often forced to choose between high sensitivity and high specificity. For assessing rare exposures, high sensitivity is usually less important, since with low specificity the number falsely classified as exposed would be considerable and thus make it more difficult to establish associations. For common exposures, high sensitivity is usually more important, since low sensitivity would have this same undesirable effect.

In many research contexts, repeated assessment of the exposure of cohort study participants during the follow-up period is advantageous, since exposure may vary with time and a single assessment at baseline may not accurately reflect the level of exposure that may contribute to the development of disease. New biostatistical models for analyses of data from repeated measurements in relationship to survival data (Asar et al. 2015) are of value in connection with epidemiological research. Repeated measurements of exposure can also capture potential trigger effects, which may also be examined advantageously using a case-crossover design involving assessment of exposure of the same individual on different occasion, with the cases serving as their own controls (Maclure 1991). It should be noted that high validity with respect to exposure assessment does not necessarily correspond to high internal validity of the epidemiological study itself. With a cross-sectional study design, for example, it may be impossible to confirm that the exposure preceded the outcome.

3.4 Measurements of multiple exposures with or without hypotheses concerning interactions

Along with the rapid development of novel biochemical techniques for exposure assessments, which often provide considerable amounts of data, epidemiological research is evolving towards dealing with a growing number of exposure parameters simultaneously. Clarity concerning whether the study is exploratory or based on a

hypothesis(es) is crucial to interpretation of the findings. Analyses of multiple variables with no clear hypothesis are more likely to produce random, spurious associations.
Realistic assessment of combined exposure to a wide range of chemicals (mixtures) or other stressors poses a considerable challenge, especially when the levels of contaminants co-vary, making it difficult to discern the effect of an individual substance. On the other hand, measurements of several contaminants may be required to obtain a clear pattern concerning a specific exposure (Lampa 2015). Synergistic or antagonistic interactions may also occur. Furthermore, concentration-response curves may be non-linear.

To an increasing extent, epidemiological research on the causes of specific diseases involves investigations of potential synergistic or antagonistic effects. Testing hypotheses concerning interaction requires, however, a careful choice of analytical model, since this influences the results obtained. Interaction on the risk-ratio scale does not necessarily imply interaction on the risk-difference scale and vice-versa (Ahlbom and Alfredsson 2005, Knol et al. 2009). In addition, a large study population is usually required to draw definitive conclusions about interactions; analyses of gene-environment interactions in prospective cohort studies may, for example, require the involvement of several hundred thousand individuals (Collins 2004). In this context, methods for making detailed power calculations have been developed (VanderWeele 2015).

One obstacle to analysing interactive effects are the difficulties involved in examining the combined effects of multiple exposures, including not only physical and/or chemical factors, but also social factors that may modify the response to other factors. Currently, no models for analysis of more than two interacting factors have been established, although such models have been proposed and are under development (VanderWeele 2015).

3.5 Research and developmental needs

Successful recruitment of participants in epidemiological surveys and research designed to improve public health is important, but such participation is currently declining. Accordingly, better understanding of the factors that influence response rates is desirable in order to design more efficient and cost-effective studies. As the social climate changes and novel techniques for exposure assessments are developed, different survey modes and approaches to recruitment must be compared in terms of efficiency. In addition, new procedures for compensating for distorting effects of low response rates need to be developed.

In addition to a satisfactory response rate, accurate exposure data are necessary for drawing definititive conclusions concerning exposure prevalence and its potential association to the risk for development of disease. New instruments for collecting data need to be properly validated. Modern technology and a wide-spread public interest in individual health management allow participatory collection of data via smart-phones and

similar devices, a field in its early development that requires both exploration and validation.

The development of novel molecular approaches to assessing multiple biological markers and techniques for measuring exposures digitally may facilitate multiple and repeated measurements. Extensive comprehensive databases and more multidisciplinary work may help address public health needs in the near future. However, methodological development designed to address questions concerning multiple exposures is sorely needed.

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4. Early Life Exposure Assessment

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4.1 Introduction

The importance of the early life environment for child health and development, as well as health later in life, is becoming increasing clear. In particular, the consequences of poor nutrition during gestation have been shown repeatedly to result in both immediate and long-lasting adverse health effects (Barker et al. 2013). Furthermore, recent research indicates that the impact of maternal stunting, a result of chronic malnutrition, on child development continues even into the next generation of children (Walker et al. 2015).

In a similar way, exposure to toxic agents during pregnancy may severely affect children's growth and development (Selevan et al. 2000). From conception to birth development proceeds at a higher pace than at any time later in life, rendering the fetus susceptible to toxic insult. This is particularly true with respect to the nervous system, which develops during a longer period than most other organs (Ritz B 2008, Grandjean and Landrigan 2014).

There is also growing evidence that the early fetal programming of organs and functions, which is regulated by e.g., DNA methylation, can be affected by toxic exposures. In particular, recent studies indicate sex-specific fetal epigenetic effects of both arsenic and cadmium (Kippler et al. 2013, Broberg et al. 2014). This may constitute a link between the early-life exposure to toxic levels of these metals and impaired health much later in life. Probably, the combination of inadequate nutrition and exposure to multiple toxic agents is particularly problematic. Several clinical trials have shown that nutritional interventions may benefit child development (Grantham-McGregor et al. 2014); however, none has evaluated the concurrent exposure to common dietary toxicants.

Since several organs, including the brain, lungs, and immune system, continue to develop after birth, early childhood is another critical period of exposure to many environmental toxicants or other chemicals (Selevan et al. 2000). Small children are also more prone to be affected by chemicals as they consume more food and drinking water and inhale more ambient air per kilo body weight than adults. They may also be more exposed to environmental pollutants through soil, dust, toys and other consumer products.

Together, these considerations emphasize the need to care for mothers and young children in order to secure future public health (Barker et al. 2013). A prerequisite in this context is high-quality data on exposure to pollutants, chemicals and other stressors during fetal and child development and other potentially critical windows of exposure, as well as suitable biomarkers of exposure, and sensitive measures of adverse health

effects, all of which are evaluated most effectively in longitudinal mother-child cohorts. With modern analytical instrumentation we have shown that it is feasible to measure multiple toxic and essential elements simultaneously in large number of samples of most exposure media, allowing assessment of combined exposure and potential interactions as well. Below, we describe the various approaches to exposure assessment during early life exposure assessment. Several examples concern toxic metals, our primary area of expertise. In addition, metals are useful model substances, since many affect various stages of early development.

4.2 Fetal exposure

4.2.1 Indirect assessment via maternal exposure

Exposure of the fetus to chemicals can often be estimated based on the mother´s exposure. Assessment of concentrations in drinking water, various food items, and ambient air, is essential to evaluate compliance with standards and guidelines. Such data can also provide some information on the level of maternal exposures, especially on a group level, but that generally requires comprehensive assessment of each exposure media. There is usually a marked variation in the concentrations of toxicants in different food items, air particles, dust and soil that must be taken into consideration, both at the individual and population level. Still, the concentrations in the exposure media do not necessarily reflect the actual intake or uptake of the chemicals in the body. Absorption in the intestine may vary with, e.g., the nature of the food, degree of exposure, nutritional status, and stage of pregnancy. Also, the absorption in the lungs of inhaled toxicants may vary with the type and degree of exposure.

The use of exposure biomarkers, such as maternal blood, urine, and hair (Figure 4.1), usually provides a more accurate measure of the actual internal exposure (internal dose) of the mother. Biomarker concentrations also reflect the exposure through all routes and from all sources, and are, thus, ideal for initial screening. Identification of routes and sources of exposure can then be focused mainly on the chemicals of elevated exposure. For many chemicals, however, the association between maternal biomarker concentrations and fetal exposure remains to be determined.

While the assessment of exposure via food, inhaled air and consumer products requires considerable involvement of specialized teams, samples of, e.g., blood and urine can be collected relatively easily at the antenatal care units. However, since the physiological changes during pregnancy may induce profound alterations in the kinetics of many toxicants, the timing of the sampling may be critical. For example, the gastrointestinal absorption of cadmium increases during pregnancy due to the up-regulation of the divalent metal transporter 1 (DMT1), the main iron transporter (Kippler et al. 2009). Gastrointestinal absorption of cobalt and manganese appears to increase during pregnancy as well. In addition, the plasma volume begins to increase already during the first trimester, before the start of the red blood cell expansion, and this is the reason for a lower cutoff for anemia defined by hemoglobin during late gestation. Thus,

concentrations of the chemicals in either plasma or whole blood may cause underestimation of the actual exposure.

Other alterations in maternal physiology during pregnancy that may affect the toxicokinetics of absorbed chemicals include an increase in body fat and total body water; reduced plasma concentrations of proteins, especially albumin; enhanced cardiac output and blood flow to the kidneys and the utero-placental unit; as well as lowered blood pressure. Usually, renal blood flow and the glomerular filtration rate (GFR) increase during the course of pregnancy and reabsorption in the proximal renal tubuli may change. Due to these changes in renal function, employing urinary concentrations of pollutants as markers of exposure may be less reliable during late pregnancy.

In light of such changes, it is recommended to collect blood and urine samples in early gestation, preferably during the first trimester or even preconception. A second sampling, e.g., early in the third trimester may be considered if changes in exposure or toxicokinetics, including the absorption rate and metabolism need to be evaluated. For example, the metabolism of inorganic arsenic, a common, highly toxic and carcinogenic pollutant in drinking water and certain food items, becomes much more efficient during pregnancy (Gardner et al. 2011). Sampling during early pregnancy is also recommended in connection with studies of epigenetic alterations, as discussed further below.

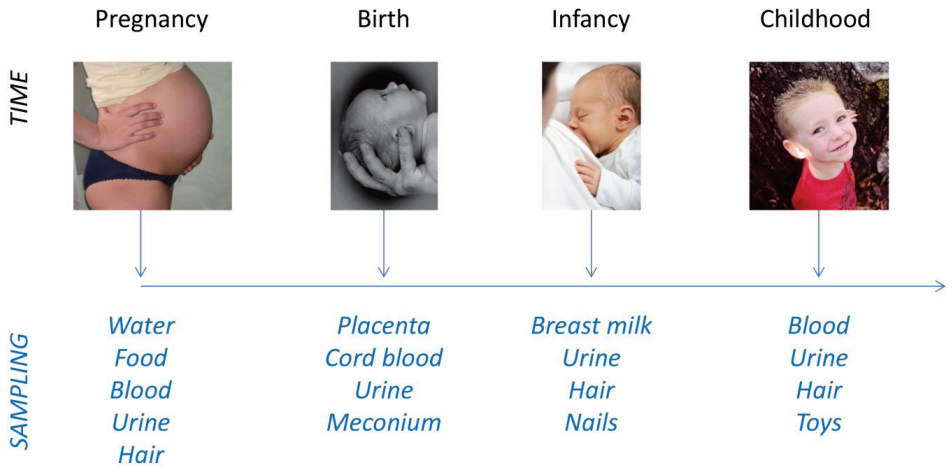

Figure 4.1. Collection of samples for exposure assessment during early life.

4.2.2 Biomarkers of fetal exposure

A more direct measure of fetal exposure may be obtained through the concentrations in umbilical cord blood, which can be quite different from those in maternal blood,

depending on the rate of transfer across the placenta. However, a concentration of a chemical in cord blood reflects exposure specifically at the time of birth, and it may differ from a potentially more susceptible earlier phase of the fetal development. Sampling of cord blood is somewhat more complicated than that from the mother, as it may interfere with the delivery process. It may be performed by direct venipuncture of the umbilical vein, which supplies the fetus with oxygen- and nutrient-rich blood from the placenta, or of one of the two umbilical arteries that return the deoxygenated blood and waste to the mother. Alternatively, mixed venous and arterial blood may be collected by pouring the cord blood from the cut cord into an open blood collection tube after clamping. The increasingly common practice of delayed clamping of the umbilical cord, to prevent iron deficiency anemia in the infant, may increase the risk of coagulation and thereby hamper blood sampling.

When evaluating fetal exposure based on concentrations in umbilical cord blood, the presence of fetal hemoglobin and high hematocrit must be given due consideration. The hematocrit decreases from about 50% at full-term birth to less than 40% by the end of the first month of life (Jopling et al. 2009). For pollutants that accumulate in the red blood cells, such as several toxic metals, the high hematocrit in the newborns may influence the blood concentrations at birth and during the early postnatal period.

Blood spots obtained in connection with newborn screening programs have been tested for the presence of certain environmental pollutants, e.g., perfluorinated compounds (Spliethoff et al. 2008). They are less suitable for determination of trace elements, since in our experience the paper used for the blood spots often contains such elements (e.g., cadmium and manganese) in concentrations that may invalidate the exposure assessment. It should be noted that, although the punch size may be homogeneous, the amount of blood contained within each punch may differ markedly.

Another measure of fetal exposure may be obtained through the concentrations in the first urine produced after birth (Concha et al. 1998), e.g. in the obstetric clinic. As later in infancy, the urine of newborns can be collected using commercially available urine collectors. In order to avoid contamination, the collectors should be tested free of the chemicals under investigation before use. The area around the urethra should be carefully washed with e.g., contaminant-free cleansing wipes. Usually, infant urine is quite diluted, and for the purposes of comparison, the concentrations measured need to be adjusted on the basis of osmolality or specific gravity. Adjustment on the basis of creatinine is not recommended for children, since excretion of creatinine changes to a great extent with age and nutrition (Nermell et al. 2008).

Collection of meconium, the first stool passed by a newborn, is another, less frequently used method for estimating the prenatal exposure (Delano and Koren 2012). Meconium begins to form in utero in the beginning of the second trimester and accumulates thereafter. As with the feces of children and adults, pollutants in meconium represent mainly the non-absorbed fraction. Thus, while this approach may provide some estimate

of exposure, especially to pollutants with low gastrointestinal absorption, it does not accurately reflect the internal fetal dose.

The amniotic fluid surrounding the fetus within the amniotic sac is usually available for chemical analysis only in the case of women who undergo amniocentesis for prenatal diagnosis of, e.g., chromosomal abnormalities. When the kidneys begin to function at approximately gestational week 16, the fetal urine also begins to contribute to this fluid. Accordingly, fetal ingestion of amniotic fluid and excretion back into this same medium may result in accumulation of pollutants, increasing fetal exposure. For example, the amniotic fluid of smokers was found to contain 25-fold higher concentrations of cotinine than that of non-smokers, while the corresponding ratio for maternal urine was six (Machado Jde et al. 2014). Similarly, accumulation of arsenic, lithium and boron in amniotic fluid has been indicated by the observed very high concentrations of these elements in the first urine produced after birth (Harari et al. 2012).

4.3 The placenta

As the interface between the mother and fetus, the placenta plays a key role in fetal growth and development (Fei et al. 2013). It has critical functions in the transfer of nutrients from the mother to the fetus and waste from the fetus back to the mother. It also regulates the transfer and production of certain hormones and progression of pregnancy, including fetal programming (Gabory et al. 2013).The potential use of the post partum placenta as a non-invasive biomarker of exposure is gaining acceptance because placental concentrations of many toxicants correlate with those in maternal and/or fetal blood (Iyengar and Rapp 2001). Usually, collection of the whole placenta immediately after delivery and the subsequent clinical examination does not interfere with the delivery process. Placenta tissue has also been utilized to identify early toxic effects of exposure to environmental contaminants, including lead, mercury, cadmium, arsenic, pesticides and perfluorinated compounds (Concha et al. 1998, Iyengar and Rapp 2001, Odland et al. 2004, Kippler et al. 2010, Esteban-Vasallo et al. 2012, Gorrochategui et al. 2014, Punshon et al. 2015). Accordingly, placenta is becoming more and more important for assessment of both chemical exposure and potential toxic mechanisms.

To date, no general guidelines concerning sampling of the placenta for analysis of chemicals have been formulated. Initially, the placenta needs to be drained from as much blood as possible or rinsed from blood with a selected solution, and then weighed and measured. If frozen directly, the placenta should preferably be maintained flat in a suitable container. It needs to be tested whether the distribution of certain pollutants in the placenta is homogenous (Esteban-Vasallo et al. 2012). For example, a wedge, starting from the cord and cutting outwards could be cut out and divided in sub-samples to be analysed. Other approaches to avoid inhomogeneous sampling would be to either *i)* mix the whole placenta or a large part of it into a tissue homogenate from which a sub-sample can be analyzed, or *ii)* to collect several sub-samples throughout the placenta to be analyzed both separately and jointly (pooled sample).

At an early stage it is also important to decide which specific tissue(s) in the placenta should be sampled. In some studies, the decidua basalis, chorionic plate, connective tissue, and large blood vessels have been collected separately, whereas in other cases the cotyledon has been sampled. In most cases the separation of specific placental tissues within the placenta requires pathology expertise. Whatever method is used for placenta sample collection, it is of utmost importance to avoid contamination, e.g., from chemicals in the equipment used for treatment, sampling and storage.

4.4 Childhood exposure

4.4.1 Breast milk

Breast milk contains all the nutrients required for infant development, although not always in sufficient amounts. Exclusive breast feeding is often recommended up to 6 months of age, but less than 40% of the infants in both developing and developed countries are breast-fed for this long (http://www.who.int/en/). In Sweden about 12% of the mothers exclusively breastfeed their babies until six months, which is one of the highest rates in Europe.

The composition of breast milk differs in three distinct stages: colostrum, transitional milk, and mature milk. Colostrum, the first milk produced, is thicker and creamy in color and contains high concentrations of protein, fat-soluble vitamins, minerals, and immunoglobulins. The transitional milk, produced several weeks after the colostrum period, contains high levels of fat, lactose, and water-soluble vitamins. Finally, the mature milk consists of 90% water, carbohydrates, proteins, and fats. The first milk consumed during a session of feeding, the so-called foremilk, contains mainly water, vitamins, and proteins, while the subsequent hind-milk contains higher levels of fat. For comparison of the concentrations of chemicals in the breast milk, the time that elapses between delivery and sampling, as well as the time-point within a session of feeding (usually after the baby has been fed) should be consistent.

Because most infants are breast-fed exclusively during the first months of life, the concentrations of pollutants in breast milk provide a reliable indication of the first period after birth. Therefore, breast milk may be a suitable medium for exposure screening in long-term environmental surveillance programs. In particular, most fat-soluble chemicals are quite readily transferred to breast milk (Lignell et al. 2011). A recent review of persistent organic pollutants (POPs) in breast milk showed generally decreasing concentrations in Sweden (Fang et al. 2015). This was concluded based on the long temporal trend studies, so far performed only in Sweden and Japan. However, the concentrations of hexabromocyclododecane appeared to increase in both of these countries. The highest concentrations of polychlorinated biphenyls (PCBs) and "dioxins" in breast milk are found in more highly industrialized countries, while the highest concentrations of pesticides are present in Africa and Asia and of polybrominated diphenyl ethers (PBDEs) in the United States. Although water-soluble chemicals accumulate to a lesser extent in breast milk, a recent study of toxic and essential elements

in the breast milk of Swedish women revealed pronounced inter-individual differences in the concentrations of both the essential elements cobalt, chromium, manganese, and molybdenum and the toxic metals arsenic, cadmium, lead, antimony, and vanadium (Bjorklund et al. 2012), which should be followed-up over time. Evaluating the concentrations in different geographical areas is obviously needed in order to obtain a measure on the national level.

4.4.2 Children's food and drinking water

In developed countries infant formula is the most common substitute for breast-feeding below four months of age (Freeman et al. 2000), after which approximately two-thirds of European infants are reportedly fed some solid foods. Although the EU limits marketing of infant food products for infants younger than four months, in some EU countries complementary items of food, most often fruits, vegetables or cereals, are given to infants as young as one month old (Freeman et al. 2000).

Substitutes for breast milk must provide sufficient energy and nutrients to support rapid infant growth during the first months of life. Recent research on formula composition has focused mainly on protein and energy content, as well as a few nutrients and vitamins (Agostoni and Domellof 2005), whereas most essential trace elements have received very little attention. Moreover, infant formula and foodstuffs may contain pollutants naturally present in or as contaminat of the raw materials used, or if may contain pollutants introduced in connection with food processing.

Recent studies have identified elevated concentrations of arsenic and cadmium in rice- and soy-based infant food in Europe (Meharg et al. 2008, Ljung et al. 2011). In 2015 the Swedish National Food Agency published a more comprehensive study of arsenic in rice and rice products in Sweden (http://www.livsmedelsverket.se/) and recommended on the basis of the frequently elevated concentrations, that parents should not give rice cakes or drinks to children under six years of age. It is, indeed, an urgent task for both the research community and food producers to find methods to decrease the levels of both arsenic and other toxic pollutants in food, most importantly in food for infants and children. Such approaches may include the development of strains of rice that take up less arsenic and other toxic metals, growing conditions that minimize the uptake of toxic substances, extraction techniques that remove arsenic from rice during production, and cooking procedures that reduce the content in the ready-to-eat food.

The daily intake of manganese, an excess of which may be neurotoxic, by infant formula present on the Swedish market was found to vary from ten up to several hundred times the intake by breast-feeding (Ljung et al. 2011). The water (well water) used to mix powdered formula may add significantly to the manganese concentrations. In addition, one portion of infant food was also found to provide significantly more arsenic, cadmium, lead and uranium, but less calcium, copper and selenium, than one feeding of breast milk. Clearly, more rigorous regulation of the levels of pollutants in infant formula and child food is warranted.

4.4.3 Toys and environmental factors

Children explore their environment through fingering, sucking and tasting. The enjoyment of tactile play renders them more prone to exposure to toxic agents in soil, dust, toys, and other consumer products than adults (Moya and Phillips 2014). There is an obvious need to test the potential presence of environmental pollutants in children's every-day environment, especially of home and on the playgrounds (Kolossa-Gehring et al. 2012). Moreover, toys and other consumer products that children come in close contact should be free of hazardous compounds and, indeed, toxic agents are banned from children's toys produced in or imported to the EU. Chemicals known to cause cancer or affect DNA or reproduction are banned or allowed to occur in very low concentrations only (see e.g. http://www.kemi.se/).

4.4.4 Biomarkers of child exposure

As in the case of the mother, the concentrations of many chemicals in the blood and urine of a child provide a more accurate estimate of actual intake than the concentrations in one or more sources of exposure. While blood sampling by venipuncture may be problematic to obtain in young children - both the child and the parents may object to it - a finger prick (or in neonates, often a heel prick) is often accepted. It has been shown possible to obtain reliable blood concentrations of toxic metals using a capillary blood microsampling technique, provided that the skin of the finger is careful cleaned prior to blood sampling, preferably using diluted acid solution (Berglund et al. 1994). However, the small blood volume obtained with this technique may not be sufficient for certain types of chemical analysis.

Child urine is often easier to obtain than blood, but the risk of contamination is higher. In the case of infants and non-toilet-trained children, the use of urine collectors is often preferable. Urine may also be collected directly from the stream into a suitable container, in which case it may help to tap with two fingers just above the bottom of the baby's tummy.

Urine collection pads, e.g., made of cotton-wool, or disposable sanitary towels, into diapers may be inappropriate, depending on the quality of the cotton-wool amd the chemicals of interest. For example, some types of cotton wool release certain trace element (Kippler et al. 2010) and the influence of the cotton on other pollutants must be tested. With potty-trained children, urine sampling in a potty or other plastic container (tested free from contamination), or in a plastic bag placed in the potty may be suitable (Hamadani et al. 2011).

Whatever method is chosen for urine collection, the area around the urethra must be clean (wet wipes) and post-sampling contamination of the sample must be avoided. Dust from contaminated cloths must also be avoided. With older children, who can pass urine when asked, collection of a midstream urine sample reduces the risk for contamination further.

The concentrations of environmental contaminants in human hair and nails are increasingly utilized to assess individual exposure (Delano and Koren 2012, Davis et al. 2014). In particular, several trace elements have an affinity for the sulfhydryl groups of keratin in tissues such as nails or hair, where they may therefore be present at much higher concentrations than in plasma, blood or urine. Hair offers several advantages in human biomonitoring, particularly of children, as it is non-invasive, inexpensive, easily accessible for sampling, and can be stored and transported without difficulties. The main disadvantage here is the considerable risk of external contamination from both dust, washing water, soap/shampoo and other cosmetic products. In addition, handling of the sample prior to analysis may be problematic, not the least with respect to weighing of small amounts of hair (10-100 mg). Handling of hair is subject to considerable static electricity, why a static electricity eliminator is required in the balance.

Although the ratio of surface area to weight is much smaller for nails than for hair, there may be high risk of contamination, especially in the case of small children, who often play in soil and dirt. With young children nail clippings, including those from toenails, may be quite small, requiring sensitive and reliable cleaning and analysis. Also the nails of newborns may be contaminated, e.g. by chemicals present in the amniotic fluid (see above). Because of such shortcomings, the validity as exposure biomarkers must be evaluated by comparison with other biomarkers.

Analysis of teeth is also employed to estimate long-term exposure to certain metals and organic chemicals, but the sample preparation need some consideration depending on the analytical method used for quantitation. With modern techniques, including laser-ablation inductively coupled plasma mass spectrometry, it is possible to measure metal concentrations in the pre- and postnatal dentine of children's shed deciduous teeth to obtain a temporal map of the early life exposure (Austin et al. 2013, Mora et al. 2015).

4.4 Early life epigenetics

The genome is programmed with the help of epigenetic marks to express appropriate sets of genes in particular tissues at specific time-points during an individual's life (Gluckman et al. 2009, Gabory et al. 2011). Epigenetic marks, defined broadly as dynamic modifications of the genome other than of the DNA sequence itself, can affect the regulation of gene expression and can be transmitted through mitosis (Berger 2007). These epigenetic marks include methylation of cytosine residues in DNA (when the cytosine is followed by a guanine, a so called CpG site), post-translational modifications of histone proteins, and small non-coding RNA molecules (referred to as miRNAs or microRNAs) that may interfere with gene transcription and/or translation (Suzuki and Bird 2008, Pasquinelli 2012). Specific pathological pathways are associated with distinct epigenetic signatures that can be detected early in disease progression.

Unlike the DNA base sequence, which is highly stable, epigenetic processes can react rapidly to endogenous and environmental signals (Figure 4.2). Substantial experimental

evidence and, to some extent, *in vivo* evidence, e.g. for smoking (Joubert et al. 2012), indicate that epigenetic marks serve as a memory of exposure to an unfavorable environment early in life, inducing long-term alterations in gene expression that may lead to disease later in life (Radford et al. 2014). This concept is known as the developmental origin of health and disease (DOHaD), or Barker hypothesis (Gluckman et al. 2009, Barker et al. 2013).

It has been shown that arsenic, cadmium and lead exposure in early pregnancy are all associated with distinct alterations in DNA methylation in the newborn, but the effect differs between metals (Kippler et al. 2013, Broberg et al. 2014, Engström et al. 2015, Vilahur et al. 2015). Moreover, these epigenetic effects of arsenic and cadmium differ by sex, which is in line with previous findings of sex-specific arsenic and cadmium toxicity. Exposure to arsenic is associated with decreased DNA methylation across the genome in the neonate, particularly in boys and particularly when the fetus is exposed at an early stage in development (Broberg et al. 2014). Cadmium is associated with increased genome-wide methylation in boys and decreased genome-wide methylation in girls, an effect that is linked in part to low birth weight (Kippler et al. 2013). In contrast to the effects of arsenic and cadmium, lead is associated with gene-specific methylation at the *GP6* (platelet activator glycoprotein VI) gene-promoter, a change which may be related to the adverse effects that lead is known to exert on the cardiovascular system (Engström et al. 2015). Moreover, specific methylation patterns associated with exposure to cadmium are still present in young children (4.5 years after birth), indicating long-lasting effects of fetal metal exposure through epigenetics (Kippler et al. 2013). However, the clinical relevance of such findings remains to be elucidated.

The use of epigenetic markers as biomarkers of exposure has so far limited applications and more research is needed to clarify their dynamics, both early and later in life, before they can be regularly used for exposure assessment. When collecting samples for analysis of DNA methylation, the same precautions must be taken as when sampling for DNA analysis in general. However, as the intracellular epigenetic marks differ between different cell types, it is recommended to sample homogenous cell populations, obtained, for example, by sorting the various cells in maternal or cord blood. This is not a problem with extracellular epigenetic markers, such as miRNAs that circulate in plasma. On the other hand, miRNAs are more unstable than DNA methylation and excessive freezing and thawing, as well as contact with RNAses, should be avoided.

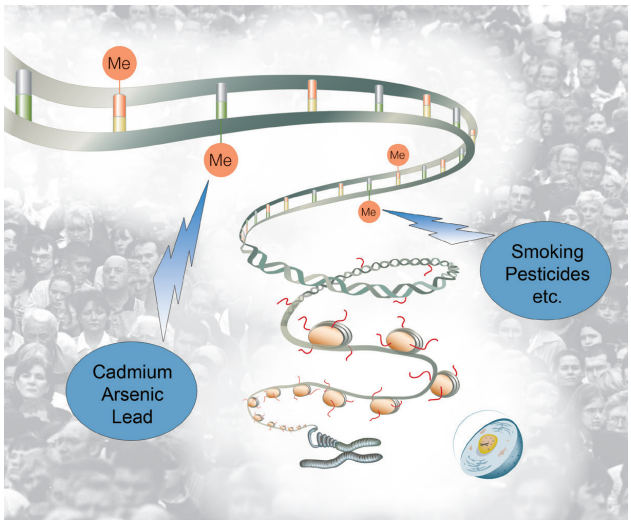

Figure 4.2. Epigenetic marks include methylation on the cytosine residues of DNA, which regulates gene expression of the cell. Toxicants that have been shown to affect such methylation are shown.

4.5 Research and developmental needs

In order to improve living conditions and also achieve sustainable development, as outlined in the new global sustainable development goals (SDGs; https://sustainabledevelopment.un.org/), it is essential to detect and mitigate the most critical environmental factors that cause the most serious impairment of the health and development of children (Walker et al. 2011, Barker et al. 2013, Grandjean and Landrigan 2014). The need to promote maternal and child health is an integral part of SDG 3, "Ensure healthy lives and promote wellbeing for all at all ages", but also of the goals of the U.S. EPA (Makris et al. 2008) and the Swedish Environmental goal "A Non-Toxic Environment". Thus, it is essential to consider the most vulnerable segments of the population, such as fetuses and children, in health risk assessment. Comprehensive health risk assessment for these early stages in life has been performed for only a few environmental pollutants and much more data on which to base reliable reference values and recommendations is required.
In particular, more research is needed in the following areas:

- There is an urgent need to develop large mother-child cohorts for longitudinal evaluation of exposure to environmental pollutants and other chemicals and potentially associated health effects. It is essential that exposure is measured repeatedly, starting already during early pregnancy or even before conception, in order to identify critical windows of early life exposure (Selevan et al. 2000). Preferably, the fathers' exposure should also be assessed. These cohorts must be designed for the measurement of multiple toxicants to allow evaluation of mixed exposures generally present in children´s food, ambient air and external environment.

- Suitable biomarkers of exposure to the most important toxicants encountered at different developmental stages, including fetal development, should be developed. The evaluation of exposure biomarkers requires detailed knowledge concerning the toxicokinetics of the toxic agents (including metabolism and the influence of individual genetic constitution) at various stages of development - knowledge which is presently quite rudimentary.
- The extent to which epigenetic changes during early life can be utilized as biomarkers of exposure and their ability to predict future disease must be clarified.
- In the health risk assessment, it is essential to identify the most susceptible population groups. Therefore, it our knowledge about the factors that influence susceptibility, such as genetic predisposition, gender and nutrition, need to be improved.

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5. Spatial and Temporal Assessment of Environmental Factors

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5.1 Introduction

Several environmental factors show different levels in different places and individual exposure may be assessed in various forms of spatial analysis, often focusing on the place of residence. Moreover, many of the relevant sources and levels change with time, making temporal analysis important as well. In the present chapter we describe approaches to such assessment of population exposure to air pollution, community noise and certain other factors, providing examples of techniques and applications. Advantages and limitations will be discussed as well as research needs.

An early example of the use of *spatial* exposure assessment and analysis is the outbreak of cholera that occurred in London in the mid-19th century. The existence of the infectious agent *Vibrio cholerae* was unknown and it was generally believed that cholera was caused by "foul air". Thanks to the efforts of John Snow the new cholera cases could be mapped together with the water supply networks, which originated from different pumps. The handle of the suspected pump at Broad Street was removed and the outbreak ceased.

London also provides another example, this time showing a *temporal* exposure assessment and analysis. In the 1950's coal was the dominant means of home heating in Great Britain. During a couple of weeks in December 1952 it was more than usually cold, causing air pollution to reach extremely high levels. It was noted that morbidity and mortality increased during this episode, in a way that closely matched the increase in air pollution.

In spite of substantial efforts to improve the air quality in many cities during the decades following the London smog disaster, in the 1980's it was proposed that - the now much lower – levels of ambient air pollution still could cause a long-term increase of general mortality. A cohort was assembled from six cities in eastern United States and a *spatial* difference in long-term exposure was shown to be closely related to survival of the cohort members (Dockery et al. 1993). When these same cities were revisited several years later, adding a *temporal* perspective, the excess mortality appeared to have decreased in line with air quality improvements.

Potentially health-hazardous ambient levels of pollutants are common, especially in urban areas, and may display high spatial and temporal variability. The total individual exposure is a product of very complex relationships and interactions between environmental and human systems, rendering assessment of exposure difficult (Steinle et al. 2013). Ideally, a population health study should take into account all environments and behaviors of each individual that contribute to exposure. This is often difficult, for both practical and economic reasons, and a common approach is to focus on the environment surrounding the individual. Both in the London smog and in the Six cites studies all inhabitants in a specific urban area were treated as equally exposed. While this may be appropriate in some situations it is clearly quite far from true individual exposure, and inadequate in long-term studies when there is substantial spatial variation also within an urban environment. High-resolution spatial modelling, using information on sources or on other factors that are related to the sources, as e.g. proximity to major roads, is a major step forward. Such techniques have been applied successfully for analyzing long-term health effects from air pollution in population studies, and these methods are now copied into spatial noise assessment as well as explored for other factors, such as temperature, greenspace and water contamination.

Example: Urban Heat Islands

Hardening of a surface decreases cooling by evaporation and generally increases the heat capacity. City environments are therefore typically hotter that the surrounding land. This *spatial* difference is referred to as the Urban Heat Island (UHI) effect. How severe this effect is was studied in Amsterdam, using land surface temperature (LST) data derived from satellite images (Landsat 60 m/120 m). The results indicated significant UHI effect and that the LST in the city is 10-20° C higher than in the surrounding rural areas. High LST and high population vulnerability were combined in particular of the western part of the city, due to the lower quality of life and poorer energy efficiency of the buildings.

The findings from Six cities and similar *spatial* studies have been used extensively for risk assessment for long-term effects from air pollution, by matching with similar-type exposure assessment for the specific population. The findings from (short-term) *temporal* analyses are of value for elucidating mechanisms, as well as for identifying susceptible groups and candidate pollutants. However, they are also often used for risk assessment of air pollution, despite the fact that a WHO working group strongly advised against this practice; the main reason being that these studies are unable to fully capture all time-relations between exposure and outcome, which may lead both to over- and underestimation of the population risk (WHO 2001).

5.2 Common methods

5.2.1 Temporal models

Temporal models of the effects of changes in exposure over time on a health outcome do not take spatial differences into account, i.e. during each unit of time, exposure is assigned uniformly across the region of study.

Time series data for health studies may include both exposure and outcome data. Exposure data may e.g. be air pollution or daily water turbidity, and outcome e.g., deaths or gastro-intestinal problems during. The time unit is often one day, but also shorter and longer time units are used. Such data are useful for studying exposures with short-term variation that can be expected to induce an effect rapidly. In simplified form, temporal analyses answer the question as to whether short-term variability in exposure is associated with short-term variability of effects on your health. In some cases such as e.g., climate change or other general changes in living conditions, much longer time scales may also be of interest, but these are not dealt with here.

Temporal analyses rely on continuous monitoring of exposure and outcome, typically making use of surveillance systems and frequently updated registries. The advantage of regular exposure monitoring is that it provides information for long periods of time with, in general, some level of quality control. Important individual factors that may influence the observed relation between the exposure and the outcome, as e.g. smoking status or age, can in general, be considered stable from day to day. Although the data on exposure and outcome necessarily involve the same unit of time, outcomes induced over periods longer than this unit, so-called lag effects can also be examined. If there are indications that physiological response occurs after, e.g., several days, associations to exposure occurring several days earlier can be explored.

The series of daily ozone levels in Stockholm presented in Figure 5.1 reveals very strong seasonal variations that are considerably greater than the daily fluctuations around the seasonal curve. Daily hospitalizations and deaths also exhibit a seasonal pattern. Therefore, attempts to study the associations between daily exposure to e.g., ozone exposure and daily deaths it becomes important to specify a time series model that absorbs enough of the seasonal variability while maintaining the daily variability. The analytical models further need to adjust for long-term time trend and meteorological factors that may be related to both levels of air pollutants and the outcome, such as temperature and relative humidity.

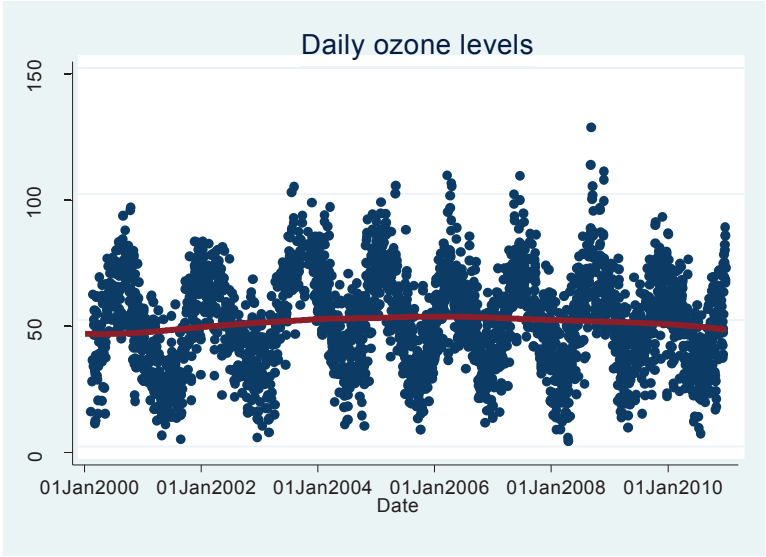

Figure 5.1. The average daily level of ozone in Stockholm urban background during the years 2000-2010 (the line indicates trend; data provided by East Sweden Air Quality Management Association).

This type of temporal analyses has certain obvious limitations. Since long-term series of data on exposure to relevant compounds or summary measures are required, in practice this approach is limited to parameters monitored for regulatory purposes. Thus, it is, in general, difficult to study "new" pollutants not previously shown to be harmful. However, sometimes data concerning environmental factors monitored for other reasons (e.g., temperature and humidity) may well be available.

Furthermore, the relationship between temporal pattern of a pollutant monitored central and the individual's pattern of exposure may be weak. Individuals move between more or less polluted areas and may experience levels that differ totally from those measured outdoors at one or a few monitoring points. The validity of the assumption of spatial homogeneity of exposure over the time interval being investigated thus depends on the region studied and the characteristics and spatiotemporal distribution of the exposure.

For example, in the case of $PM_{2.5}$, which generally contains a sizable amount of particles that originate from long-range transport, changes in levels from day to day (temporal variability) may demonstrate a much higher level of exposure contrast than the spatial

contrasts (spatial variability) in a city during the same time interval. Consequently, the temporal model will reveal virtually all of the health effects of daily exposure to $PM_{2.5}$. In contrast, in connection with exposure to NO_2 in Stockholm, geographical variations must also be considered (Bellander et al. 2012).

Another limitation of this approach is the lack of data on the individual level, making it impossible to relate the health effects of interest directly to factors that influence susceptibility, other than age and sex, which is generally available in the outcome registries. Other difficulties include how to deal with missing observations in a time series, how to combine more than one time series of environmental data, and how to apply such data to non-urban populations.

One example of time series analyses is the Air Pollution and Health: A European approach (APHEA) study, in which associations between daily levels of PM_{10} and overall and/or cardiovascular mortality were investigated in several European cities. Surveillance data on PM_{10} were collected from strategically placed air pollution monitors and daily counts of death were retrieved from registries. The data for each of the 10 cities were first analyzed separately and then meta-analyzed. Results showed a roughly 0.7% increase in risk of cardiovascular death for each 10 $\mu g/m^3$ increase in PM_{10} during the preceding two days (Zanobetti et al. 2003).

As mentioned above, time series data concerning mortality must be used with caution for risk assessment. For instance, the "harvesting effect", a minor forward displacement of time of death, is of limited significance for public health. One example is the heat wave in Moscow in 2010 which led to nearly 11,000 extra deaths during five weeks (Shaposhnikov et al. 2014). This was followed by an unusually low number of deaths during the next several months, so that the net excess mortality observed with a longer follow-up was only about 5,000 deaths (Shaposhnikov et al. 2015). Similar harvesting effects have been observed in several other studies.

5.2.2 Spatial models

A map of pollutant levels or some environmental factor that may be related to exposure at different locations, may not always be immediately useful for population exposure assessment, but can be of value when designing studies of individual exposure. For example, maps illustrating levels of metals in moss may help identify an important source of exposure, but do not in themselves provide information concerning population exposure, since the most import route for human exposure to most metals is dietary. A spatial representation of cases of and/or risk for disease may also provide basis for sampling of the environment, e.g., the identification of spatial clusters of arsenic-related fetal loss and infant death in Bangladesh (Sohel et al. 2010).

Models that combine measurements with geographical and other local features for statistical analysis may be referred to as *geostatistical*. The basic assumption here is that the relation between the observed pollutant level and the spatial correlates at the

measurement points is representative for the entire region of interest. In the case of air pollution this approach is called Land Use Regression (LUR) and was originally proposed by David Briggs (Briggs et al. 2000).

This approach is attractive in that it requires only limited data. At the same time, it is necessary to have a carefully designed measurement study to get the observed pollutant levels. Geographical data of various kinds may even be retrievable from international databases such as the European CORINE land cover database initiated by the European Commission in 1985. This database is managed by the European Environment Agency and includes periodic data on land-use It contains geographically related data on artificial surfaces including urban fabric and agricultural areas, but also forests, wetlands and water bodies up to 2006, so far (Büttner et al. 2012). In such studies the geographical data are used as the independent variables in a regression analysis with the observed values as the dependent variable. Often simple linear regression is used. The measurement campaign required for such analysis is generally the most time-consuming and expensive aspect, often limiting the number of observations to less than 100. This puts a restrain on the number of independent variables that can enter the regression (Austin and Steyerberg 2014). In cases involving traffic-related air pollutants typically one or two traffic-related variables enter together with one or two variables characterizing population density or land use. In spite of the seemingly simplistic nature of this approach, quite high correlations are often observed, even when independently observed data are used for evaluation.

Among the several limitations involved, the most serious is probably the difficulty to transfer the results of one specific spatial analysis to another geographical region or another period of time in the same region, although this has been done successfully in some cases.

Example: LUR in the ESCAPE study

Within the European Study of Cohorts for Air Pollution Effects (ESCAPE), land use regression (LUR) models for urban air pollutants were developed for Stockholm County and 35 other areas in Europe. Spatially distributed monitoring of NO_x, NO_2, PM_{10}, $PM_{2.5}$ and soot was performed by regional teams in 2009-2010, using a common protocol (Cyrys et al. 2012; Eeftens et al. 2012).

The traffic variables for Stockholm County were primarily based on the local road network provided by the Eastern Sweden Air Quality Management Association (www.slb.nu/lvf). Predictors were calculated as the inverse of the distance to the nearest road and nearest major road (Beelen et al. 2013). The total length of roads (m) was calculated in buffers of 25-1000 m radius (Su et al. 2008).

Other data concerning land use were obtained from the CORINE (Coordination and Information on the Environmental programme) land cover data for 2000 (CLC2000). The minimal mapping unit was 25ha, corresponding e.g., to a 500x500m square. The final predictor variables included urban greenspace, semi-natural areas, forest areas, high and low density residential land, industry and ports in buffer zones with radii of 100-5000 m. Population data was distributed in a 100x 100m grid. The area of surface water within buffer zones was obtained from the Swedish mapping, cadastral and land registration authority. For Stockholm County the final LUR model for NO_2 included the total length of roads within 500 m, traffic flow on the nearest street and population within 100 m and explained 82% (R^2) of the spatial variance between measurement sites (Beelen et al. 2013).

5.2.3 Spatio-temporal models

When the sources are well characterized and the manner in which the contaminants spread is known, source-receptor modelling is possible (Bellander et al. 2001). One basic requirement here is that either the sources are independent from each other or that the relation between them is known. Sufficiently precise geographical input data may allow spatial resolution to only a few meters.

Typically, sources are characterized first of all by their geographic location in three dimensions, as points, lines or areas. For air pollutants, a typical point source is a chimney, a line source a road and an area source a harbor. The source strength needs to be described, often as average annual emissions, which is generally sufficient for assessment of average long-term levels. When shorter time periods are of interest, information concerning temporal variations in each source is of value.

The spread of the contaminant away from the source should be described mathematically, employing a physical model. For air pollution usually Gaussian plume dispersion is often used. In most cases, spreading is dependent on factors that vary with time. Air pollution levels are e.g. heavily influenced by meteorology also when all sources remain the same. The same can be said about noise and snow cover. Location is also important, e.g., the level of air pollution the reduced ventilation in a narrowly configured street space is important, and for noise it is essential to consider reflections from surrounding buildings.

Example: Dispersion modelling of air pollution in Stockholm

An emission database was assembled for Stockholm County in 1993, primarily for air quality management. It was the basis for one of the first spatio-temporal assessments of air pollution for epidemiology in the world (Bellander et al. 2001). NO_2 was used as an indicator/representative of road traffic exhaust and SO_2 for heating of individual houses. The temporal aspect was created by retrospective assessment of differences in the emission database, successively back to 1960. The first application was in a case-referent study of lung cancer (Nyberg et al. 2000), which mapped study subjects' home addresses to modeled outdoor levels. It has later been updated and used for several other investigations, e.g., on myocardial infarction (Rosenlund et al. 2006). In connection with the Swedish birth cohort BAMSE (Barn Allergi Miljö Stockholm Epidemiologi; Children, Allergy, Environment, Stockholm, Epidemiology), that follows over 4 000 children, this emission database has been used to examine respiratory and other outcomes. Accordingly, elevated levels of traffic-related pollutants during the first year of life were associated with sensitization against allergens in pollen and persistent wheezing at 4 years of age (Nordling et al. 2008), as well as with enhanced risk for symptoms of asthma up to 12 years of age (Gruzieva et al. 2013), and long-term negative consequences on pulmonary function (Schultz et al. 2012, Schultz et al. 2016).

It takes considerable time and effort to collect and maintain a database of all sources of a specific pollutant. With air pollution, and noise as well, data on road traffic flow and composition of vehicle types is key and in Sweden provided to a large extent by the municipalities. Since it is virtually impossible to perform on-site counting of traffic on every road in a municipality, much of this data comes from models based on measurements on a small number of road segments. Such modelling is based either on generalization from measurements on a stratified sample of road links (Bowling and Aultman-Hall 2003) or on the construction of an origin-destination model that is adjusted to correspond to observed traffic flows (Beser and Algers 2001).

Another complication in this connection is that the source strength of the traffic fleet changes with time. Both the composition of vehicles and emissions from different types of vehicles change. Of course, the accuracy of the road network maps is also important, in particular for noise assessment, and here the quality has improved considerably in recent years. While digitized meteorological data have been available for extended periods, digitized high-resolution topographic data have been made available more recently, greatly facilitating noise assessment.

Thus, source-receptor modelling requires large amounts of high-quality data, as well as considerable computational power. Therefore, dynamic modelling of this sort has been employed to a lesser extent than, e.g., geostatistical modeling, even though the advantages of the former are clear. With historical databases, levels can be assessed for preceding decades with high geographical resolution and e.g. future traffic scenarios can be evaluated.

Example: Exposure to traffic noise and annoyance

Digital noise maps produced by Member States of the EU as required by the Environmental Noise Directive (END) depict estimated levels of noise from road and railway traffic and industrial sources. We examined whether these maps can be utilized for assessment of exposure to traffic noise exposure in connection with population health studies (Eriksson et al 2013).

Individual annoyance due to noise was based on responses to relevant questions included in the 2007 Swedish National Environmental Health Survey (NEHS07). This particular study focused on 2,496 respondents aged 18 to 80 years and living in three Swedish cities (Stockholm, Gothenburg and Malmö). Additional information concerning their housing and its orientation in relation to the environment (e.g., windows facing roads and railways, or gardens) was obtained from this same survey.

For each respondent, noise levels were assessed at the most exposed façade and at the entrance to the building he/she was living in, using the END maps and survey data. By taking the additional information on apartment orientation into account, noise levels could also be assessed at the façade of the participants' actual dwelling.

The proportion of people who reported being annoyed in the NEHS07 agreed well with the proportion predicted to be disturbed by noisy road and rail traffic, as indicated by the established exposure-response functions (Miedema and Oudshoorn 2001).

The results show that the END maps could be used to assess exposure to traffic noise in connection with population health studies. Additional information on e.g., apartment location within a building helped to refine this assessment.

5.3 Validation of spatial modelling of individual exposure

The validity of a spatial assessment of a pollutant can be studied in various ways, the most obvious being to compare the estimates made with actual corresponding observations. When individual exposure is assessed in this manner, it is of great interest to make comparisons to individual measurements. In modeling there is additionally the possibility to study the validity of in-data and other components of the model.

Exposure assessments of air pollution for purposes of epidemiological studies have been improved considerably by the enhanced availability of relevant temporal and spatial data, computing power, and procedures for statistical analysis. Nonetheless, this remains a challenging task and the validity of the models employed is often poorly investigated. However, spatial modelling of outdoor levels of air pollution has been subject to some validation.

For example, the annual levels of NO_2 due to road traffic in Stockholm obtained from a dispersion model averaged 64% of those measured at the front-doors of 487 addresses during one month (Nordling et al. 2008). Despite the temporal mismatch involved, the correlation (r) was 0.72. The agreement between land use regression and dispersion modelling was studied for as many as 13 urban areas in Europe (de Hoogh et al. 2014). For NO_2 there was good correlation between the estimates, particularly with dispersion modelling at higher spatial resolution. For particulate matter in air, the agreement was considerably lower.

Commonly used approaches to quantifying human exposure involve pollutant concentrations obtained from stationary monitoring sites or from spatial models, with the underlying assumption that the individuals in the population do not move. However, every individual exhibits a unique pattern of activity, e.g., time spent indoors, and outdoors in the vicinity of traffic. Thus, it seems likely that exposure levels calculated in this manner reflect actual exposure to only a certain degree, and the short- and long-term uncertainties result in an underestimation of health risks.

Most long-term studies of air pollution thus only take into account residential location and ignore relative amounts of time spent indoors and outdoors, as well as temporal pattern of activity. Personal measurements and biological monitoring are expensive, make demands on personnel and can be a burden to individuals. As a consequence, comparisons of modeled to monitored exposure are few in number and generally involve small populations.

However, the relationship between individual exposure to air pollutants as indicated by personal monitoring and a spatial model has been investigated in a few cases. Personal exposure to NO_2 was measured for 7 days in 247 adults in Stockholm County, and compared to dispersion-modeled estimates of annual outdoor NO_2 levels from road traffic. The between-person variation in individual exposure was to 20% and 16%

explained by NO_2 from road traffic at home and at work, respectively (Bellander et al. 2012).

5.4 Remote sensing

Most models of air pollution described previously have focused on urban areas, neglecting less densely populated areas. Combined assessment of the effects of short- and long-term exposure to air pollution on health is also still lacking.

In recent years, increased availability of satellite data on daily levels of air pollution for large areas (including less densely populated areas) has opened new possibilities for overcoming these problems by advanced statistical modelling that matches such satellite data with parameters of land-use, emission and population (Kloog et al. 2012).

Example: Modelling UV radiation from the sun

Exposure to ultra-violet (UV) radiation from the sun or artificial sources such as sunbeds is associated with an elevated risk for skin cancer. Outdoor levels of solar UV depend on the height of the sun, thickness of the cloud cover, and the amount of ozone in the atmosphere. The STRÅNG model for levels of solar UV radiation (as well as for global radiation and photosynthetically active radiation) was originally developed by the Swedish Meteorological and Hydrological Institute, SMHI, using a grid size of 22 x 22 km (Landelius 2001). The spatial resolution was increased to 11 x 11 km in 2006 and estimates of UV levels may be downloaded freely from a website (http://strang.smhi.se).

In a study on lifeguards and farm workers, the personal exposure indicated by polysulphone film dosimeters correlated reasonably well 24-hour STRÅNG estimates (r=0.54; Liljendahl et al. 2013), indicating that this model in combination with time-activity information might be helpful for assessing UVR exposure in connection with health studies.

5.5 Monitoring time-activity patterns

Assessment of personal exposure is evolving quickly due to advances in sensitive sensor technology that enable researchers to monitor exposure to air pollutants in real-time, as individuals go about their activities at various locations. GPS devices (now incorporated into most smartphones) allow monitoring of movement through different microenvironments and, in combination with portable monitors, can evaluate exposure to pollutants accurately with respect to time and location. Such monitoring measures concentrations with high spatial and temporal resolution, in contrast to stationary indoor

and outdoor monitors, that usually average data for a given unit of space (Steinle et al. 2013). With such approaches, not only can long-term exposure and the proportion of a population exposed to levels above a certain threshold be calculated, but the frequency of short-term exposure above a certain level can also be monitored. The latter information may provide novel opportunities to examine the effects of short, but frequent peaks of exposure on health outcomes.

5.6 Research and developmental needs

- Further development and validation of dispersion models for estimation of levels of different particulate components (such as PM_{10}, $PM_{2.5}$ and soot) in ambient air with high geographical resolution including contributions from various local sources (primarily road traffic and burning of biomass) are required. It is crucial that the quality of such data be as high as possible.
- Although soot in air (black carbon, black smoke, elemental carbon, etc.) is considered a health-relevant measure of air quality, this parameter has not been systematically measured or modeled, and the relation of models to individual exposure needs to be assessed.
- To provide a basis for epidemiologic research and risk assessment hybrid models combining dispersion modeling, land use regression and satellite-based techniques should be developed to allow estimation of air pollution over large populated areas with high spatial and temporal resolution.
- The models for evaluation of noise from different sources, such as road and railway traffic, as well as aircraft, both outside and inside buildings should be improved. For purposes of research on the effects of long-term exposure, retrospective data are required as well. Some of the data needed for assessment of air pollution and noise generated by road traffic are similar and could preferably be obtained simultaneously.
- The influence of commuting by different modes of transport (i.e., bus, subway, car, walking and biking), as well as of other microenvironments such as road tunnels should be explored.
- Increasingly human exposure to green space, especially in urban environments, is being linked to different aspects of health. Several underlying mechanisms have been proposed and future epidemiological studies should address multiple aspects of exposure to both green and blue space (water). In this context it will be important to distinguish between, e.g., seeing, spending time in, or being physically active in or close to green, spaces with lower levels of, e.g., air pollution and noise. Appropriate methods should be developed in close connection to recent approaches to monitoring human activities in urban environments, such as assessment of "walkability", i.e. how the urban layout affects the possibilities to walk between different locations.
- In all spatially related epidemiological analysis there may be a problem with spatial confounding, i.e., other risk factors to the studied health outcome co-vary spatially with the parameter assessed. Most often, relatively coarse measures of, e.g.,

neighborhood socioeconomy are employed. This may be sufficient when spatial co-variation is not very pronounced, as with air pollution in Sweden. However, with respect to green- and blue space, more sophisticated approaches may be required.

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6. Occupational Exposures

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6.1 Introduction

Representative monitoring and assessment of occupational exposure to airborne pollutants, vibrations or noise must deal with a number of methodological problems. In the case of chemical substances, exposure may be defined as the potential for human contact by inhalation, deposition or direct contact with the skin or eyes, and/or by ingestion. The four major components of assessment of the distribution and determinants of occupational exposure that may affect workers' health are: the study design, data collection, data analyses (statistical modelling), and interpretation.

Assessment of exposures that lead to disease only after a long period of latency is especially challenging. For this purpose, retrospective data are required and these are often collected utilizing indirect methods, since data on individual levels of exposure is often missing. Ideally, quantitative measurements of both external concentrations (e.g. in air or on the skin) and of internal dose (in blood, plasma or urine) would facilitate such assessment, but few studies have access to such measurements. Assessing the exposure by human biomonitoring (discussed in detail in Chapter 2) is a cost-effective approach to obtaining a large amount of high-quality data.

The choice of methodology for the assessment of exposure depends both on the study design and resources available. In cross-sectional studies where current exposure is of interest, direct procedures are preferable. For retrospective assessment of exposure based on incomplete data, indirect approaches are necessary.

6.2 Direct methods for assessment of exposure

Assessment of occupational exposure on the basis of measurement of individual exposure generally provides an accurate estimate of the actual situation, provided that the measurements are performed under normal working conditions and reliable methods and monitors are utilized in a suitable manner.

6.2.1 Measurement strategy

To detect the actual dose-response relationship between an exposure and health outcome, the study must be designed very carefully. Various measurements of exposure following an appropriate strategy and linked to proper statistical evaluation of variability are required. Standards and guidelines for designing measurement strategies to monitor compliance with limits set for occupational exposure have been formulated (CEN 1995, British Occupational Hygiene Society 2011).

The variability of exposure will depend heavily on factors such as distance from the source(s) of emission and the working procedures performed, (e.g. whether these are continuous or intermittent), but also on environmental conditions such as temperature, air currents and weather. Since exposure is closely related to the tasks performed, it will inevitably vary among workers at the same location (Kromhout 2002). The various purposes of sampling include monitoring for compliance with permissible occupational limits of exposure, and studying a health outcome. To cope with variability in the measurements, specific strategies for sampling must be decided on before monitoring starts.

The first step in assessment of occupational exposure is to describe factors that may influence exposure in the workplace, including the presence of chemicals, the production processes, work tasks, layout of the facility, ventilation, sources of emission, periods of exposure, and work load. Secondly, the type of measurements to be performed must be decided, i.e., worst-case scenario, close to the source of emission, or screening of samples. Normally measurements of exposure are expensive and the project budget limits the number that can be made.

In many cases, it is relevant to measure the average level of an exposure agent during a full-day shift, for comparison with occupational limits of exposure (OELV). However, it may sometimes be more appropriate to determine the presence and frequency of peak exposures, e.g., when concerned about individuals with asthma, who may respond to peaks rather than a low and consistent exposure.

The question as to which workers accurately represent the exposure at the workplace must be given careful consideration. If the sole purpose is to evaluate whether the OELV is exceeded, straight-forward determination of the worst-case scenario is adequate. For other end-points more representative measurements must be performed.

The most obvious approach is to select members of the entire workforce at random. Another popular method is to divide workers into sub groups that can be expected to differ in exposure (e.g., on the basis of work tasks, job titles or similar exposure groups (SEG)) and then sample any member of each such group (Nieuwenhuijsen 2003). However, this strategy has been criticized since even among workers who share the same tasks and environment, exposure may vary widely.

When employing the similar exposure group (SEG) approach, several measurements must be performed on each worker, in order to evaluate the variability within-workers, as well as between-workers. Furthermore, to draw valid conclusions more complex statistical methods, such as mixed models of exposure, must be applied to the data (Rappaport et al. 1993, Rappaport et al. 1995, Kromhout 2002). A third option is to categorize workers a priori into three groups assumed to be subjected to low, medium and high exposure, and then sample these groups in the ratio 1:3:5. The rationale here is that exposure in the highly exposed group is likely to vary to a greater extent, so that more samples are required (Loomis et al. 1994).

6.2.2 Monitoring air

When performing measurements on air, individual sampling in the breathing zone is usually preferred since area sampling often results in lower concentrations and is thereby a less accurate measure of personal exposure. Depending on the substance of interest, several different sampling techniques, usually categorized as *active* or *passive* are available. *Active sampling* can either be *integrated*, providing an average concentration of the substance in the air per unit time, or involve *real-time measurements,* where variations in this concentration, including peak values, with time are monitored. *Passive* sampling results in an integrated concentration.

In connection with *active sampling* air is pumped through a collection unit, usually a sampling head with filter. Different sampling devices are used to collect particles of different sizes. The most common being the 37-mm open-face/close-face cassette and inhalable dust sampler. In Sweden, "total dust" concentrations, determined with the open-faced 37-mm filter cassette have traditionally been used to evaluate compliance with OELVs. However, this sampling procedure does not always correlate well to the fraction of particulate matter inhaled. A better approximation of inhaled dust can be obtained with the IOM sampler (Institute of Occupational Medicine) (Liden et al. 2000). To evaluate the finest particles reaching the cilia of the respiratory tract, the fraction of respirable dust should be sampled (Vincent 1995).

Most reports on parallel usage of the IOM and "total dust" samplers have found an average dust ratio between these two of approximately 1-2 (Davies et al. 1999, Harper et al. 2004, Kriech et al. 2004, Lee et al. 2011) although values as high as 6 have been observed (Spear et al. 1997). With respect to different chemicals/substances attached to the particles, for example brominated flame retardants (Julander et al. 2005) and metals (Julander et al. 2014) similar or even higher ratios have been described. The variation in this ratio is related directly to the size distribution of the particles and the homogeneity of the particulate aerosol, as well as the placement of the samplers (Davies et al. 1999, Liden et al. 2000). There is now sufficient evidence to discontinue use of the traditional "total dust" sampler and start employing only the inhalable fraction or, for even finer particles, the respirable fraction, for comparison with OELVs. The sampling head can also contain a material designed to collect the gas phase, which is suitable for measuring compounds not readily adsorbed to a filter, e.g. those with high volatility (Julander et al. 2005).

In the case of measuring exposure to nanomaterials, standardization has not yet been achieved. Although nanomaterials are covered in principle by REACH (the regulation of chemicals in the European Union), it is not yet clear how the law applies to such materials. Numerous ongoing projects, both at the national and international level, are attempting to assess the risks associated with nanomaterials including, including Project FP7 NANOREG, in which Sweden is participating. In general, different types of nanoparticles appear to exert very different toxic effects on cells and animals

depending for instance on their composition, but also on their size and shape (Karlsson and Fadeel 2014).

Passive sampling, primarily used for gaseous substances, is based on diffusion and does not require a pump. Researchers at our institute are presently performing passive sampling to assess the occupational exposure of, e.g., kitchen staff and fire-fighters. An advantage to this approach is easy self-operation of measuring devices.

The third alternative, *real-time measurements* using, e.g., direct logging instruments such as Respicon, DataRam, P-Trac or Grimm, allows variations in exposure to be monitored in real-time. This approach is suitable for studying processes that generate dust and for identifying sources of exposure. One example is presented in Figure 6.1, which involves granulation of plastics at an electronics recycling facility and clearly shows various peaks in the exposure to dust. The levels of "inhalable dust" and "thoracic dust" fall immediately during the lunch break, while that of the respirable fraction does not.

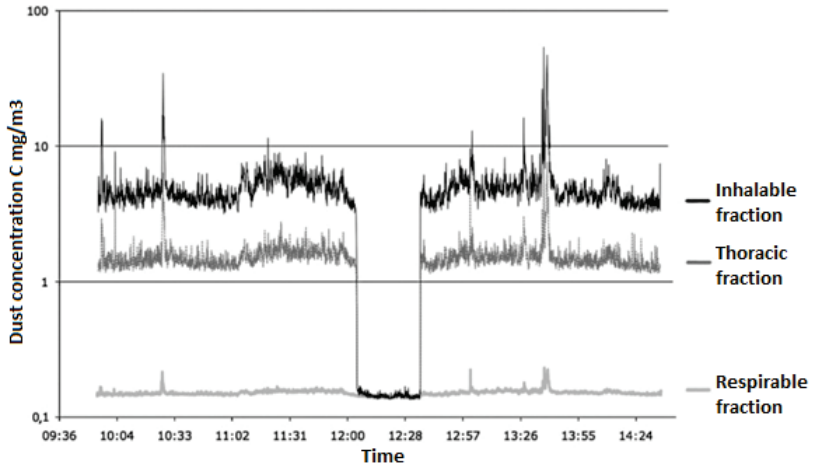

Figure 6.1. Real-time measurement of dust concentrations in a facility that recycles plastic in electronic devices using a Respicon sampling device. The three different fractions of dust were collected on filters simultaneously. Peaks of exposure, as well as virtually exposure-free periods are seen for the two larger fractions (inhalable and thoracic) but the respirable level of dust is constant.

6.2.3 Monitoring noise

The level of noise in the working environment, which is regulated by the Swedish Work Environment Authority (Buller, AFS 2005:16), is usually measured as "Daily noise exposure level", i.e., the equivalent A-weighted level of sound pressure during an 8-hour workday. Workplace noise is often expressed as dB(A).

Noise is normally measured with personal sampling equipment and measurements during the working day should include quiet periods for optimal accuracy. Since software programs provide exposure during different intervals of time, it is possible, when desired, to retrospectively exclude breaks.

When the aim is to attenuate noise or select appropriate hearing protection, stationary measurements of the sound pressure level at various frequencies are performed at one or several locations. If this level is relatively constant at any given setting, a map of areas with noise levels above the action- and limit-values can be constructed. In certain settings reverberation is important, since long reverberations can camouflage sound and be extremely annoying, especially for individuals with hearing impairment.

Even noise below the limits for exposure can interfere with concentration and communication or make it difficult to perceive warning signals, and have been associated with an elevated risk of cardiovascular disease. Individuals with hearing impairment, elderly workers and those who speak a foreign language may have special needs for low background levels of noise.

Sound with a frequency of 20-200 Hz is referred to as low frequency sound, and below 20 Hz, as infrasound. Infrasound and low-frequency noise are generated by, e.g., diesel engines, ventilation, technological and chemical processes, transportation and construction activities (Bolin et al. 2011). Although infrasound is hard for humans to perceive, it can disturb rest and sleep, influence psychology performance and behaviour, and cause annoyance (Fritschi et al. 2011). Since routine noise dosimeters filter out low-frequency sound almost completely, a more advanced monitor is required for such measurements.

Although ultrasound (i.e., sound with a frequency, above 18,000 Hz) is beyond the upper limit of human hearing, it may still cause hearing damage. At the same time, unlike infrasound and low-frequency noise, ultrasound is easy to screen out, and effective hearing protection is available.

Since 2007, Swedish legislation recommend that pregnant women should not be exposed to noise louder than 80 dB(A), due to its adverse health effects (Basketter et al. 2005). For the mother, these include elevated blood pressure, stress and fatigue and, for the foetus, an enhanced risk for hearing impairment, slower growth and stress. Low-frequency noise, in particular should be avoided, since this is not attenuated by body tissues and may even be amplified inside the body.

In Sweden, 15% of women employed report occupational exposure to noise so loud that they cannot have a normal conversation during at least one fourth of their working day (Arbetsmiljöverket 2011). Research ongoing at our institute indicates that such exposure during pregnancy is associated with an increased risk of small size for gestational age and low birth weight, as well as future hearing dysfunction. Dose-response associations indicate that pregnant women should not be exposed to medium or high levels of noise at work (Selander et al. 2015).

A Job-Exposure Matrix (JEM) based on hundreds of measurements of exposure to noise in various work environments and in connection with various types of activity has been developed at IMM (Sjostrom et al. 2013). The noise levels were classified into three groups, and an estimated level of exposure provided for different occupations. This JEM has been validated and recently expanded to include five exposure groups. It is readily available to our collaborators and already being used by several research teams.

6.2.4 Monitoring vibrations

Vibrations (movements relative to a static equilibrium and expressed as accelerations (m/s^2)) are prevalent in many occupations and regulated by the Swedish Work Environment Authority (Vibrationer, AFS 2005:43). Workers who handle electrical machines (e.g., construction workers, cutters, vehicle repair men, and welders), experience hand and arm vibrations as do those in occupations involving high-frequency vibrations, such as dental technicians and dentists. Moreover many professional drivers (e.g., forklift trucks, road vehicles for transporting goods, buses and coaches), are subjected to whole-body vibrations. Among the approximately 350 000 workers exposed occupationally to hand and arm vibrations, vibration injuries, especially nerve damage, are common. Indeed, an increasing number of the patients seen at clinics specialized in occupational medicine have symptoms due to vibrations at work.

Daily exposure to vibrations involves both magnitude and duration. In the case of strong shocks, a special standard is applied in order to avoid underestimation of the risk. A vibration monitor connected to an accelerometer attached to a hand-held machine can quantify vibrations. Although not standard, special gloves with built-in accelerometers are easy to use. For measuring whole-body vibrations, a seat plate with an accelerometer placed on the seat of the machine/vehicle is a convenient approach.

Manufacturers must declare levels of vibration, which is an indirect means of estimating exposure. However, the values declared may not be relevant to the work tasks under investigation. Exposure to vibration is also influenced by a number of other factors, including the weight of the tool, tool maintenance, the hardness of the material being processed, and temperature.

Researchers at IMM are involved in building a modern database on exposure to vibration for purposes of assessment and research. The concept is to modernize an existing database by incorporating a calculator of exposure to vibrations. New vibration data for modern tools will be added, and novel features that facilitate assessment developed.

We also plan to develop small, simple, wireless devices for measuring hand-arm vibrations. Importantly these devices will also record the duration of exposure. In this way uncomplicated, time-efficient and accurate measurement of vibrations during the entire working day will be achieved.

6.3 Methods for indirect assessment of exposure

In connection with large population studies, measurements of exposure may not be the most efficient and cost-effective approach. In addition, in retrospective evaluations of exposure studies during the past several decades, the data required may be incomplete or partially or entirely missing. In such cases, indirect measures of exposure including, e.g., questionnaires, job exposure-matrices (JEMs), and statistical modelling, must be employed. In recent decades procedures from basic self-assessed exposure to computerized modelling have been developed for this purpose (see Chapter 3 for additional information on epidemiological methods for assessment of exposure).

6.3.1 Self-assessed exposure

Although self-assessed exposure, where workers fill in a questionnaire and/or are interviewed about their occupational exposures, is comparatively simple and inexpensive, validated questions must be posed. This approach is limited to exposures that the subjects can perceive in relatively objective manner and relate to a level, e.g., a threshold for detection of an odour. Due to recall bias, self-assessment of exposure may result in both systematic and random misclassification.

6.3.2 Assessment of similar groups

Traditionally, occupational and work-related tasks have been used as a basic surrogate for exposure, usually utilising data from company personnel records or reports by the study subjects. Although such data are normally reasonably accurate, (Teschke et al. 2002) they cannot identify specific agents as risk factors. Another problem here is that the effect of a particular agent may be missed if only a few individuals are exposed. This approach entails primarily random misclassification.

6.3.3 Expert assessment

Estimation of exposure by experts (e.g., occupational hygienists) is useful in connection with the assessment of occupational exposure in retrospective community-based studies when measurements of exposure are lacking (Stewart and Stewart 1994). The experts interpret the measurements available and/or or fill in the gaps. The advantage over self-assessment is that the expert understands the mechanisms of exposure and can evaluate which agents and levels of exposure are of potential significance in connection with a range of jobs.

However, in large studies experts may not be familiar with all jobs during the period of interest. They base their estimates on job-title and occupation, together with supplementary information such as interviews of key individuals, measurements of exposure and the scientific literature on hygiene. The quality can be enhanced with detailed data on working conditions, and by focusing on agents that are well known and easily sensed. The fact that this procedure is blinded, i.e., the expert has no information concerning the case-control status, reduces the risk for systematic misclassification.

At IMM, we have utilized expert assessment in connection with epidemiological evaluation of relationships between, e.g., myocardial infarction and occupational exposure to motor exhaust (Gustavsson et al. 2001), exposure to asbestos fibres and lung cancer, (Gustavsson et al. 2002) and silica dust and lung cancer (Wiebert et al. 2014).

6.3.4 Job-exposure matrix (JEM)

A Job-exposure matrix (JEM) provides an efficient tool for assessing exposure in large populations. JEMs list occupations on one axis and the exposure agents to which workers are potentially exposed on the other (time may be on a third axis) and the cells of the matrix indicate "yes" or "no" for exposure. In more sophisticated models the presence, intensity, frequency, and/or probability of exposure to a specific agent in a specific job are also shown in these cells. The information in the cells is usually based on measurements, but if data are missing expert judgement, extrapolation from adjacent cells and/or statistical modelling can be employed.

Generic JEMs include jobs undertaken by the entire population, and the early ones constructed in the 1980s were based primarily on expert judgement. Since then, their validity has been improved in various ways, such as by ensuring that jobs are correctly coded (Kennedy et al. 2000), utilising measurements of exposure available in databases to complement expert assessments (Kauppinen et al. 1998), and combining assessment of exposure from several studies (Lavoue et al. 2014). The advantage of JEMs over self-assessed exposure is that the former involve no risk for recall bias. However, the specificity and sensitivity of generic JEMs are relatively low and JEMs do not take the variability of exposure in association with a given job into account.

When the coding of jobs in the JEM does not conform to that of the cohort, translation is necessary. Crosswalks from one coding system to another are provided by, e.g., SCB (Statistics Sweden) and ISCO (International Standard Classification of Occupations). Normally, information is lost during this process, due to splitting and merging of job categories. Accordingly, to minimize misclassification experts familiar with both the coding-systems and occupational exposure should perform these translations (Burstyn et al. 2014).

At IMM, we have utilized JEMs for epidemiological assessment of exposure since the 1980's and have developed generic JEMs for, e.g., carcinogens (Plato and Steineck 1993, Kauppinen et al. 2009), dust and smoke (Wiebert et al. 2012), allergens and irritants (Wiebert et al. 2008), and motor exhaust (Ilar et al. 2014).

6.3.5 Tools for modelling exposure

Several computerized tools for modelling exposure have been developed in recent years. When measurements are incomplete or missing, exposure can be assessed using a combination of disparate information within a statistical framework provided by the most simplistic, conservative models, i.e., ECETOC (ECETOC 2012), and Stoffenmanager

(Marquart et al. 2008). For more detailed evaluation of substances or preparations, more advanced models, i.e., the ART should be applied (Tielemans et al. 2011).

In the case of such more elaborate models, expert knowledge on inter- and intra-individual variability in exposures, as well as measurements of exposure in specific contexts are incorporated into a calibrated mechanistic model of exposure. Such models can provide central estimates and intervals for different percentiles of the distribution of average full-shift and long-term exposures. These estimates can be improved by the inclusion of more data. Modelling is of value in connection with designing preventive measures at workplaces, finding hazardous operations and identifying areas where exposure can be expected to be elevated. It is, however, essential that these tools are handled properly, and in combination with measurements (Landberg et al. 2015).

Table 6.1. A summary of the advantages and disadvantages of tools used for indirect assessment of exposure in connection with occupational science.

Exposure assessment method	**Advantages**	**Disadvantages**
Self-assessed	Simple and inexpensive	Systematic and random misclassification
Similar groups	Data often accurate, cost-effective	Specific agents not identified as risk factors, variability in association with a job not taken into account the, risk for random misclassification
Expert assessment	Low risk for systematic misclassification	Expensive and labour intensity
Job-exposure matrix (JEM)	Efficient for large populations, no risk for recall bias	Variability in association with a given job not taken into account, risk for random misclassification
Exposure modelling	Effective use of measurements, no risk for recall bias	The various models available have their own individual weaknesses

6.4. Research and developmental needs

Exposure levels in the work environment are usually several times higher than in the general environment, and, therefore, it is especially important to assess occupational exposure in order to avoid illness, as well as expenses for health care and sick leave. Measurements of occupational exposure are essential to reliable assessment and should be performed by personnel proficient in measurement strategy and sampling methods, and who can interpret the findings. When measurements are missing or large populations are being examined, indirect approaches, such as job-exposure matrices and modelling, must be utilized.

The following areas concerning occupational exposure require more attention:

- To assess occupational exposure, measurements are required analogous to the data hosted by IMM in connection with a National and regional health-related environmental monitoring program (HÄMI). Since no corresponding program for the work environment presently exists, there is no basis for setting priorities for a national work-environment plan. We see a great need for a National health-related work-environmental monitoring program, where measurements can be gathered in a Swedish national occupational exposure measurement database. Sweden already has a large collection of occupational measurements that need to be processed and compiled in a database, thus being made available to researchers.

- The theoretical framework concerning usage of measurements and dose modelling to determine personal exposure metrics in association with epidemiological studies must be developed further, and existing information analysed more fully, especially with respect to the dose-response relationships needed for hazard evaluation. At present, epidemiological studies rely heavily on cumulative exposure, even though it is well known that this may not be proportional to the risk for disease. Methods for planning measurements and choosing the most appropriate statistical models for evaluating individual exposure within a group are needed. Disentangling the effects of the duration and intensity of exposure may be one step in this direction.

- Epidemiological studies of chronic diseases often require assessment of past exposures, but historical measurements are often scarce, so that modelling is often applied. Improved and transparent procedures for accurate assessment of past exposures are highly desirable.

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7. Indoor Air Exposure

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7.1 Indoor air quality (IAQ) in relationship to building materials and other sources

Indoor air quality (IAQ) refers to the air within buildings, especially with respect to the health and comfort of the building residents. This term encompasses the occurrence and/or excess of, for example, carbon dioxide, carbon monoxide, volatile organic compounds (VOCs) microbial volatile organic compounds (MVOCs), particulate matter, microbial contaminants (mold, bacteria), and radon. IAQ is part of indoor environmental quality, which includes also physical and psychological aspects of life indoors such as lighting, visual quality, acoustics, and thermal comfort (temperature, humidity, air drafts). The present chapter focuses on exposure to chemicals in indoor air, in particular VOCs and MVOCs.

People living in Sweden spend roughly 90% of their time indoors and should ideally have access to clean air, even when surrounded by walls, floors and ceilings. However, indoor air is always contaminated to some extent by environmental pollutants originating from interior surfaces and building constructions.

Since modern buildings contain many different types of building products based on a wide variety of materials and chemicals, the potential for exposure to complex mixtures of pollutants in indoor air is considerable. The levels of pollutants emitted directly from building materials, referred to as primary emissions, generally decrease with time and odor is rarely a problem since odorous materials would not be successful on the market.

Certain primary emissions may react with other chemicals in air, such as ozone, leading to the formation of new chemicals or particles, so-called secondary emissions. Chemical reactions that give rise to secondary emissions may also occur when different materials are combined and/or when materials become damp (Uhde and Salthammer 2007).

Moisture in building materials often elevates the level of primary emissions and leads to secondary emissions as well. In addition, dampness supports microbial growth and may thereby give rise to a variety of emissions from bacteria, actinobacteria and mold, for example spores and toxins. Secondary emissions arising from chemical reactions and microbiological sources may be odorous.

Altogether, many different types of indoor air pollutants originating from buildings may result in deterioration of IAQ and cause symptoms in residents, typically from the upper airways and with asthmatics being more sensitive (WHO 2009). However, since the

levels of chemicals in indoor air are low and the levels of mold are often lower than outdoors, it is generally difficult to identify the individual agents responsible for health effects. This situation is quite different from indoor occupational exposures in industrial settings which are generally anticipated, regulated by occupational exposure limits (OELs), and controlled (see Chapter 6: Occupational exposure).

In some cases however, it is possible to identify the pollutants that cause symptoms from the eyes, skin and airways in non-industrial indoor environments, such as offices, schools and homes. Many of these exceptional cases involve allergies towards specific agents, such as pets and house dust mites, and even towards certain chemicals in building materials or interior coatings, such as colophonium (used, e.g., in linoleum flooring) and methylchloroisothiazolinone (MCI), methylisothiazolinone (MI) and other isothiazolinones (present, e.g., in water-based paints) (Fall et al. 2015, Schwensen et al. 2015) (see Chapter 8: Skin exposures to hazardous chemicals).

In addition to building materials, the IAQ is strongly influenced by other sources of emission, such as furnishing, equipment and household products, cooking and unventilated use of fireplaces, as well as by the habits of the residents, such as smoking. Moreover, pollutants from the outdoor air, for example radon from the ground and exhaust fumes from traffic, may reduce the IAQ.

7.2 Assessment of exposures and health hazards in relationship to IAQ in Western countries

Attempts are being made to identify the chemical contaminants of major concern in the indoor air of residences, as a foundation for ongoing efforts to manage residential IAQ. One investigation, concerning the United States and other countries with similar life-styles, focused on indoor levels of VOCs, semi-VOCs (SVOCs), metals, and chemicals defined as criteria pollutants in amendments to the 1990 Clean Air Act, i.e., nitrogen dioxide (NO_2), ozone, carbon monoxide (CO), lead, sulfur dioxide (SO_2), and particulate matter with an aerodynamic diameter of $\leq$ 2.5 μm ($PM_{2.5}$) (Logue et al. 2011). Five activity-based emissions were identified as potential acute health hazards, i.e., $PM_{2.5}$, formaldehyde, CO, chloroform, and NO_2. Nine pollutants were identified as priority hazards, on the basis of the robustness of measured concentration data and the fraction of residences that appeared to be impacted, i.e., acetaldehyde, acrolein, benzene, 1,3-butadiene, 1,4-dichlorobenzene, formaldehyde, naphthalene, NO_2, and $PM_{2.5}$. A follow-up study that aimed to assess the chronic health impact came to the conclusion that $PM_{2.5}$, acrolein, and formaldehyde accounted for the vast majority of the loss of disability-adjusted life-years caused by the indoor air pollutants examined, with impacts on a par with or greater than those estimated for secondhand tobacco smoke and radon (Logue et al. 2012).

Only a few of the potentially hazardous pollutants mentioned above, including radon, originate primarily from indoor sources and none exclusively from building materials or surfaces. As an example, formaldehyde, a constituent of the carbamide glue used in several building materials, including particle board, is also used as an additive to inhibit microbial growth, e.g., in hygiene products and cosmetics.

Concerning VOCs, these authors (Logue et al. 2011) compared their results with data in a previous review (Brown et al. 1994) to assess temporal changes in concentrations. For the most part, these concentrations had fallen by at least 50%, although some remained the same or had risen, i.e., those of certain light solvents (acetone, ethanol and methyl ethyl ketone) and VOCs associated with deodorizers, insecticides, and cleaning (limonene and 1,4-dichlorobenzene). The earlier study (Brown et al. 1994) also reported a large elevation, often by an order of magnitude or more, in the concentrations of VOCs in new homes, whereas in the later article the concentrations of most VOCs in most new homes were reduced or only slightly higher (Logue et al. 2011).

These apparent reductions in VOC concentrations were proposed to reflect lower levels of chemical emissions from new materials, as well as improvements in home ventilation (Logue et al. 2011). Similar to this situation in the United States, indoor concentrations of VOCs have decreased with time in Sweden (Boverket 2010a).

7.3 Assessment of exposures and health hazards in relationship to IAQ in Sweden

A survey of the status of the Swedish housing stock and IAQ conducted in 2007-2009 included measurements of selected pollutants, some of which may cause symptoms in the airways and eyes, e.g., NO_2, formaldehyde and the total of VOCs (TVOC). Regarding NO_2, which is in general derived from outdoor sources such as burning and combustion, indoor concentrations were lower than outdoors and the outdoor concentrations have declined with time. The mean levels of formaldehyde and TVOC are generally higher indoors but in the case of formaldehyde still less than one fourth of the guideline value of 100 µg/m^3 recommended by the WHO (WHO 2010). The mean concentration of TVOC was 230 µg/m^3, representing a 20-45 % reduction in comparison to a previous survey reported in 1993 (Boverket 2010a).

It was noted already several years ago that the levels of VOCs in indoor air, which captures a large proportion of the gaseous chemicals in air at room temperature, are generally far below toxicological effect levels, even in buildings where many residents experience problems with the IAQ. Thus, the concentrations of VOCs that cause irritation are for the most part at least 100-1000 times lower than the corresponding OELs. Recent observations suggest that asthmatics may be as much as ten times more sensitive to irritating airborne particles and chemicals, and combinations thereof, than healthy

subjects (Johansson et al. 2016). Nonetheless, it remains difficult to explain the relationship between IAQ and symptoms perceived by some residents.

Thus, with the exception of radon, chemical and microbiological measurements generally fail to detect levels of indoor pollutants that might be responsible for adverse health effects. However, since such measurements do not usually reflect how the IAQ is perceived by residents (see above), it has been suggested that the definition of good IAQ should take into account the extent to which human requirements are met, i.e. that the air does not have a negative impact on health but also that it is perceived as acceptable, or even better, as fresh and pleasant (Fanger 2006). In other words, good indoor air should not cause disease, discomfort or dislike. With this definition, also used in the following, odor will generally be incompatible with good IAQ.

7.4 Potential inclusion of odor in assessment of exposure and health hazards related to IAQ

Odor is an inherent aspect of IAQ. In an early report on the sick building syndrome by the World Health Organization (WHO) odor was described both as a symptom of disease and as an exposure of high relevance for future research (WHO 1986). The sick building syndrome, today referred to as unspecific building-related illness, encompasses a variety of symptoms originally reported mainly from the Scandinavian countries and the United States and described as follows: eye, nose and throat irritation; sensation of dry mucous membranes and skin; erythema; mental fatigue; headaches, high frequency of airway infections and cough; hoarseness, wheezing, itching, and unspecific hypersensitivity; nausea, dizziness (WHO 1983).

More recently, a review from the WHO describes mold odor as a sufficient single indicator of dampness and mold in buildings, which can be associated with various diseases of the airways, including asthma and allergy (WHO 2009). The mold odor is considered to indicate co-emission from hidden mold growth of some elusive and unidentified pathogenic factor(s) responsible for this negative impact on health. The working group that produced the WHO report suggested that “the individual species of microbes and other biological agents that are responsible for health effects cannot be identified. This is due to people often being exposed to multiple agents simultaneously, to complexities in accurate estimation of exposure and to the large number of symptoms and health outcomes due to exposure” (WHO 2009).

One thing to consider here is that the exposures in damp buildings may vary considerably, for example due to differences in climate and the types of buildings and materials. In addition, the perception of risk associated with dampness in buildings may differ between populations in different countries. Therefore, it is conceivable that the mere perception of mold odor, in combination with the knowledge that living in damp

buildings constitutes a health risk, may in some cases contribute to the development of symptoms in residents through various psychobiological mechanisms.

In the experimental setting, it is generally difficult for exposed individuals to discriminate the chemosensory perception of odor from that of low levels of irritating chemicals (Wolkoff et al. 2006). From a toxicological perspective, odor is not usually considered to be an adverse effect. However, in its guidance document the Scientific Committee on Occupational Exposure Limits (SCOEL) of the European Commission discusses that not only symptoms such as ocular or nasopharyngeal discomfort or irritation but also nuisance due to smell might be regarded as an adverse effect and thereby serve as a basis for setting a health-based OEL (SCOEL 2013).

Nonetheless, development of criteria concerning nuisance from malodors remains difficult due to the essentially subjective nature and extensive inter-individual variability in such perceptions. However, public perception of the risk associated with mold odor, or any other disliked odor, could be decisive for the outcome of exposure. This was shown recently in a population survey and in an experimental study where hazard information and perceived pollution were more reliable predictors of reported symptoms reports than the actual exposure concentration (Andersson et al. 2013, Claeson et al. 2013). This raises the question as to where, when and how the perception of a relationship between air pollution and health hazards evolved. We suggest that in Sweden this perception evolved some decades ago, when the levels of indoor air pollutants were higher than today.

In this context, it is of interest that a recent survey of Swedish buildings still reports higher indoor air concentrations of formaldehyde and TVOCs in houses built in the 1960s and 1970s, i.e., at the time when the concept of hazardous new buildings developed, than in houses built before and after this time (Boverket 2010a). Therefore, building practices and materials that had and may still have an impact on IAQ in Swedish buildings should be evaluated (Figure 7.1).

7.5 Temporal changes in Swedish building practices and materials of relevance for IAQ

By today´s standards, living conditions in Sweden in the first decades of the 20th century were poor. The population density in cities was high and dwellings were overcrowded, resulting in poor hygienic and sanitary conditions. After the Second World War, the Swedish economy developed rapidly and authorities took large-scale initiatives to improve housing conditions.

In 1961, the rate of building increased and reached a peak between 1965 and 1974, as the result of a government decision that led to the construction of more than one million new homes in a country with, at the time, approximately 8 million inhabitants (Figure 7.1). In

addition, many non-residential buildings were erected in new or reformed urban districts and many old buildings were renovated (Vidén 2012). During this unprecedented expansion of the Swedish building stock, new types of houses were constructed, often on moist ground, and new building techniques and materials came into practice (Figure 7.1).

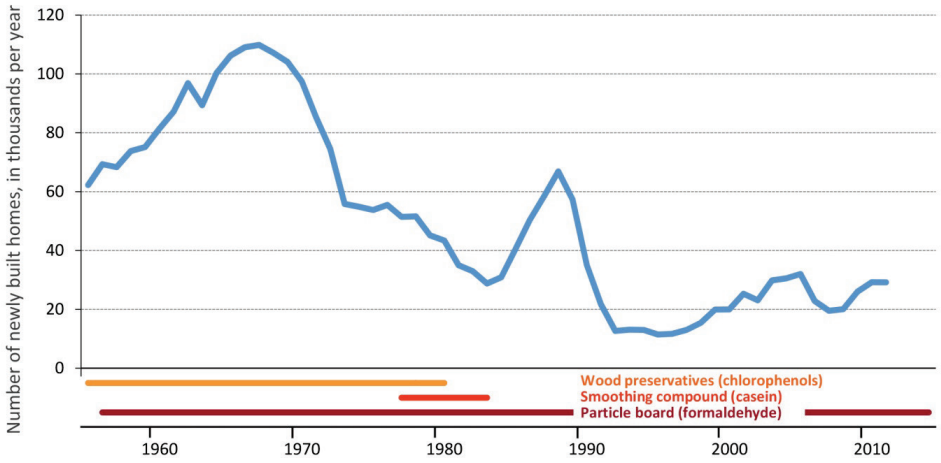

Figure 7.1. The number of homes built over time in relationship to the use of three building components that have caused, and may still cause, problems with IAQ.

In response to the global energy crisis in 1973-1974, various steps were taken to conserve energy in Swedish houses. Buildings that were more thoroughly insulated and air tight became standard, often with re-circulation of air and reduced rates of ventilation. The number of buildings with IAQ problems increased rapidly in the 1970s.

In the middle of the 1970s Swedish authorities recognized different types of health hazards in the indoor environment, including legionella disease (from air moisturizers), radon and its daughters (from the ground or building blocks containing certain minerals), and formaldehyde (from carbamide glues in various types of board, e.g., particle board). Concerning formaldehyde, indoor air levels up to 2000 µg/m^3 were reported (Byggforskningsrådet 1976), i.e., well above the threshold of sensory irritation (SCOEL 2008) and the current Swedish OEL of 370 µg/m^3. The general population became more aware of potential health hazards in newly constructed or renovated buildings and major efforts to identify hazardous microbiological, physical and chemical agents in sick buildings were made.

Sweden became a leader in this new field of research. In 1984, the Third International Conference on Indoor Air Quality and Climate was held in Stockholm, sponsored by the

Swedish government. A paper at the conference (from the Swedish National Testing Institute) describes an odor problem that had already received wide attention in Sweden. The paper presents mold in building constructions as a problem that appeared in the 1970s and was increasing. The residents in affected houses suffered from various degrees of medical complaints and were troubled by an offensive odor (Samuelsson 1984). After the conference, a WHO meeting was arranged in Stockholm and the subsequent report introduced odor as the result of moisture and fungal growth (WHO 1986). This WHO report also put more emphasis on odor compared to the preceeding and first report on the sick building syndrome (WHO 1983).

In both WHO reports, the detrimental role of inadequate ventilation was emphasized and even today, ventilation is one of the very first factors to be examined in connection with complaints about IAQ. Shortly thereafter, Swedish authorities, other national stakeholders and experts in the field of indoor environment published a joint report that attempted "to cover all that is known about building related health problems", addressing mold and mold odor in several chapters (Planverket 1987).

The chapter dealing specifically with "chemicals in building materials and in products for operation and maintenance" covered the following typical problems:

- Glued wood products (formaldehyde emissions).
- Self-leveling smoothing compounds containing casein (emission of odorous amines and ammonia resulting from the breakdown of casein due to alkaline moisture in concrete).
- Plastic polymers, in e.g. flooring, wall-paper and paints (various emissions, including phthalates).
- Insulating material, asbestos in ventilation channels and mineral wool fibers (sometimes emitting odorous substances when exposed to moisture).
- Bedrock, ground and certain building materials (radon).
- Enemal paint, paint and glue (emission of various compounds, including glycol ethers and formaldehyde).
- Flooring of wood and linoleum (emission of, e.g., terpenes).
- Cleansing agents (emission of, e.g., solvent naphta).
- Polish on floors (emission of, e.g., glycolethers and acetic acid).

Several additions to this list were published as case reports in 1990 by the Swedish National Testing and Research Institute (Gustafsson 1990), including:

- PVC flooring (emission of 2-ethyl-1-hexanol resulting from the breakdown of phthalates due to alkaline moisture in concrete).
- Plasticizers and flame retardants in board (emission of PCB).
- Wood impregnated with creosote (emission of naphthalene from the exterior panel).
- Smoothing compound containing casein (suggesting that the odor is due to the presence of 2-aminoacetophenone in extremely low concentrations).

These indoor air pollutants are recognized by Swedish authorities, and several of them are mentioned in a recent report from The National Board of Health and Welfare (Socialstyrelsen 2006). The textbook case of odor emitted from self-leveling concrete containing casein is described in an international review on the impact of reaction products in building materials on IAQ (Uhde and Salthammer 2007). Interestingly, this review included another type of reaction product overlooked by Swedish authorities and researchers, i.e., chloroanisoles formed in damp conditions by microbial metabolism of chlorophenols present in wood preservatives. The chlorophenols protect damp wood against structural damage by wood rot fungi but they apparently do not hinder the production of chloroanisoles from microbes present on the surface of treated wood (Lorentzen et al. 2016).

7.6 Chlorophenols as a cause of mold odor and IAQ complaints

Chloroanisoles have extremely low odor thresholds and may be perceived as mold odor. Accordingly, the growing interest in mold in connection with IAQ during the 1970s and 1980s may actually reflect, at least in part, the ubiquitous use of chlorophenol-containing wood preservatives from 1955-1978, a period overlapping the record rates of building from 1961-1975 (Figure 7.1).

Any negative effect on IAQ associated with chlorophenols at the time when the concept of the sick-building syndrome developed appears to have been attributed to other exposures, such as mold and formaldehyde, and the extensive use of chlorophenols (with chlorodioxin contaminants) seems never to have been recognized as a potential health hazard in Sweden.

The situation is very different in Western Germany, where chlorophenols (mainly pentachlorophenol, PCP), were widely used in building constructions and on indoor surfaces and furniture, promoted by a chemical company. This resulted in serious health symptoms in the population, which were referred to as wood preservative syndrome. As stated by the Commission on Human Biomonitoring of the Federal Environmental Agency, PCP came to be regarded as a “major environmental chemical”. As a consequence, monitoring of blood levels of PCP in the general population was incorporated into a program begun in 1987, two years before suspending the usage of PCP, and these levels have declined with time (Heudorf et al. 2000, Schulz et al. 2007).

Chlorophenols were used on indoor surfaces also in Sweden, but their use was promoted in other parts of the building construction as well and, from 1970, impregnation was even recommended as an alternative to traditional moisture barriers. This replacement of moisture barriers by chlorophenols created perfect prerequisites for microbial production and emission of chloroanisoles (Lorentzen et al. 2016), and may explain the observation that mold odor was uncommon in houses built before the 1970s and that structural damages due to rot declined and were replaced by the problem of sick houses (Samuelsson, 1984).

Most products containing chlorophenols had their licenses withdrawn in 1977-1978 (Produktkontrollnämnden 1977) and were replaced by products with other preservatives. Today, indoor concentrations of chlorophenols and chloroanisoles in Swedish buildings are several orders of magnitudes less than those associated with adverse effects in animal toxicity tests. Nonetheless, mold odor in buildings may often be due to chloroanisoles which can be formed in the absence of any apparent mold growth. In light of this, the use of mold odor as an indicator of mold growth is not reliable in Sweden. Furthermore, associations of mold odor with various symptoms, such as unspecific building-related illness and asthma, may not actually be related to mold (Lorentzen et al. 2016).

7.7 Mold and problems with IAQ

Concentrations of mold spores in indoor air are generally lower than outdoors and not elevated in damp buildings. This is because spores are often trapped by the ventilation system and microbial growth in damp Swedish buildings usually occurs in the building construction from which spores, fungal fragments, actinobacteria and bacteria cannot easily reach the indoor air. In light of these considerations, and the fact that allergy to mold is uncommon, it appears unlikely that direct exposure to mold spores and other microbes is of relevance to IAQ other than in rare cases.

In addition to microbial particles, microbial growth can result in emission of MVOCs some of which pass through building materials, including plastic film used as a moisture barrier, and thereby contaminate the indoor air. However, the recorded concentrations of various MVOCs in indoor air are far below toxicological effect levels (Korpi et al. 2009) but certain MVOCs including geosmin and 2-methylisoborneol (Korpi et al. 2009), as well as chloroanisoles (Lorentzen et al. 2016), have extremely low odor thresholds and may thus cause deterioration of IAQ. Accordingly, species of microbes that emit specific odorous chemicals may be of relevance to IAQ, even though there is no evidence that any specific microbe in building materials is of particular concern with respect to asthma or other diseases associated with damp buildings (WHO 2009).

7.8 Research and developmental needs

As reported in this chapter, new types of building materials have in several cases given rise to emissions that have made IAQ unacceptable to numerous residents. Currently, there are ongoing efforts in Europe to regulate and reduce chemical emissions from new building materials (Kemikalieinspektionen 2015, Kephalopoulos et al. 2013). However, these efforts do not address changes in material composition due to, e.g., dampness or ageing. Consequently, it is probably impossible to prevent such problems completely. Nevertheless, joint efforts by stakeholders in the building sector to reduce emissions from new building materials and to control and enhance rates of ventilation, have gradually lowered indoor levels of pollutants. Despite these apparent improvements, the

frequency of self-reported adverse health effects related to the indoor environment seems not to be declining (Socialstyrelsen 2009).

This paradox may be explained by some as yet unidentified indoor air pollutant whose level has not decreased and/or a combination of pollutants. In addition, other factors not considered here, e.g., air humidity and temperature, may be important in this context. Another explanation could be that residents of today make higher demands on IAQ, due to increased awareness of potential health risks and the conception that the risk is substantial.

This public perception of substantial risk is reinforced by information from authorities, e.g., the National Board of Housing, Building and Planning recently stated that 36% of the Swedish building stock is affected by moisture and water damage in a manner that might influence indoor environment and health (Boverket 2010b). This implies that a large portion of the population is at risk and the cost for remediation to avoid this risk has been estimated as more than 100 billion SEK (Boverket 2010b).

However, estimates of the risk to health and fraction of the population at risk are highly uncertain, in part because etiological factors and disease mechanisms have not yet been elucidated. International meta-analyses of associations between health effects and dampness/mold are of questionable relevance to the situation in Sweden since these are based on indicators of dampness in buildings, which means that the extent of mold damage and chemical emissions may vary considerably with time and between countries, as well as with climate and other aspects of geography. Although Scandinavian studies should be more relevant several of these have not corrected for socioeconomic status (Mendell et al. 2011). Moreover, Swedish studies that use mold odor as an indicator of dampness and mold may be unreliable since a considerable portion of Swedish buildings is affected by chloroanisoles, which may be perceived as, but do not necessarily indicate, mold.

To improve rational assessment of exposures via indoor air in Sweden, we identify the following research needs:

- Review of available information and updating the base of knowledge concerning IAQ, taking into account the methodological quality of studies and use of wood preservatives containing chlorophenols.
- Further examination of wood preservatives and chloroanisoles as environmental factors, attempting in particular to distinguish between odor due to mold growth and odor due to chloroanisoles.
- Review and development of novel methods for the identification of odors associated with specific typical building problems (e.g. chloroanisoles and possibly 2-aminoacetophenone).

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8. Skin Exposure to Hazardous Chemicals

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8.1 Introduction

In addition to protecting the body against external physical and chemical threats, the skin fills of a number of other vital functions, such as maintaining water inside the body, controlling body temperature, synthesizing vitamin D, storing energy, absorbing shock, and providing tactile sensation. The skin is our largest organ, accounting for approximately 10% of total body mass.

The chemical substances/mixtures, materials, metals/alloys, cosmetics and other products that are part of our daily lives may all, in various contexts, come in contact with the skin from the day of our birth until we die. Such contact can be intentional (e.g., when using a product), accidental (contamination), or involve processes that are less obvious (e.g., airborne deposition). As a target and/or route of transport for hazardous substances, the skin may be affected, and moreover systemic effects may occur. The most frequently occurring local effects are caused by skin sensitizing and irritant chemicals.

8.1.1 Local effects

The most common adverse effect of exposure of the skin to chemicals is allergic or irritant contact dermatitis, while contact urticaria, photo-contact dermatitis, burns, oil acne and lichenoid reactions may also occur. Moreover, skin cancer can be caused by chemical exposure, for example, to arsenic, creosote or PAH. The present chapter focuses on exposure of the skin to sensitizing substances that give rise to contact allergy, which afflicts many individuals, highly warranting prevention by reduction of exposure.

Contact allergy, a delayed type of hypersensitivity (type IV hypersensitivity), involves two major phases, *sensitization (induction)* and *elicitation*. Allergic contact dermatitis, the clinical disease caused by re-exposure to the sensitizer (elicitation), typically occurs at the site of re-exposure. Metals, in particular nickel, is the most common group of chemicals causing contact allergy, followed by fragrances and preservatives. Other frequent sensitizers are rubber and plastic chemicals, and hair dyes. A single exposure to extremely potent sensitizers, such as epoxy resin, certain preservatives and p-phenylenediamine, may induce contact allergy. Less potent sensitizers, such as nickel and many fragrance substances, frequently cause contact allergy and allergic contact dermatitis because they are difficult to avoid, resulting in repeated exposure. So far, more than 4000 substances have been identified as contact allergens.

Approximately 20% of the adults in the general population develop contact allergy to at least one substance (Thyssen et al. 2007). Moreover, at least 15% of the adolescents in Sweden have been sensitized to one or more contact allergens (Lagrelius et al. 2016). Contact allergy is a lifelong condition, requiring avoidance of further skin exposure to the allergen, to minimize the risk of developing contact dermatitis.

Unlike contact allergens, skin irritants act through chemical reactions directly on the skin, impairing its barrier function. Irritant contact dermatitis may result from acute exposure to highly irritating substances (corrosives such as acids, bases, and oxidizing and reducing agents), repeated exposure to lipid solving chemicals (organic solvents), or chronic cumulative exposure to relatively weak irritants (water, surfactants). Some substances in tar, plants, and medicaments may cause phototoxic skin reactions, when the skin is concomitantly exposed to UV light.

8.1.2 Systemic effects

Many chemicals can, to varying extents, pass through the skin and enter the systemic circulation, where they may give rise to acute and/or chronic systemic adverse effects on health.

All organic solvents can be absorbed through the skin as a consequence of their high solubility and mobility in the lipid phase of the stratum corneum (the outermost layers of the skin). Glycol ethers, amphiphilic organic solvents often used in paints and cleaners, are efficiently absorbed through the skin. They can penetrate the skin by dissolving both in lipids of the stratum corneum and hydrophilic layers of living skin cells in the epidermis. Vaporized organic solvents may also penetrate the skin (Rauma et al. 2013). Long-term exposure to organic solvents affects mainly the central nervous system, and accidental skin exposure may be acutely toxic, even fatal.

Acute and chronic adverse health effects caused by exposure to pesticides are a major public health concern in developing countries. Such exposure through the skin may result in acute, even fatal reactions, primarily in the case of organophosphorous compounds (Ngo et al. 2010). Paraquat, a chlorinated pesticide, is highly toxic and may lead to multiple organ failure following dermal absorption.

Systemic exposure to many other chemical substances as a consequence of dermal absorption has been considered to be relatively minor. However, exposure of the skin to metals, such as cobalt, has been shown to give rise to systemic exposure (Scansetti et al. 1994). Furthermore, nano particles of metal appear to remain in the viable skin, where they release metal ions (Larese Filon et al. 2013).

8.2 Factors affecting skin exposure

The many factors that influence skin exposure and consequent effects can be grouped into the physical and/or chemical properties of the substance, factors related to exposure, and/or the host (Figure 8.1).

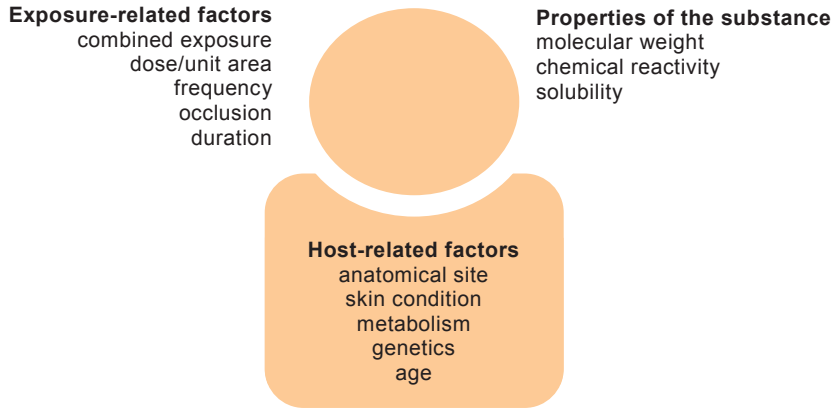

Figure 8.1. Some of the factors that influence skin exposure and consequent effects.

8.2.1 Repeated low-dose exposure

The relative significance of repeated low-dose skin exposure for local and systemic effects is not yet fully understood. Both the frequency and duration of such exposure may, for example, influence the properties of the skin barrier and threshold for elicitation. Repeated low-dose skin exposure is likely to be as important as single high-dose exposure, especially with respect to the development of allergic contact dermatitis (Jensen et al. 2005, Paramasivan et al. 2010). One explanation for this is that with time many sensitizers may accumulate in the skin. Furthermore, it has been proposed that each low-dose exposure stimulates the immune system slightly, eventually leading to an immune response that results in sensitization. Recent investigations performed at IMM provide further evidence of the impact of repeated low-dose exposure to two common allergens, the preservative methylisothiazolinone (MI) and nickel (Erfani et al. 2015, Yazar et al. 2015).

8.2.2 Combined exposures

Skin is exposed simultaneously to different mixtures of chemicals from single or multiple products and/or different sources. The combined exposure may involve sensitizers, irritants and/or substances that facilitate the uptake of, for example, a sensitizing chemical. A number of substances present in many consumer products, such as terpenes and surfactants, efficiently enhance penetration of the skin. Combined exposure to sensitizing and irritant substances may reduce the thresholds for sensitization and

elicitation, as demonstrated in the case of sensitizing fragrance substances and certain vehicles (Bonefeld et al. 2011, Pedersen et al. 2004).

8.2.3 Skin surface physiology and filaggrin mutations

The condition of the skin surface is affected both by external factors (temperature, humidity, chemicals, moisturisers, etc.) and host-related factors (e.g. genetic predisposition, atopy, mutations in filaggrin). The physiological condition of skin influences the uptake of chemicals, as well as consequent adverse outcomes.

Several non-invasive measures of skin physiology have proven to be valuable complement to visual scoring in the assessment of disease severity in patients with active atopic dermatitis (Holm et al. 2006). Such quantitative information is essential to both research and risk assessment. Hydration (electrical impedance/capacitance, transepidermal water loss) is one aspect of the skin barrier that influences the absorption of ionized chemicals (Kalia and Guy 1995, Fluhr et al. 2006). In addition, the topography of the skin (roughness and coefficient of friction) is directly related to its surface area, and thereby of importance for exposure and absorption (Lodén et al. 1992). Wrinkles, ridges, chapped skin, scaling, fissures, and the like increase the surface area. Moreover, hydration and deficiency of filaggrin affect the structure and, consequently, surface area of the skin.

Filaggrin proteins are crucial for the structure of stratum corneum and the skin barrier function. Mutations in filaggrin proteins are a major risk factor for development of atopic dermatitis and extremely dry skin (xerosis) (McAleer and Irvine 2013). It is not yet known, however, whether such mutations are also an independent risk factor for irritant contact dermatitis, contact allergy or allergic contact dermatitis.

8.3 Assessment of skin exposure to hazardous chemicals

Skin exposure can be assessed in the outer environment or at the source, at the surface or in the layers of the skin, and/or systemically employing various methods and models (Table 8.1).

Table 8.1. Some of the methods available for assessment of skin exposure.

Point of assessment	Measurement	Experimental procedure
Source	Screening, market surveys, labels, questionnaires	Release/migration tests
Surface of the skin	Removal techniques, visualization	Surrogate skin techniques
Skin accumulation/penetration	Tape stripping, skin punch biopsy, biomonitoring	Diffusion cells, mathematical modelling
Local effects	Repeated open application test, serial dilution patch test	Animal models

8.3.1 Assessment of sources of exposure

The information in the scientific literature concerning exposure of the skin to irritants, sensitizers or other hazardous chemicals is often based on self-reports by interviews or questionnaire, or visual observation. Investigation of the immediate environment at the workplace, and in the home or other place of leisure is also important, as are market surveys of chemical products, cosmetics, clothing, protective equipment and other items that come into contact with the skin. Ingredient labels, safety data sheets, and other product information, as well as simple screening tests and chemical analyses are frequently used in the clinic, in surveillance programmes at work places, and in research, to assess sources of hazardous exposure. Characterization of the release from materials, typically metals and alloys, in artificial sweat solutions, can also be of value in this connection.

8.3.2 The surface of the skin

Recently, interest in assessing what actually is deposited onto the skin has been increasing. Except in the case of pesticides, such methods are not yet commonly used, particularly not with respect to skin sensitizers.

Efficient sampling without causing harm is required for taking samples from the surface of the skin. The three major approaches available at present involve: removal, surrogate skin or visualization.

One of the most common removal techniques is wipe sampling, which can be performed for several different substances, depending on the solvent utilized. For inorganic substances, such as metals, acid (acid wipe sampling) or water (finger immersion sampling) may be appropriate. Acid wipe sampling has been used to measure the amount of metal on the skin of individuals in different occupations (Lidén et al. 2008, Julander et al. 2010), as well as in connection with experimental handling of tools (Julander et al. 2011) and coins (Lidén et al. 2008, Julander et al. 2013).

Hand washing or hand rinsing are usually preferred for sampling larger areas of skin for, e.g., organic substances such as pesticides or hair dyes. An organic solvent such as isopropanol or a buffer solution may be employed (Fenske 2000, Lind et al. 2004).

All such methods demand proper subsequent extraction of the wipe or wash sample, followed by chemical analysis, e.g. by inductively coupled plasma mass spectrometry (ICP-MS), high-performance liquid chromatography (HP-LC) (sometimes in combination with MS), or gas chromatography-mass spectrometry (GC-MS). The result is often expressed as amount per unit area ($\mu g/cm^2$), an important metric for evaluating thresholds for irritation, sensitization and elicitation concerning contact dermatitis.

If the skin dose itself is not the main parameter of interest, a fluorescent tracer (e.g., laundry whitener) that does not harm the skin can be added to the solution/chemical mixture under evaluation. Then, after the worker performs his/her tasks, exposed areas

can be visualised from the fluorescence produced by illumination with UV light in a semi-dark room, as has been done for pesticides exposure (Aragón et al. 2006).

When sampling from the surface of the skin itself is not possible, surrogates such as patches, whole-body coveralls or textile gloves can be employed (Hines et al. 2011). Patches placed directly on the skin, on clothes or under protective clothes allow scanning of large areas for contamination, but provides only an estimate of the actual skin dose.

8.3.3 Accumulation on and penetration through the skin

Determining the amount of a substance present in the skin generally requires greater effort. The two standard dermatological procedures presently available, tape stripping and skin punch biopsies, are used by clinicians to examine patients for other purposes. Monitoring of biomarkers of exposure is another approach to assessing penetration of the skin by a chemical, as are laboratory experiments and mathematical models.

Tape stripping involves repeated applications of tape to the same area of skin. If uptake of a substance in skin is to be evaluated, at least 10 tapes are needed from the same area. The method has been utilised to evaluate the penetration of nickel salts, copper, and certain organic substances into the skin (Hostynek et al. 2001, Hostynek et al. 2006). To follow the substance in deeper layers, stripping must be continued until the glistening living epidermal cell layers is reached. This approach is reliable, but invasive, giving rise to a superficial wound. Therefore, it should be used with caution and not normally as a tool for assessing exposure in the workplace. With workplace assessment, tape should be applied no more than three times; this does not harm the skin and allows work to continue, but provides no information about dermal uptake.

Skin punch biopsies, another invasive technique that requires local anaesthesia, allow different layers of the skin to be evaluated for chemical penetration and transport. The biopsy can be evaluated using various microscopic techniques, either as a whole piece or after slicing into thin sections. This approach has been utilized to trace nano-particles following application of sun screens (Monteiro-Riviere and Riviere 2009).

The *diffusion cell* is a standardized *in vitro* technique for characterizing dermal penetration of chemicals (OECD 2004). A piece of skin (usually from a human being or pig) is clamped in the cell. The outer side of the skin is exposed to the chemical or solution of interest in the donor compartment. The concentration of the substance in the receptor compartment (usually filled with a saline solution mimicking blood) is measured. The absorption kinetics can be followed by taking samples from the donor and receptor medium at different time intervals. The amount of the chemical that is retained in the skin can also be measured.

Systemic exposure via the skin can be detected by monitoring the levels of chemical substances in blood or urine (or sometimes hair and nail clippings) or following other biomarkers of exposure (see chapter 4: Early life exposure assessment).

Other procedures for measuring the skin accumulation/penetration of chemicals include Fourier transform - infrared photoacoustic spectroscopy (FTIR-PAS), photothermal deflection spectroscopy (PDS), confocal Raman microscopy (CRM), fluorescence techniques (FLIM, TPM) and ^{14}C labelling.

8.3.4 Assessment of local effects of skin exposure

Elicitation studies employing, e.g., the repeated open application test (ROAT) or serial dilution patch test, can reveal dose-response relationships and identify elicitation thresholds in contact allergy (Fischer et al. 2009). These approaches focus on the ability of allergens to actually cause the clinical disease, allergic contact dermatitis. The study participants are typically patients who formerly suffered from dermatitis and are already sensitized to the substance of interest, and are therefore not placed at risk.

The *ROAT,* designed to mimic realistic exposure, involves humans and may also involve realistic product compositions (vehicles) and patterns of exposure (frequency and duration of application, etc.) Typically, a square with 5-cm sides is drawn on each forearm, and the vehicle containing the allergen is then applied to one arm and the vehicle alone to the other (Hannuksela and Salo 1986). Preferably, this test should be continued up to 3 weeks to ensure detection of all positive reactions. Finally, reactions are scored according to a scale using cut-off criteria for positive reactions (Johansen et al. 1998). The ROAT has been modified and adapted to different types of exposure and research questions (Zachariae et al. 2006, Yazar et al. 2015).

The elicitation phase can also be examined with the *serial dilution patch test.* This is the method utilized in the clinic for diagnosis of contact allergy, the only difference being that the allergen of interest is tested at different doses, typically ranging over several orders of magnitude (Johansen et al. 2015).

For several decades now, animal testing has been an important tool for assessing the ability of chemical substances to cause skin sensitization (induction). The three generally accepted test methods are the *guinea pig maximisation test* (GPMT), the *Buehler test* in guinea pigs, and the *local lymph node assay* (LLNA) in mice (OECD 1992, OECD 2010). For registration of chemicals in REACH, LLNA is the method of first choice. In addition to identifying a sensitizer/non-sensitizer, the LLNA (which has an inherent dose-response design) and the GPMT (with a modified protocol) can be used for assessment of the dose-response relationship.

With these models, the sensitizing potency, i.e., the strength of a sensitizer, can be categorized as extreme, strong or moderate (Basketter et al. 2005). In the case of LLNA, potency categorization is based on the EC3 value, i.e., the concentration of a substance required to elicit a three-fold increase in lymph node cell proliferative activity. Such animal data is also used for sub-categorization (1A and 1B) of classified skin sensitizers in accordance with the EC regulation on classification, labelling and packaging of substances and mixtures (CLP Regulation).

Exposure in these tests of induction is much different from the real-life situation, which has led to the introduction of compensatory assessment (uncertainty) factors. However, this approach to quantitative risk assessment has not yet been proven to be reliable.

8.4 Research and developmental needs

In everyday life, we are exposed repeatedly to combinations of hazardous chemicals, often at relatively low doses, many times per day, and usually throughout our entire lives. A major challenge for the future will be to accurately assess the effects of this type of exposure, which differs substantially from experimental approaches and models of today. Meeting the challenges involved requires the development of novel approaches to the assessment of skin exposure to hazardous chemicals. In this context the properties of the substance/mixture/material/product, skin physiology, and accumulation on and penetration of the skin, must be linked to mechanisms and effects. To achieve this, improved procedures for the detection and quantification of various substances on and in the skin are needed. Recent improvements allowing non-invasive measurements *in vivo* hold great promise.

- Skin exposure must be quantified to better understand the associated risks.
- Endogenous factors that influence susceptibility to sensitization need to be identified.
- Development and application of new and better methods for measurement of skin exposure are crucial. Techniques for measurements in skin, to understand uptake and accumulation of contact allergens, should preferably be *in vivo* and non-invasive.
- The impact of skin exposure to “cocktails” of allergens and other substances originating from different sources must be elucidated.
- The relative importance of the duration, frequency and level of exposure to skin sensitizers and irritants, largely unknown at present, must be determined to support health risk assessment and prevention.

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9. Exposure Extrapolation between Cells, Animals and Humans: Focus on Nanoparticles

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9.1 Extrapolation between humans, animals and cell cultures

There is growing recognition that more efficient methods and strategies to assess the exposure and risks associated with the wide array of chemicals to which humans are exposed are needed. A recent addition to this array of "chemicals" are manufactured nanoparticles (<100 nm), with various chemical compositions, sizes, and toxicological profiles (Karlsson et al. 2014) At present, toxicological hazard and risk assessment relies largely on animal experimentation. Such animal tests are designed to identify the toxic effects that a chemical can cause (hazard identification) as well as to determine the toxic potency, i.e. the quantitative relationship between the level of external exposure (administered dose, environmental concentration) and the toxic effects (dose-response assessment). Traditionally, *in vitro* toxicology has been used primarily to explore mechanisms. More recently, however, novel *in vitro* testing strategies designed to reveal early toxic event that occur at realistic doses are being developed.

Ideally, *in vitro* assays should not only classify compounds according to their mode of action, but also provide data for more *quantitative* applications such as hazard ranking and risk assessment (US EPA 2015). Furthermore, the use of animals for testing cosmetics is no longer allowed. If *in vitro* methods are to be used for toxicological hazard and risk assessment as well as for safety evaluation, it must be possible to extrapolate the information they provide on both the toxic effects and potencies to real-life scenarios. *In vitro* systems consisting of single types of cells, co-cultures of cells, or organ-on-a-chip models, do obviously not reflect the complexity of the human body where many organs and tissues are interlinked via the circulatory system. Moreover, each individual tissue and organ is composed of various types of cells arranged in a complex and specific architecture. Physiologically based pharmacokinetic (PBPK) models, mathematical descriptions of the kinetics of absorption, distribution, metabolism/degradation and excretion (ADME) of substances, are useful for translating the results obtained with *in vitro* models into predictions about toxic effects in humans. One advantage here is that these predictions do not require additional studies on animals or human. PBPK models for chemicals and pharmaceuticals have been successfully applied to correlate exposure with target dose, extrapolate from low to high dose, extrapolate results on animals to humans and explore variability in populations.

In the case of nanoparticles, on the other hand, only a few PBPK models have been described to date (e.g. Pery et al. 2009, Sweeney et al. 2015, Bachler et al. 2015) and these cannot extrapolate findings on one type of nanoparticle to another type. An additional limitation is that most of these models do not take humans into consideration. To improve nano PBPK models rendering them more general, appropriate *in vitro* and *in vivo* procedures for establishing key parameters and rate limiting processes are required. At the same time, for (nano)particles the lung is the main organ of interest, and focus on lung allows the use of several models for prediction of deposition and clearance of inhaled particles in humans as well as dose comparisons between the *in vitro* and *in vivo* situation.

In the present chapter we discuss extrapolations and comparisons between *in vivo* and *in vitro* assays with a focus on lung exposure and the effects of nanoparticles, providing a number of examples. A central question concerns how real life human exposures can be related to the doses employed in *in vitro* systems (Figure 9.1). Furthermore, we describe and discuss advanced *in vitro* exposure systems for nanoparticles and the research needed for the application of data from *in vitro* assays in risk assessment.

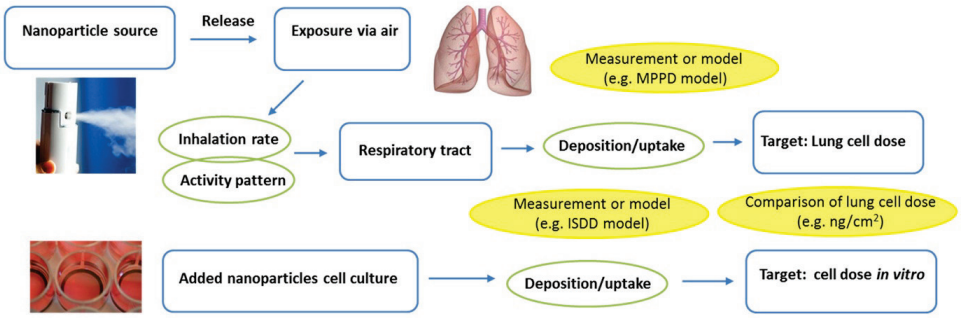

Figure 9.1. Schematic illustration of human and *in vitro* exposures to nanoparticles. One of our aim is to improve extrapolation of exposures *in vitro* to e.g. an occupational environment.

9.1.1 In vitro-in vivo correlations for assessing toxicity of nanoparticles: importance of the dose metric

With greater and greater usage of *in vitro* studies as a basis for hazard ranking and risk assessment of nanoparticles, it is vital to determine whether the results of such *in vitro* assays can predict *in vivo* outcomes. One of the first attempts to explore such correlations was performed by Sayes and co-workers (2007), who studied the inflammatory responses of rats exposed to crystalline and amorphous silica as well as to zinc oxide nanoparticles. The inflammatory effects of the same substances on rat macrophages and epithelial cells exposed *in vitro* were also studied and it was concluded that no clear correlation between

the *in vivo-in vitro* exposures could be observed. Several critical factors can contribute to such discrepancies.

The *dose* is a key factor in comparisons between *in vitro* and *in vivo* assays. Exposure via inhalation is typically characterized and reported in terms of concentration of particles in the air (mg/m^3), or total dose exposure per animal weight (mg/kg) for intratracheal or pharyngeal aspiration studies. In the case of *in vitro* studies, mass of nanoparticles per unit volume (mass/mL) is often used. Rather than expressing the *in vitro* dose in units of mass, Donaldson and co-workers investigated *in vivo-in vitro* correlations by expressing the dose as the surface area of the particles (BET area) per volume of liquid (cm^2/mL). Nine different nanoparticles (CeO_2, TiO_2, carbon black, SiO_2, NiO, Co_3O_4, Cr_2O_3, CuO, and ZnO) were examined and acute pulmonary inflammation in rats exposed via instillation was compared to cytotoxicity, expression of pro-inflammatory cytokine, and haemolytic potential *in vitro*. Agreement between the assays was found to depend on the mechanism of toxicity. The *in vitro* assays predicted the *in vivo* toxicity of the ZnO and CuO nanoparticles, which dissolve and release ions, whereas the inflammatory response caused by other mechanisms (with CeO_2, NiO, Co_3O_4) was more difficult to predict *in vitro* (Cho et al. 2011).

The need to consider the *cellular target dose* when comparing the toxicity of different nanoparticles in different systems has been highlighted in recent years (Teeguarden et al. 2007). This dose is seldom considered, which is likely to contribute significantly to the poor correspondence between *in vitro* and *in vivo* responses. Teeguarden and co-workers tested the hypothesis that this correspondence could be improved in the case of nanomaterials by expressing the *in vitro* and *in vivo* dose in the same manner, i.e. as the amount of material associated with cells (target cell dose). Mice were exposed through the nose-only to an aerosol (19.9 mg/m^3) of iron oxide nanoparticles for four hours, target cell doses were calculated and markers for inflammation analyzed. The dose was normalized by dividing the deposited mass in a given region by the corresponding surface area. The dose was also calculated by dividing the total dose deposited in a given region by the number of macrophages present. In parallel, epithelial cells and macrophages were exposed *in vitro* to the same material in liquid suspension for four hours, and the levels of inflammatory markers, as well as the cellular dose determined. These investigators found that more nanoparticles per cell were required to induce inflammation in the alveolar epithelial cells *in vitro* than *in vivo*. However, in the case of macrophages the correspondence between target cell dose that triggered inflammatory processes *in vitro* (8-35 pg/cell) and *in vivo* (1-100 pg/cell) was good.

Indeed Donalson and colleagues (2008) have reported similar findings for so-called low-solubility, low-toxicity particles (in this case TiO_2 and $BaSO_4$) of both nano- and larger sizes. Their hypothesis was that the cellular dose in the proximal alveolar region (PAR) of the lung that initiates inflammation can be predicted from *in vitro* studies. The PAR region includes the terminal bronchioli as well as 400-600 µm section immediately beyond the junction between the bronchiole and alveolous. Deposition of particles within this region is likely to be high due to the transition from a relatively narrow airway with

airflow to an exponential increase in volume and surface area with no airflow. In that study, the surface area of the PAR region in rats, as well as the particle dose in that region leading to inflammation in the rats, were calculated. This revealed a value of approximately 1 cm^2 (particle area)/cm^2 (lung PAR area) as the critical threshold for the onset of inflammation. Interestingly, a similar threshold of 1 cm^2/cm^2 for the onset of inflammation *in vitro* (IL-8 release following exposure of A549) was clear. These investigators concluded that in the case of low-solubility, low-toxicity particles, the threshold dose for stimulation of IL-8 gene expression *in vitro* predicts the threshold dose for stimulation of neutrophil influx into the lungs *in vivo* (Donaldson et al. 2008).

9.2 How can the cellular dose be assessed?

As discussed in the previous section it is important to quantify the cellular target dose for comparison of *in vitro* and *in vivo* assays. One obvious reason is that the nominal concentration in the medium can differ substantially from this dose. In contrast to soluble chemicals, nanoparticles can settle, diffuse and aggregate in various ways depending on their size, density and surface chemistry, as well as the properties of the culture medium and this can affect the particle dose reaching the cells at the bottom of the culture dish.

The cellular dose can be measured quantitatively using e.g. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) or Atomic absorption spectroscopy (AAS). ICP-MS, an analytical technique used to quantify elements, combines a high-temperature ICP (Inductively Coupled Plasma) source with a mass spectrometer. The ICP source first converts the elements in the sample to ions and these ions are then separated and detected by the mass spectrometer. AAS is based on the absorption of light by free atoms in the gaseous state for quantitative determination of elements. Although we and others have employed such approaches to assess the cellular dose of nanoparticles (e.g. Gliga et al. 2014), this can be quite time-consuming and, moreover, it is difficult to distinguish between nanoparticles taken up by the cells and those simply attached to the cell membrane. One the other hand, even in the absence of uptake, nanoparticles in contact with the cell membrane can exert effects, by interfering e.g. with receptors and ion channels. Another limitation of these analytical procedures is their inability in general to distinguish between the nanoparticle itself and ions released from the nanoparticles.

An alternative approach is to estimate the *delivered dose* by modelling. Hinderliter and co-workers (2010) introduced the "In vitro Sedimentation, Diffusion and Dosimetry (ISDD) model" that incorporates both Stokes Law (sedimentation) and the Stokes–Einstein equation (diffusion) to estimate the movement of particles to the cells. One critical aspect here is the effective density and diameter of particle agglomerates in suspension (Hinderliter et al. 2010). The nominal concentration in the medium expressed on the basis of mass (µg/mL) and target cell doses expressed as the mass, particle surface area or particle number basis can differ by several orders of magnitude. As a consequence, *in vitro* hazard assessments that utilize only the nominal mass as an exposure metric can result in extensive error in cases where the number or surface area of

the particles in the cells, i.e., target cell dose, determine the response. At the same time, although the ISDD model estimates the dose delivered (per unit time), once in contact with cells the cellular uptake is often an active process that can be influenced by the type of cells as well as e.g. cell density. Obviously, lower toxicity may be observed at higher cell density, since the dose per cell will be lower.

9.3 How can the internal lung exposure in vivo be estimated?

To determine the dose deposited *in vivo*, analytical approaches such as magnetic particle detection (MPD) and ICP-MS of the whole lung, can be utilized. However, assessing the regional distribution of particle deposition is much more challenging. For this purpose electron or optical microscopy can be performed on tissue sections, but this provides only semi-quantitative data and requires considerable resources (even for assessing small regions of the lung).

The difficulty of evaluating regional, tissue and cellular levels of particles experimentally has led to the development of computational models designed to accomplish this for rats and humans (ICRP 1994, Asgharian et al. 2001) and, more recently, also for mice (Asgharian et al. 2014). The *multipath particle deposition model (MPPD)*, now widely used in the research and regulatory science communities, calculates the deposition and clearance of monodisperse and polydisperse aerosols containing particles ranging in size from ultrafine/nano-sized (<100 nm) to coarse particles (20 µm) in the respiratory tracts of rats and human adults and children (deposition only). Such models provide an inexpensive and rapid estimate of the internal tissue dose, which often is the missing link between external measures of exposure and response.

It should be noted, however, that current lung dosimetry models assume uniform deposition on all bronchial airway surfaces, i.e. that all epithelial cells receive the same average dose, an assumption that can be questioned. Indeed, Balashazy and co-workers (2003) computed the patterns of particle deposition in airway bifurcations and found that for micron-sized particles the local deposition in the tracheobronchial region can increase doses several hundredfold; while in the case of nanoparticles the deposition at these bifurcational 'hot spots' was enhanced from about 5- to 60-fold (Balashazy et al. 2003, Oberdörster et al. 2009). Therefore, when estimating cellular doses, a factor of ×10 is sometimes applied to account for the uneven distribution of nanoparticles (Paur et al. 2011).

9.4 Comparison of doses in vitro and human exposure via inhalation

In connection with comparison of *in vitro* exposure to *in vivo* exposure of animals or humans, it is clearly important to be able to compare the doses. Here, we describe some estimates of human lung exposure/dose (mass or particles per lung surface area) that can be compared to *in vitro* exposures. More detailed calculations are presented in the text boxes.

Example 1: A worker exposed to silver nanoparticles

What is the monthly (4-week) deposition in the lung of a worker exposed to silver (nano)particles at a level of 289 µg/m^3? These calculations are based on same assumptions made by Wang et al. *i.e.* that the ventilation rate of a healthy human adult in a working situation is 20 L/min (=9.6 m^3/8-h-day), the deposition fraction is 30% and short-term clearance is neglible.

Calculations:

Monthly deposition: 0.289 (mg/m^3) × 0.3 (deposition fraction) × 9.6 (m^3/day)×5 (days per week) × 4 (weeks)= 16.64 mg/person

Deposition per lung surface area assuming a total surface of 100 m^2: 16.62/100= 0.166 mg/m^2 surface, i.e. approx. 0.017 µg/cm^2. To account for uneven deposition: ×10 = **0.17 µg/cm^2**

Assuming a 70% long-term clearance, 10 years of exposure could lead to: 0.166×11 (months/year)×10 (years)×0.3 (remaining after clearance)= 5.48 µg/cm^2

The first example involves a worker exposed to silver nanoparticles at a concentration of 289 µg/m^3. Employing the same assumptions and calculations as Wang et al (2011, see text box), the deposition after 4 weeks can be estimated as 0.017 µg/cm^2, which, with a factor of 10 to account for uneven deposition results in 0.17 µg/cm^2. This value is in the same range as the daily deposition in a more extreme situation where a worker is exposed to 5 mg particles/m^3 (the Swedish limit for respirable dust in the work environment). With the same assumptions as in Paur et al (2011, see the text box) this daily deposition can be calculated to be approximately 0.38 µg/cm^2.

If instead considering nanoparticles in an urban environment, for example at street level on a busy street in Stockholm, the number of particles (dominated by nanoparticles) can be around 25.000 per cm^3. Calculating similar as Geiser and Kreyling (2010), the daily cellular exposure at the alveolar surface can be around 20 nanoparticles/cell. Assuming a long-term clearance of 70% (Paur et al. 2011), this would lead to 21,900

nanoparticles/cell after 10 years. If instead the mass deposition is considered, a concentration of 25 µg/m^3 (nano)particles result in estimated daily and 10-year depositions to be 1.13 ng/cm^2 and 1.24 µg/cm^2, respectively (see text box with example 3).

9.5 Can in vitro-in vivo correlations be improved by using advanced systems for in vitro exposure to nanoparticles?

As discussed above, in most *in vitro* studies cells are exposed to particles suspended in a liquid (i.e. mixed into the cell medium). This will lead to interactions between the cell culture medium and the nanoparticles and to agglomeration of the nanoparticles, which could affect the biological response. Another disadvantage of submerged cell exposure to nanoparticles is that the motion of nanoparticles in liquids is primarily driven by random diffusion. Consequently, under submerged conditions a substantial fraction of the nanoparticles will either remain in the medium or be lost to the lateral walls of the cell culture vessel, thereby altering the cellular dose. As already emphasized, the behavior of nanoparticles in cell culture media could be a major explanation for reported differences in nanoparticle toxicity (Teeguarden et al. 2007).

Example 2: Daily deposition in a worst case occupational scenario

What is the daily deposition for a worker exposed to 5 mg/m^3 dust? These calculations are based on the same assumptions as in Paur et al (2011) *i.e.* an inhaled air volume of 25 m^3, alveolar lung surface of 100 m^2 and a deposition efficiency of 30%.

Calculations:

Daily deposition: 5 (mg/m^3) × 0.3 (deposition fraction) × 25 (m^3/day)= 37.5 mg/person

Deposition per unit of lung surface (assuming a total area of 100 m^2): 0.375 mg/m^2 surface, i.e. 0.0375 µg/cm^2. To account for un-even deposition: ×10 = **0.37 µg/cm^2**

If assuming 70% long term clearance, 10 year of exposure could lead to: 0.37×225 (workingdays/year) × 10 (years) × 0.3= 250 µg/cm^2

An alternative approach is to use direct exposure of the cells at the air-liquid interface (ALI). In this case, the cells are cultured on transwell membranes with no cell medium covering the cells, thus enabling cell exposure to an aerosol of particles. Morover, such exposure is more comparable to inhalation of nanoparticles. The cellular dose can be

analyzed on-line using a Quartz-Crystal Microbalance (QCM); estimated by e.g. measuring input and output concentrations; or quantified by chemical analysis (e.g. ICP-MS or AAS).

A variety of ALI cell exposure systems have been described in the literature (e.g. Aufderheide and Mohr 1999; Lenz et al. 2009; Elihn et al. 2013). Most of them rely on diffusion and/or gravitational settling for deposition and since nanoparticles tend to follow the air stream, obtaining appropriate deposition onto the cells can be challenging (Elihn et al. 2013). One option to increase the deposition onto cells is to use electrostatic deposition, where the particles are first charged and deposition enhanced by an electrostatic force generated beneath the exposure chamber. Most of the procedures described in the literature have been developed in-house, but systems such as the CULTEXs exposure technology (http://www.cultex-laboratories.com) and the VitroCell system (http://www.vitrocell.com) are also commercially available.

We and others have generated an aerosol of (nano)particles with e.g. a high temperature-oven (for metal nanoparticles), a rotation brush generator (for aerosolizing a powder) as well as by using the PreciseInhale system (http://www.inhalation.se). This latter platform aerosolizes milligram-amounts of powders, portion by portion, into small volumes of concentrated aerosol. The generated aerosols are ready for immediate exposure of cell cultures, animals and eventually also humans. The key features of this system include a high energy aerosol generator that de-agglomerates powder materials to concentrated aerosols, combined with a computer-controlled dosing system that administers the substance based on aerosol concentration and, for animal studies, measured ventilation pattern of the study subject (Selg et al. 2013). One advantage here is the low amounts of substance consumption, which allows e.g. investigation of particles collected on filters. The ability to use the same aerosols for exposure of both cell cultures and animals is of particular value when evaluating *in vitro-in vivo* correlations. Such correlations can involve both pharmaco/toxicokinetic- and pharmaco/toxicodynamic endpoints and constitute part of a more comprehensive 3R strategy.

9.6 Advanced in vitro cell models

The *in vitro* lung cell models exposed via ALI can be more or less advanced. The construction of 3D models and co-culture systems enable cell-matrix and cell-cell interactions, and are thereby more similar to the physiological situation. Since lung tissue contains more than 40 different types of cells, mimicking the microenvironment *in vivo* requires *in vitro* systems involving specialized cells arranged in a realistic architecture (Rothen-Rutishauser et al. 2008). Pulmonary fibroblasts maintain structural integrity and tissue homeostasis by producing the extracellular matrix (ECM) and growth factors. Moreover, these cells are active participants in inflammatory responses via the local release of cytokines (Marshall et al. 2015). Other cells of interest that can be incorporated include macrophages and neutrophils, which are recruited to the lung in response to particle exposure (Rothen-Rutishauser et al. 2005). We and others (e.g. Choe

et al. 2006) have during recent years developed 3D models with both human primary cells and cell lines originating from the airway wall. One such example is a 3D-model composed of human fibroblasts together with human primary bronchial epithelial cells obtained from either healthy subjects or individuals with respiratory diseases such as chronic bronchitis. After establishing the model, culturing at the air-liquid interface allows the epithelial cells to differentiate into ciliated cells, mucus producing cells and

Example 3: Daily and long-term deposition of (nano)particles from city air

What is the daily and long-term (10year) deposition of (nano)particles from a typical city air environment assuming concentrations of 25.000 nanoparticles/cm^3 or 25 µg/m^3 PM2.5? These calculations are based on the assumption that a healthy, adult, moderately active individual inhales 15 m^3 and particle deposition and lung surface is the same as in previous examples. Furthermore, it was assumed that the alveolar surface contain 2×10^{10} epithelial type I cells, 3×10^{10} epithelial type II cells, and 6×10^9 alveolar macrophages, *i.e.* a total of 5.6×10^{10} epithelial surface cells (Geiser and Kreyling 2010)

Calculations:

Daily deposition (number of particles per lung cell): 25×10^9 (nanoparticles per m^3) × 15 (m^3) × 0.3= 112.5×10^9 nanoparticles.

Deposition per lung surface cell: $112.5 \times 10^9 / 5.6 \times 10^{10}$ = 2 nanoparticles/cell, to account for a possible un-even distribution in the lung (due to "hot-spots"), the dose locally can be ten times higher = 20 nanoparticles per cell.

Assuming an approximately 70% long-term clearance, 10 years of exposure could lead to: 20×365×10×0.3= 21.900 nanoparticles/cell.

Daily deposition (mass of PM2.5 per cm^2 lung surface): 25 (µg/m^3) × 0.3 (deposition fraction) × 15 (m^3/day)= 112.5 µg/person. Deposition per lung surface assuming a total of 100 m^2: 1.125 µg/m^2 lung surface, i.e. 0.1125 ng/cm^2. To account for un-even deposition: ×10 = **1.13 ng/cm^2**

Assuming an approximately 70% long-term clearance, 10 years of exposure could lead to: 1.13×365×10×0.3= **1.24 µg/cm^2**

basal cells. The normal bronchial epithelium consists of 50-70% ciliated cells, up to 30% basal cells, up to 25% goblet cells, and 11% Clara cells. Confocal-, scanning- and transmission electron microscopy can be used to document the appearance of 3D models grown under air-liquid interface culture condition and confirm the presence of all above mentioned cell types. Upon treating these models with IL-13, the number of mucus-producing cells increases, mimicking chronic bronchitis (Atherton et al. 2003). Such

models incorporating multiple cell types allow cell- to cell interactions and cross-talk to be examined. In addition, models of normal mucosa and chronic bronchitis-like mucosa as well as models with cells from different patients enable comparison of responses.

9.7 Research and developmental needs

Clearly, dosimetry needs to be given more consideration in the future. More specifically, research in the following areas is highly warranted:

- Investigation on how *in vitro* assays can be used for better prediction of *in vivo* outcomes, including *e.g.* improved dosimetry for nanoparticles.
- Development of *in vitro* assays for improved predictions of *in vivo* outcome following nanoparticles. Exposure at the air-liquid interface and more complex cell models represents steps forward but comparison to and validation in relation to traditional exposures *in vitro* and *in vivo* are required.
- Determination of which *in vivo* assays can be replaced by *in vitro* assays for purposes of risk assessment.

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10. Exposure Assessment in Regulatory Risk Assessment and Training

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10.1 Different regulatory frameworks

Regulations concerning chemical safety are formulated for specific chemicals. Human exposure in connection with various uses of a chemical may differ considerably, e.g., intentional administration of drugs and unintentional exposure via contaminated food. Accordingly, legislation in the area chemical safety, including the requirements for data concerning both hazards and exposure varies considerably. The primary areas for regulation of chemical safety are occupational exposure, biocides, plant protection products, food additives and contaminants, food-contact materials, pharmaceuticals, medical devices, cosmetics, ambient air and noise. During the last decade more and more focus has been directed at exposure assessment and some examples are described briefly below.

10.1.1 Chemicals/REACH and CLP

When REACH, the European legislation on chemicals, came into force in 2007, the responsibility for exposure assessment was transferred from governmental authorities to the company(ies) producing the chemical or importing it for sale on the European market. The same company is also responsible for exposure and safety assessment in connection with all down-stream uses of their chemical, which requires cooperation and exchange of information with all down-stream users. Scenarios for all types of exposures (occupational, in consumer products, environmental) must be developed if 10 tonnes or more of the chemical are produced annually. The European Chemicals Agency (ECHA) provides guidance on how to develop such scenarios and assess exposure.

Restrictions in REACH designed to protect human health from unacceptable risks posed by exposure to chemicals, may limit or ban the usage of substances, mixtures or articles. Upon request from ECHA experts provide scientific opinions on proposed restrictions.

According to the *regulation on classification, labelling and packaging (CLP)* and REACH, industry bears the responsibility for hazard classification and hazard communication. Classification into categories and subcategories is based on intrinsic hazards (potency) of chemicals and classification of certain hazardous chemicals must be harmonised (legally binding). The extent and frequency of human exposure is taken into account when assigning skin and respiratory sensitisers to subcategories. ECHA provides guidelines on how to apply the CLP criteria.

10.1.2 Occupational exposure limit values

Occupational exposure limit values, which are set by the Swedish Work Environment Authority (Arbetsmiljöverket) and legally binding in Sweden, aim to protect workers from negative health effects throughout their entire working life. These limits are based on scientific evidence from epidemiological, toxicological, experimental and industrial hygiene studies, in combination with economic and technical considerations. Scientific criteria documents have long been produced by the Swedish criteria group for occupational exposure limits. The Work Environment Authority also relies on assessments made by the Nordic Expert Group, a Nordic collaboration, as well as by the European Union's Scientific Committee on Occupational Exposure Limit Values (SCOEL). The Work Environment Authority is currently considering discontinuation of the Swedish criteria group.

The exposure limits (Hygieniska gränsvärden), published in the "Work Environment Authority's series of statutes, defines levels that should not be exceeded, expressed either in terms of an 8-hour time-weighted average ("nivågränsvärde"), or a 15-minute average ("takgränsvärde").

10.1.3 Additives and contaminants in food

The European Food Safety Authority (EFSA) is responsible for evaluating the risks of chemical compounds in food, including those added intentionally (food additives, flavourings, food-contact materials, pesticides, biocides and residues of veterinary drugs) and environmental contaminants, to health. For compounds added intentionally added, the producer is required to provide data that are usually relatively extensive on both hazard and exposure. In addition, member states often monitor the levels of prioritized contaminants in food, as well as in humans (biomonitoring). Together with data on food consumption by different populations, this allows probabilistic analysis that characterizes variations in exposure. Food safety is also an area where methodology for exposure assessment is developing rapidly, one example being the new Horizon 2020 project EuroMix that aims to achieve experimental verification of a tiered strategy for risk assessment of mixtures of multiple chemicals in this area.

10.1.4 Medicinal products

Safety assessment of medicines has a long tradition of rigorous toxicity and clinical testing. Exposure assessment in this area is relatively simple compared to other classes of chemicals since individuals/patients usually take their medicines in the dose prescribed by a doctor or indicated on the product, making calculation of individual dose/exposure straight-forward. Moreover, also in contrast to other areas, internal dose levels are measured in connection with drug development and sometimes also in patients as well.

10.1.5 Cosmetics

Cosmetic products are intended for application on the skin for cleaning, perfuming, altering appearance, and other purposes. They are regulated by the Cosmetics Regulation, and should be safe. Substances may be prohibited, restricted, or allowed in cosmetic

products. In this context, the European Commission Scientific Committee on Consumer Safety (SCCS) provides risk assessments based on toxicological evaluations and routes of exposure (e.g., dermal, by inhalation and oral). Groups at particular risk include hairdressers exposed heavily to skin sensitizers and irritants; individuals allergic to skin sensitizers (who can react to very low levels present in cosmetics); and those with atopic dermatitis, who are more sensitive to irritants and, possibly, to skin sensitizers.

10.1.6 Air pollution

The legally binding limits ("Miljökvalitetsnormer", MKN) to ambient levels of air pollutants in Sweden are implemented by the Swedish Environmental Protection Agency on the basis of directives from the European Commission, and the Swedish limits are with few exceptions identical to the European. These limits are based on health science, as well as other considerations by member states and lobbying organizations, and it is generally recognized that they do not provide complete protection from adverse health effects. Recommendations based solely on considerations of health are issued both by the World Health Organization (WHO) in its "Air Quality Guidelines" (AQG, currently under revision), and, in some instances, by IMM. The Swedish Parliament has adopted long-term environmental goals ("Miljömål") concerning, among other things, air quality based at least in part on the recommendations of the WHO and/or IMM recommendations.

Air pollutants that are translocated between countries are also regulated at the global level through the 1979 Geneva Convention on Long-range Transboundary Air Pollution, which has been extended by several specific protocols issued by the United Nations Economic Commission for Europe (UNECE).

10.1.7 Noise

The European Commission has issued an "Environmental Noise Directive" (END) requiring member states to publish maps of and management actions plans for noise for regions with more than 100 000 inhabitants, as well as for areas around major traffic systems, once every 5 years. However, this directive does not set any limits or target values for levels of noise. Health-based recommendations have been issued by the WHO and are presently being updated. In Sweden, these actions of the several agencies involved in regulating noise are coordinated by the Swedish Environmental Protection Agency.

10.2 Training

There is need for training in exposure assessment. Professionals who work with relationships between exposure and health both at national and international agencies, in industry and in academia require training as well that encompasses basic epidemiology and toxicology.

IMM regularly coordinates training in health risk assessment organized through several European projects. Furthermore, at IMM there is a long tradition of organizing educational programs in epidemiology and toxicology for both doctoral and master students.

10.2.1 RA-COURSES

RA-COURSES was a EU-funded Marie Curie Conferences and Training Courses project coordinated by IMM from 2007-2011. Its overall objective was to provide a coherent European programme of courses in health risk assessment. The ten courses given included two on exposure assessment.

Chemical exposure assessment analysis and modeling

The objectives of this course, given at Umeå University in collaboration with IMM in 2009, was to introduce its participants to the field of exposure assessment, with a specific focus on chemical analysis and various modelling techniques. The topics included were environmental pollutants, analytical chemistry, and strategies for sampling, modelling and interpretation of data. The quality of data concerning environmental concentrations and processes for exposure assessment were explored as integrated aspects of risk assessment protocols.

Dietary Exposure Assessment

This course, given by RIVM (Bilthoven, the Netherlands) in collaboration with IMM in 2010, focused on databases concerning concentrations of chemicals and consumption of food, as well as probabilistic modelling. Its objective was to familiarize the participants with dietary exposure assessment to toxic compounds and, in particular, with probabilistic modelling in this context. The topics covered were as follows: the importance of exposure modelling in connection with risk assessment; models employed data on levels of chemicals and food consumption for dietary exposure assessments; probabilistic modelling, especially at the pan-European level; new developments in modelling of exposure; and intake modeling in the field of nutrition.

In connection with a workshop on training in exposure assessment organized under the RA-COURSES project in 2009, it was proposed that a package of at least four modules is required to provide in-depth training:

1. A course covering the basic concepts involved in exposure assessment.
2. A course covering the concepts of emissions, sources, release and distribution of chemicals in the environment, in combination with environmental modelling.
3. A course on external exposure, covering dietary exposure, dermal and inhalational exposure routes as well as occupational exposure.
4. A course on internal exposure, covering PBPK modelling, biomonitoring, etc.

10.2.2 TRISK

TRISK, a project funded by the EU from 2009-2012 and coordinated by University of Milan with IMM as one of the partners, involved a pilot training programme in human

health risk assessment consisting of eight one-week courses, an applied project and a final examination. One of the courses was on exposure assessment.

Exposure analysis in risk assessment

The objectives of this course, organized in 2010 by Utrecht University in the Netherlands in collaboration with IMM, was to acquaint the participants with the principles of exposure assessment and its role in toxicology and risk assessment. The topics included were: direct measurement of levels of exposure levels obtaining representative measurements, analyzing the data obtained and the fundamentals of exposure modelling.

On the basis of the experience from this project, as well as input from various European stakeholders guidelines for a European Training Programme in Human Health Risk Assessment were developed. These guidelines suggest four core training themes: risk assessment in connection with risk analysis, effects assessment, exposure analysis and risk characterization. The following topics were identified for inclusion in exposure analysis:

- Different procedures for exposure assessment (e.g., models, deterministic and probabilistic assessment).
- Routes, types and patterns of exposure.
- Direct and indirect assessment of exposure.
- Estimations of exposure involving varying degrees of complexity.
- Modelling in the absence of direct measurements.
- Use of environmental monitoring and human biomonitoring for exposure assessment.
- Biomarkers of exposure.

10.2.3 Specialised courses on advanced aspects of risk assessment for EFSA panel members and staff

IMM coordinated the project entitled "Specialized training courses on certain aspects of food safety risk assessment" for members of EFSA Panels/Scientific Committee, which was also open to EFSA scientific staff. EFSA funded this project, which ran from 2012-2015. Its overall objective was to organize and give high-quality training courses designed to meet the needs identified by EFSA. The three different training courses given were: Evidence base for risk assessment, Variability and uncertainty in risk assessment, and Exposure assessment.

Exposure Assessment

The objectives of the course on exposure assessment, given by RIVM in the Netherlands, were to promote understanding of dietary exposure assessments to a wide range of chemicals. This course encompassed the collection, harmonization, limitations and inclusion of data in dietary exposure assessment; the various methodologies currently

used by EFSA to assess exposure; and examples of such methodology, as well as future developments in modelling dietary exposure.

10.2.4 Doctoral programs

At present three doctoral programs in this area are being coordinated by IMM:
1) Environmental Factors and Health
2) Epidemiology
3) SINGS (The Swedish INterdisciplinary Graduate School in Register-Based Research)
In addition to courses, seminars and workshops for doctoral students at Karolinska Institutet, these programs are also open to doctoral students at other universities, in particular the courses within the SINGS program, which is funded by the Swedish Research Council. The Environmental Factors and Health program includes a course that focuses specifically on exposure assessment. Other courses included in these programs integrate concepts concerning exposure assessment.

Biologically based exposure assessment for epidemiology

The objective of this course, given in 2012, organized by IMM and taught primarily by a member of the School of Public Health at Harvard University, was to examine the theoretical basis of exposure-response models, as well as to bridge the possible gap between exposure assessors and epidemiologists. The topics included were: integration of exposure assessment and epidemiology; design and analysis of field studies; and implications for future research directions, in risk assessment, and disease prevention.

10.2.5 Other training courses in exposure assessment

Very few additional training courses in exposure assessment are offered regularly by IMM and it has also been difficult to find such courses offered by different universities and institutes. The newly established WHO Chemical Risk Assessment Network, in which IMM is participating, now provides a database covering courses in risk assessment presently being given. This database includes only the following two courses in exposure assessment:

Exposure Assessment in toxicology organized by Utrecht University

The objective of this course is to introduce the principles of environmental and human exposure assessment in toxicology and their role in risk assessment. After completing this course, the student: should understand the importance and complexity of such assessment; be able to assess exposure to chemicals in the environment with the help of fate models based on partitioning relationships; and be able to interpret measurements of environmental exposure and evaluate the scientific literature in this area critically.

Advanced Exposure Assessment organized by Utrecht University

The main theme of this course is advanced exposure assessment in connection with environmental and occupational epidemiology. The methodology for such advanced assessment is introduced, discussed and applied in lectures, case studies and practical

computer exercises. The specific themes covered are GIS & geodata, land use regression, dispersion modelling, personal exposure monitoring and occupational exposures.

EUROTOX, the Federation of national societies of toxicology in Europe
EuroTox organizes annual conferences that include one-day Continuing Education Courses. During the past four years four such courses on exposure assessment have been organized:

- New challenges in modeling human exposure to chemicals.
- Dietary exposure assessments – current scenario and emerging issues.
- Emerging innovative methods and technologies for biomonitoring of xenobiotics.
- Co-exposure risk assessment: approaches and options for prioritization and refinement.

10.3 Developmental Needs

Exposure assessment for regulatory purposes is performed in different ways, depending on the regulatory requirements, specific type and amount of data available, and methodology employed. During the past decade more requirements have been placed on exposure assessment and new approaches have been developed, leading to an increased demand for well-trained exposure assessors. Education and training in this area must be provided not only for students and researchers at universities, but also for a wide range of professionals, working in government agencies and industry, and as consultants.

Experience from the European projects that IMM has coordinated or participated in indicates clearly that the need for such training is considerable. The applicants for the courses given have come from more than 30 different countries and been approximately three times as many as could be accepted. In general, the participants have been highly satisfied.

On the basis of our experience in organizing courses on exposure assessment a future series of training courses can be designed. These courses would begin with the basic concepts and thereafter cover the methodology and applications of exposure assessment within different areas, including dietary exposure, dermal and inhalational exposure and occupational exposure.

New courses on exposure assessment should preferably combine different perspectives and engage expertise from the various areas of research in epidemiology and toxicology. Such cross-disciplinary expertise is available at IMM. Naturally, the teaching should be of high quality and incorporate new pedagogical developments.

At present, very few courses of this nature are given. IMM has developed fruitful collaborations with experts in exposure assessment who work mainly at Umeå University, RIVM and Utrecht University. Nonetheless, the capacity for such training at

IMM and in Sweden is inadequate and more scientists in this area should get involved in this educational task.

11. Conclusions, Research and Developmental Needs

In the present report, researchers at IMM active in diverse fields within environmental health have described the state-of-the-art, together with the challenges and needs for future research in their respective areas. These needs are summarized below (section 11.1 *Specific research needs*).

Exposure assessment is fundamental to many fields related to environmental and occupational health, including toxicology and epidemiology, and is also an important component of health risk assessment and management. Exposure assessment is also useful for determining status and trends with respect to levels of environmental contaminants and chemicals in the environment and in humans. The overriding aim of exposure assessment is to protect public health and to prevent exposure to harmful chemicals or physical stressors by collecting information on their occurrence and levels in various environments and media.

This report emphasizes the need for more efficient and reliable methods for assessing human exposure, and not least the combined exposures we are all subjected to. There is a requirement for reliable, sensitive and specific biomarkers of exposure. Furthermore, trans-disciplinary collaborations between experts concerned with exposure and the health research would help improve our understanding of the influence of (combined) exposures on human health.

Certain segments of the population (such as children, pregnant women and workers) are more sensitive and/or more highly exposed to chemicals or physical stressors and require special attention in exposure assessment. Such target populations must be identified and relevant exposures quantified. In addition, chemicals to which the general population is exposed to on a daily basis e.g., air pollutants and food contaminants are also of considerable concern.

Moreover, development of novel and improved models and tools for collecting and analyzing data, as well as for handling and interpreting the large amounts of exposure information collected by new technology (e.g. remote sensing, cellular telephones, web-based systems and omics analysis) is crucial to expand exposure information with the aim to improve the understanding of where, when and how exposure occur and their health impact (NIH 2012).

Improved methods and techniques in exposure assessment are fundamental for the development in the field. Better equipment, automated sensors and robotics, as well as more in-depth analyses of human behavior and activity patterns are required, as are field simulations of exposure, source-to-exposure and dose-modeling systems (Lioy 2010; NIH 2012).

11.1 Specific research needs

11.1.1 Human Biomonitoring

Human biomonitoring has become a primary tool for chemical exposure characterization in a wide variety of contexts. The value of HBM as a tool for assessing overall exposure of humans to single or multiple chemicals in population monitoring at national level, in risk assessment, public health surveys and epidemiological research, as well as in PBPK modelling, has become increasingly apparent. Harmonization of HBM would allow better utilization of the biomonitoring data to answer questions concerning chemical exposures and potential health effects, respond to community concerns, improve environmental and public health, and to inform policy. A crucial factor in HBM is the availability of high-quality, validated, high through put analytical methods, and analytical methods for "new" or emerging chemicals.

More specifically there is a need for:

- Harmonization of HBM at the national and European levels.
- Development of reference values and health based guidance values for purposes of risk assessment and management.
- Development of biomarkers suitable for quantifying combined exposures.
- Improvement of methodologies for high-through-put analyses, including non-target analysis.

11.1.2 Epidemiology and exposure assessment – some general aspects

Successful recruitment of participants for epidemiological surveys and research aimed at improving public health is important. Today we see trends of decreasing participation. Accurate exposure assessment is also crucial for drawing firm conclusions from epidemiological studies.

Research and development needs:

- Improve the understanding of factors influencing response rates.
- Compare different survey modes and recruitment approaches in terms of efficiency.
- Develop new epidemiological and biostatistical methods to account for potential distorting effects of low response rates.
- Develop and validate new instruments for data collection to facilitate repeated measurements during a follow-up period and to obtain simultaneous measurements of multiple exposure variables, e.g. biological markers.
- Develop new epidemiological and biostatistical approaches to address research questions concerning the analyses of combinations of multiple exposures.

11.1.3 Early life exposure assessment

In order to improve living conditions and sustainable development it is essential to detect and mitigate the most critical environmental factors leading to impaired child health and development. Thus, it is essential to consider the most susceptible population groups, such as fetuses and children, in health risk assessment. Comprehensive health risk

assessment for these early stages in life is available for a few environmental pollutants only and much more data is needed in order to establish reliable reference values and recommendation.

More research is needed concerning the following areas:

- Develop large mother-child cohorts for longitudinal evaluation of exposure to environmental pollutants and other chemicals and potentially associated health effects.
- Measure exposure repeatedly, starting already in early pregnancy or even before conception, in order to identify critical windows of early life exposure.
- Design cohorts to measure multiple toxicants in order to evaluate combined exposure of chemicals and environmental pollutants present in the children´s food, ambient air and external environment.
- Develop suitable biomarkers of exposure to the most important toxicants during different developmental stages, including fetal development.
- Clarify how early life epigenetic changes can be used as biomarkers of exposure and their role as predictors for future disease.
- Improve the knowledge about susceptibility factors such as genetic predisposition, gender and nutrition.

11.1.4 Spatial and temporal assessment of environmental factors

Spatial modeling of air pollution is well established, especially for traffic-related gaseous pollutants in urban areas. Good spatial models are however still lacking for other air pollutants, and the link to personal exposure requires further development. Temporal models for long-range transport air pollution are adequate, but for other types of air pollutants they are not sufficient. Modelling of community noise is underway, while modelling of other important factors such as heat stress and access to green areas are only in early development.

In this connection more research is needed in the following specific areas:

- Development and validation of dispersion models for the estimation of levels of pollutants in ambient air, with high geographical resolution, especially of different particulate components (such as PM_{10}, $PM_{2.5}$ and soot), and contributions from different local sources, primarily road traffic and biomass burning.
- Investigation of the relation between modelled levels of air pollution and personal exposure, especially with regards to soot.
- Development of hybrid models that combine dispersion modeling, land use regression and satellite-based techniques to improve estimation of air pollution levels with respect to geographic and temporal resolution over large populated areas, as a basis for epidemiologic research and risk assessment.
- Improvement of models for estimation of noise originating from different sources, such as road, railway and air traffic, both outside and inside buildings.
- Development of methods that can distinguish between different dimensions of exposure to green-structure.

- Development of efficient approaches to resolving spatial (contextual) confounding of socieconomy.

11.1.5 Occupational exposure

Exposure levels in the work-environment are usually several times higher than in the surrounding environment, and it is important to assess exposure of workers to prevent illness and expenses for health care and sick leave. Measurements of occupational exposure are essential for a reliable exposure assessment. Measurements should be performed by personnel that is proficient at measurement strategy, sampling method, and who can interpret the results. When measurements are missing, or large populations are studied, the exposure assessors have to use indirect methods, such as job-exposure matrices and modeling tools.

The following areas of research in occupational exposure assessment need more attention:

- In order to assess the occupational exposure in the population, exposure measurements are required. IMM is host for exposure data collected in the National and regional health-related environmental monitoring program (HÄMI). There is no corresponding program for the work environment, which means that there is no basis for priorities in a future national work-environment plan. We see a great need for a National health-related work-environmental monitoring program, where measurements can be gathered in a Swedish national occupational exposure measurement database. Sweden has a large collection of occupational measurement data that also need to be data-processed and compiled in a database, and thus become available for research.
- The theoretical framework on how measurements and dose modelling are used to assign personal exposure metrics in epidemiological studies must be further developed, and existing knowledge fully implemented, especially for effective analyses of dose-response, needed for hazard evaluation. There is a strong reliance on cumulative exposure in dose-response analyses in epidemiological studies, although it is well known that disease risk may not be proportional to cumulative dose. Methods are needed for planning of measurements as well as for the most efficient statistical models to assign the personal exposure for a group. Disentangling the effects of exposure duration and intensity may be one step in this direction.
- Epidemiological studies of chronic diseases often require assessment of past exposures, while historical measurements often are scarce, and modelling techniques are often applied. Improved and transparent methods are needed for accurate assessment of past exposures.

11.1.6 Indoor air exposure

Indoor dampness and mold are considered to deteriorate IAQ in many Swedish buildings but estimates of the health risk and the fraction of the population at risk are highly

uncertain. To improve the knowledge base for rational handling of indoor air exposures in Sweden we identify the following research needs:

- Review already published studies and update the knowledge base on IAQ in Sweden, taking into account the methodological quality of studies and the use of wood preservatives containing chlorophenols.
- In new studies, include wood preservatives and chloroanisoles as environmental factors of potential importance for IAQ, and in particular consider the possibility to dissociate odor due to mold growth from odor due to chloroanisoles.
- Review and develop methods to identify odors specific for typical building problems (e.g. chloroanisoles and possibly 2-aminoacetophenone) as a basis for health risk communication and building remediation.

11.1.7 Skin exposure to hazardous chemicals

In everyday life, we are repeatedly exposed to hazardous chemicals in different combinations, often at relatively low doses, many times per day, possibly throughout our entire lives. A future challenge will be to accurately assess the effects of such exposure, which differs substantially from those employed in today's experimental systems and models. Methodology for the detection and quantification of hazardous substances on and in skin needs to be expanded and refined, in order to better understand skin absorption, accumulation and effects.

In this context, areas where more research is needed include the following:

- Skin exposure must be quantified reliably.
- Endogenous factors that influence susceptibility to sensitization need to be identified.
- Development and application of measurements of skin exposure are crucial to understanding the uptake and accumulation of contact allergens in skin. These should preferably be *in vivo* and non-invasive.
- The impact of skin exposure to "cocktails" of allergens and other substances originating from different sources is presently unknown and must be elucidated.
- The relative importance of duration, frequency and level of exposure to skin sensitizers and irritants is largely unknown and must be determined to improve assessment and prevention of health risks.

11.1.8 Exposure extrapolation between cells, animals and humans: focus on nanoparticles

In this chapter we conclude that *in vitro* assays will be used to a higher extent in risk assessments of nanoparticles (and other chemicals) in the future. There is, however, at present a lack of understanding about extrapolations and comparisons between *in vivo* and *in vitro* for exposures and effects following inhalation of nanoparticles. We conclude that dosimetry considerations are critical to enable such comparisons and need to be considered better in future studies. In the chapter several examples and calculations regarding how comparisons can be made between human exposures and exposures in *in vitro* assays are described. Finally, we suggest that more advanced exposure- and cell

models may be a way forward to increase the correlation between *in vivo* and *in vitro* assays.

More research is needed concerning the following areas:

- Investigation on how *in vitro* assays can be used for better prediction of *in vivo* outcomes, including *e.g.* improved dosimetry for nanoparticles.
- Development of *in vitro* assays for improved predictions of *in vivo* outcome following nanoparticles. Exposure at the air-liquid interface and more complex cell models represents steps forward but comparison to and validation in relation to traditional exposures *in vitro* and *in vivo* are required.
- Determination of which *in vivo* assays can be replaced by *in vitro* assays for purposes of risk assessment.

11.1.9 Exposure assessment in regulatory risk assessment and training

Exposure assessment for regulatory purposes is performed in different ways, depending on the regulatory requirements, specific type and amount of data available, and methodology employed. During the past decade more requirements have been placed on exposure assessment and new approaches have been developed, leading to an increased demand for well-trained exposure assessors. Education and training in this area must be provided not only for students and researchers at universities, but also for a wide range of professionals, working in government agencies and industry, and as consultants.

- There is a need for development of courses in exposure assessment, to be given on a regular basis for master and PhD students, researchers, as well as professionals, both nationally and internationally, preferably in collaboration with other universities/institutes.

References

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Glossary

Amniocentesis: sampling of a small amount of the amniotic fluid.

Amniotic fluid: the fluid around the baby in the amniotic sac in the uterus.

BET area: "Brunauer, Emmett and Teller" (BET) area is a method used to determine the surface area of a powder e.g. nanoparticles. It is based on measurement of the gas adsorption of the powder and results are expressed in units of area per mass of sample (m^2/g).

Biomarker of exposure: "indicator of changes or events in biological systems. Bioloigcal markers of exposure refer to cellular, biochemical, analytical, or molecular measures that are obtained from biological media such as tissues, cells, or fluids and are indicative of exposure to an agent" (WHO IPCS 2004).

Breathing zone: "hemisphere (generally accepted to be 0.3 m in radius) extending in front of the human face, centred on the midpoint of a line joining the ears; the base of the hemisphere is a plane through this line, the top of the head and the larynx" (International Organization for Standardization, 2012).

Colostrum: the first milk produced after delivery.

Critical window of exposure: the most susceptible life stage for toxic insult.

Epigenetic marks: encompass DNA methylation, histone modifications, and small non-coding RNA molecules. Epigenetic marks are important for regulation of gene expression.

Exposure: "contact between an agent and a target. Contact takes place at an exposure surface over an exposure period" (WHO IPCS 2004).

Exposure assessment: "the process of estimating or measuring the magnitude, frequency, and duration of exposure to an agent, along with the number and characteristics of the population exposed" (WHO IPCS 2004).

Hand-arm vibrations: "the mechanical vibration that, when transmitted to the human hand-arm system, entails risks to the health and safety of workers, in particular vascular, bone or joint, neurological or muscular disorders."

Hematocrit: the amount of red blood cells as percentage of the whole blood volume.

Hemodilution: increase in the plasma volume relative to red blood cells.

Inhalable fraction: "mass of total airborne particles that is inhaled through the nose and mouth" (International Organization for Standardization, 2012).

Meconium: the first feces produced by the newborn.

Occupational exposure limit value (OELV): “limit of the time-weighted average of the concentration of a chemical agent in the air within the breathing zone of a worker in relation to a specified reference period” (International Organization for Standardization, 2012).

Personal sampling: “process of sampling carried out using a personal sampler” (International Organization for Standardization, 2012).

Personal sampler: “sampler, attached to a person,that collects airborne particles in the breathing zone to determine exposure to chemical agents” (International Organization for Standardization, 2012).

Total airborne particles: “all particles surrounded by air in a given volume of air” (International Organization for Standardization, 2012), often impossible to measure because all instruments are size-selective to some extent.

Toxicokinetics: the kinetics of the absorption, distribution, metabolism, and elimination of hazardous substances by an organism.

Whole-body vibrations: “the mechanical vibration that, when transmitted to the whole body, entails risks to the health and safety of workers, in particular lower-back morbidity and trauma of the spine.”

Abbreviations

AAS - Atomic Absorption Spectroscopy

ADME - Absorption, Distribution, Metabolization/degradation and Excretion

ALI - Air-Liquid Interface

BE - Biomonitoring Equivalents

BPA - Bisphenol A

CAG - Cumulative Assessment Groups

CDC - Centers for Disease Control and Prevention

CLP - Classification, Labelling and Packaging of substances and mixtures

CO - Carbon Monoxide

CRM - Confocal Raman Microscopy

ECM - ExtraCellular Matrix

EHBMI - European Human Biomonitoring Initiative

EWAS - Environment Wide Association Studies

FLIM, TPM - Fluorescence techniques

FTIR-PAS - Fourier Transform InfraRed PhotoAcoustic Spectroscopy

GC-MS - Gas Chromatography Mass Spectrometry

GPMT - Guinea Pig Maximisation Test

HELIX - Human Early-Life Exposome

HBM - Human Biological Monitoring

HP-LC - High Performance-Liquid Chromatography

IAQ - Indoor Air Quality

ICP - Inductively Coupled Plasma

ICP-MS - Inductively Coupled Plasma Mass Spectrometry

IL-8 - Interleukin-8

IL-13 – Interleukin-13

IMM - Institute of Environmental Medicine, Karolinska Institutet, Stockholm

ISDD - In vitro Sedimentation, Diffusion and Dosimetry

JEM - Job-Exposure Matrix

LLNA - Local Lymph Node Assay

LST - Land Surface Temperature

LUR - Land Use Regression

MCI - Methylchloroisothiazolinone

MI - Methylisothiazolinone

MPD - Magnetic Particle Detection

MPPD - MultiPath Particle Deposition model

MS - Mass Spectrometry

MVOC - Microbial Volatile Organic Compound

NBP - National Biomonitoring Program

NHANES - The National Health and Nutrition Examination Survey

NO_2 - Nitrogen dioxide

OELs - Occupational Exposure Limits

PAH - Polycyclic Aromatic Hydrocarbons

PAR - Proximal Alveolar Region

PBPK - Physiologically Based PharmacoKinetic

PCP - PentaChloroPhenol

PDS - Photothermal Deflection Spectroscopy

$PM_{2.5}$ - Particulate Matter $\leq$ 2.5 µm in aerodynamic diameter

PM_{10} - Particulate Matter $\leq$ 10 µm in aerodynamic diameter

QCM - Quartz-Crystal Microbalance

REACH - Registration, Evaluation, Authorisation and restriction of Chemicals

RfD – Reference Dose

ROAT - Repeated Open Application Test

SCCS - Scientific Committee on Consumer Safety

SCENHIR - Scientific Committee on Emerging and Newly Identified Health Risks

SCHER - Scientific Committee on Health and Environmental Risks

SCOEL - Scientific Committee on Occupational Exposure Limits

SEG - Similar Exposure Groups

SNP - Single Nucleotide Polymorphisms

SO_2 - Sulfur Dioxide

SVOC - Semi- Volatile Organic Compound

TDI - Tolerable Daily Intake

TVOC - Total Volatile Organic Compound

UHI - Urban Heat Island

US EPA – US Environmental Protection Agency

VOC - Volatile Organic Compound

WHO - World Health Organization

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