Chapter 4
Modeling Complex Exposures

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Abstract This chapter deals with the aspects of modeling complex exposures, highlighting the integration of various external and internal exposure models, following the exposome paradigm. Several approaches are investigated, relating either to the assessment of the overall chemical mixture as a single compound, or applying the compound-by-compound approach. Identifying the contribution of the various pathways leading to complex exposure requires the precise estimation of the various exposure mechanisms that integrate through the three main exposure routes (inhalation, oral and skin); hence, modeling environmental fate at different scales (such as regional, local or micro-environmental scale) for capturing both far field and near field exposure is essential. Integration of exposure through various pathways and routes occurs at the level of internal dosimetry. This is also reflected in the observed biomonitoring data, highlighting the need for integrated modeling tools that allow the functional link among exposure, internal dose and biomonitoring data. Extrapolation of exposure estimates from individual data to population exposure through advanced probabilistic techniques and agent-based models, as well as the latest advances in personal sensors for tracking activity and location are also presented. The importance of these aspects is highlighted in characteristic case studies regarding indoor air mixtures and multiple pesticide exposure.

Keywords Exposure modeling · Exposome · Complex mixtures · Internal dosimetry · Probabilistic exposure modeling

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4.1 Exposome and the Need for Modeling Complex Exposures

One of the most recent developments in the field of exposure science is the introduction of the exposome concept (Wild 2005). The exposome represents the totality of exposures from conception onwards, simultaneously identifying, characterizing and quantifying the exogenous and endogenous exposures and modifiable risk factors that predispose to and predict diseases throughout a person's life span. The exposome came as a complement to the human genome; although decoding of the human genome (Schmutz et al. 2004) increased our understanding of the underlying causes of disease, the genome explains only a percentage of the population burden of disease. Indeed, according to Rappaport et al. (2014), two thirds of all people worldwide die of chronic disease (mostly heart disease and cancer) which are caused by combinations of the genome and exposome (representing all exposures – internal and external). However, disease risks attributed to the genome alone are modest, representing less than 15%, suggesting that more than 85% of risks results from exposome and interaction of the genome and the exposome. Thus, it is evident that environmental factors are equally or potentially more important than genetic traits for characterizing health risk. What is truly critical is the interaction between environmental determinants of disease with biological systems. Characterizing the exposome will carry us along towards a better understanding of the causal links between the genome, environment, and disease. To do this, both environmental exposures and genetic variation should be assessed simultaneously; properly determining and quantifying complex exposures is one of the main pillars of the current efforts towards unraveling the exposome. It is very important to keep in mind that the several responses observed at the molecular level, are the result of the combined exposures to several compounds in a dynamic, time-dependent manner. Thus, to properly identify the differences in the responses at different levels of biological organization it is critical:

- To properly account for different sources of exposure at different time resolutions and scales
- To translate these exposures into internal and biologically effective doses.

Exposome studies require novel tools to address the complexity of emerging environmental health issues. Critical for success will be the ability to bring together existing geospatial, environmental, health and socioeconomic data, and to collect new high resolution data. Innovative environmental micro-sensors, remote sensing or other community- and -omics/systems biology-based approaches can be used to describe the exposome and how it relates to the advent of multiparametric and multicausal human disease, such as endocrine disruption-related syndromes and sex-related changes (menopause), neurodegenerative or respiratory diseases. It is important to focus on: susceptibility windows during growth (including pregnancy) and development; the unequal distribution of the burden of food and environment-related disease to vulnerable populations (e.g., the young, the elderly, socio-

economic disadvantaged, and gender and ethnic minorities); and epigenetic influences.

The individual exposome is dynamic and continually changing. Indeed, all exposures and their determinants and modifiers can vary over the course of a day, not to mention over the weeks, months, and years that make up a lifetime, as our bodies, diets, risk factors and lifestyles change. Sources and levels of exposure change over time and capturing all these changes verges on the impossible in the impracticality of "high-resolution real-time" monitoring of all the exposures for the entire lifetime. Thus, the exposome has to be constructed by assessing the exposures at critical life periods through representative snapshots that act as demonstrative measures of these critical periods. Indeed, mapping the entire lifecycle of an individual may not be necessary if critical lifetime events where an individual's geospatial lifeline crosses a noteworthy environmental event (Sabel et al. 2009) are recognized and understood. Thus, one major challenge consists of identifying critical life stages that are most informative, as well as forming a picture of a person's overall exposure using sets of short-term measurements during these critical life stages, and relating these to downstream consequences. The latter may include both observable health outcomes and subtler changes in biomarkers. The most relevant exposure episodes in an individual's life could be reconstructed and linked to socio-economic conditions at critical life stages such as prenatal exposure, puberty, or the reproductively active period. Whereas exposure during all life stages may entail adverse effects, *fetuses*, *children, pregnant women* and the *elderly* are particularly susceptible. Modeling the mobility patterns of the population at risk at the individual level is challenging. There are considerable conceptual and computational difficulties involved in intersecting data on the distributions of pollutants, and/or the patterns of movements of recipient individuals or groups, reflecting the limitations of available data on environmental conditions and human distributions. Complex exposure patterns can be disentangled by fusing mobility and behavior data with the corresponding environmental data. The most appropriate such data are themselves the result of fusion of environmental monitoring data derived from the use of personal and remote sensors, including air- and satellite-borne ones; conventional monitoring systems used for regulatory compliance across several jurisdictions; and environmental modeling used to fill the observed data gaps. With the advent of geographic information systems (GIS), global positioning systems (GPS) to track individuals, and personal environmental monitoring, undertaking such analyses throughout an individual's lifetime is now possible.

Complex exposures are rarely measurable at sufficient levels of resolution and precision to allow proper exposure assessment for risk assessment purposes. For this reason, exposure modeling is necessary to support accurate exposure assessment and thus provide the currently missing link in complex mixtures risk assessment. In this context external exposure modeling needs to be coupled with internal exposure modeling to properly account for potential interactions influencing both:

- Uptake and intake rate of mixture components (dealt with individually or in chemical groups) and
- Exposure route and the relative importance of multiple routes.

The latter, in turn, influences the bioavailability of the mixture components and may determine the dominant phase of metabolism and consequently the internal and biologically effective dose of mixture components (assessed individually or in chemical classes on the basis of clustering criteria coupling physical/chemical properties, biokinetic features and toxicity profiles).

Internal and external exposure modeling brings out the hidden added value of human biomonitoring data. Human biomonitoring alone has a limited ability to identify specific exposure sources, and may not be applicable to all relevant environmental stressors (e.g., particulate matter (PM_x) or noise). Coupling it with internal exposure modeling may help to reconstruct external exposure profiles even in cases with high complexity either due to the nature of co-exposure patterns to multiple stressors or due to the complexity of the exposure patterns in time or space. Integrating human biomonitoring data, exposure models and environmental monitoring and modeling data will lead to a more comprehensive view of the exposome and of related health outcomes and will be of use in future large-scale population studies.

Information on *lifestyle/behavior patterns* (such as time-activity-location information, food consumption, use of consumer products, etc.) is needed to understand individual and population-based geospatial lifelines and the corresponding exposure profiles.

- Spatial information and initiatives to harmonize their collection (e.g., Infrastructure for Spatial Information in Europe (INSPIRE), Copernicus) have the ability to transform the way scientists and policy makers think about exposure to environmental stressors.
- At the same time, behavioral information functions as the most accessible and direct way for policy makers and risk assessors to understand and manage an individual's exposure patterns.

4.2 Complex Exposure Modeling

Complex exposures include exposures to complex mixtures of chemical stressors (e.g., mixtures of more than fifty chemicals) as well as to combinations of stressors (chemical, physical, biological) on the basis of complex exposure patterns in space and time. Modeling complex exposures requires describing and mathematically capturing both the fate and exposure characteristics of key individual mixture components and taking into account potential interactions of the latter. Thus the related models need to pertain to the combination of aggregate (all pathways and routes) and cumulative (all stressors) exposure. In practice, people live in a continuously dynamic environment, encountering different locations and performing different activities within the day, ingesting several food items and using several consumer products. Thus, individual exposure dynamics are greatly affected by personal behavior and practices such as transportation mode and nutritional habits.

Although exposure assessment historically used to pay special attention to specific exposure scenarios (e.g., a particular occupational exposure) and well defined mixtures (e.g., circumscribed chemical classes such as polycyclic aromatic hydrocarbons (PAHs) or polychlorinated biphenyls (PCBs)), now attention has been paid to the cumulative exposure to compounds or groups of compounds that pose additive or greater than additive effects. That means that when exploring the causality of an adverse outcome, e.g., endocrine disruption related outcomes, a long list of compounds needs to be addressed such as plasticizers (bisphenol-A, phthalates), pesticides, dioxins and PCBs. This implies that an exposure-driven approach should be established, seeking identification of the complex and cumulative exposures of stressors that result in combined toxicity. Thus, it is imperative to properly account for exposure of each individual compound (or sub-groups of compounds) before estimating risk based on established methods (e.g., concentration/dose addition, independent action, effect summation) or seeking for associations between exposure and disease.

One of the main dimensions of complex exposure modeling is accounting for the fate of and exposure to complex chemical mixtures (Kinerson 1987). Kinerson (1987) has identified the following possibilities to model complex chemical mixtures:

(a) Based on bulk properties of the overall mixture
(b) Based on chemical classes that are representative of the mixture
(c) Based on chemical fractions clustered by physical/chemical and biokinetic properties (the latter is of particular importance when considering the link between external exposure levels into internal and, eventually, biologically effective dose.
(d) As individual components (one compound at a time).

(a) *Model bulk properties of the mixture:* In this approach, the overall mixture of components is treated as a single component. This approach has to be carefully applied and it is appropriate only for mixtures where the properties governing environmental and biokinetic fate are very similar for the individual components of the mixture. The advantage of the method is that model estimates are obtained through a single run.
(b) *Model by (representative) chemical classes:* This approach represents an intermediate solution between the one compound and the bulk properties approach. In practice, the overall mixture is broken down into representative chemical classes, and each class is represented by one characteristic compound. Although ideally the representative compounds should be selected as being of biological significance, frequently, they are selected because their properties have been determined.

A good example of such an approach is given by the work of Pistocchi and Bidoglio (2009) who attempted to model the spatial extent of exposure to pesticides in Europe on the basis of a compound that has been considered as representative of each chemical class of pesticides based on the toxic potency of the class. Similarly, Sarigiannis et al. (2013) have derived a highly granular inventory of pesticide

emissions into the air and the corresponding bystander and farmer/applicator exposure maps across Europe. An example of this application is given later in this chapter to exemplify the advantages and disadvantages of the approach as well as possibilities to optimize the outcome by integrating models of different complexity over space and time.

(c) *Model by chemical fractions:* a similar approach to the above is to chemically fractionate the mixture, by dividing it physically into several fractions, each containing chemicals of greater similarity, based on determinants of environmental fate (water solubility, volatility, degradability, etc.). The advantage of this approach compared to modeling by chemical class is that chemicals are clustered based on their properties rather than the designated class; thus they provide more realistic estimates about the compounds included within the same fraction. Highly toxic fractions could be further fractionated and tested to determine their chemical properties in an iterative process to optimize the modeling outcome without excessive demand in computational resources. The chemical fractionation approach is discussed further in Chap. 3.

(d) *Model one compound at a time:* The most common approach for addressing complex exposure modeling is to model the fate and exposure of one single compound at a time. This includes execution of the model for each compound identified in the mixture and collation of the results for assessing the overall mixture fate. Although this approach seems ideal, sufficient data on the properties (volatilization, hydrolysis, photolysis, and biodegradation) governing environmental behavior and exposure are lacking, and there are very high requirements of modeling and computational resources for such an endeavor to succeed.

The choice of complex exposure modeling tier could be based on the availability of data to characterize the chemicals constituting the complex mixture of interest or the intended use of the modeling outcome. This follows the two-tiered approach to cumulative risk assessment of chemicals proposed by Sarigiannis and Hansen (2012). The authors suggest using a dose addition assumption to calculate a hazard index taking into account interactions as a default option for hazard quantification and risk assessment. The hazard index formulation takes into account potential non-linear effects from the interaction of mixture components if the necessary information is available, while simplifying down to dose addition if interaction data do not exist. Overall, it would give a reasonable approximation of the toxic potency of a mixture if the necessary data were available; and it would allow conservative assumptions about effects of combined exposure to multiple chemicals if no such data exist. For further detail, please read the chapter on dose addition (Chap. 9) or the chapter on component-based risk assessment (Chap. 14) in this volume. As a second tier assessment (i.e., when dealing with data-rich situations) more sophisticated tools can be used, including mechanistic, biology-based modeling that accounts for the biologically effective dose of mixture components at the target tissue and incorporates system-wide data coming from –omics technologies. The authors call this the *connectivity* approach. By the same token, exposure

modeling of complex mixtures and scenarios could be designed to follow these tiers by moving gradually from whole mixture, to chemical class, fraction and finally individual compounds considering the potential interactions amongst them.

4.2.1 Modeling Exposure Using the Intake Fraction

Intake fraction (iF) is a metric of the emission-to-intake relationship, facilitating comparisons among sources in terms of their exposure potential (Bennett et al. 2002b). For a given emission source and pollutant, iF is the cumulative mass taken in by the exposed population divided by the cumulative emissions. Considering that iF depends on several parameters affecting the emission-to-intake process, (e.g. prevalent wind, emissions strength, population density), it is expected that it would vary with location and time.

For a primary pollutant, iF can be expressed as (Marshall et al. 2003):

$$\mathrm{iF} = \frac{\text{Population Intake}}{\text{Total Emissions}} = \frac{\int_{T_1}^{\infty} \left(\sum_{i=1}^{P} C_i(t) Q_i(t)\right) dt}{\int_{T_1}^{T_2} E(t)\, dt} \tag{4.1}$$

Where, $T1$ and $T2$ are the starting and ending times of the emission; P is the number of people in the exposed population; $Q_i(t)$ is the intake rate for individual i at time t; $C_i(t)$ is the incremental concentration, attributable to a specific source at time t and $E(t)$ is that source's emissions at time t.

The mathematical expression for exposure to a release through all exposure pathways is given by the following equation (Bennett et al. 2002a):

$$\mathrm{iF(total)} = \mathrm{iF(inhalation)} + \mathrm{iF(ingestion)} + \mathrm{iF(dermal)} \tag{4.2}$$

where the term "total" indicates that intake is summed across all exposure routes.

In a multimedia, multipathway model, a source to-intake relationship is typically expressed as an intake rate (mg/kg-BW/d) per unit emission rate (mg/d). To convert from a source-to-intake relationship to an iF, the following conversion must be made (Bennett et al. 2002a):

$$\mathrm{iF} = \text{Source to Dose}\left(\frac{\mathrm{mg/kg/day}}{\mathrm{mg/day}}\right) \times \mathrm{BW(kg)} \times \text{Population} \tag{4.3}$$

Where BW is the population average body weight (kg) and Population is the size of the exposed population.

Typical values for the iF vary greatly based upon the environmental fate of the mixture of interest and the population density of the area of release; this is the reason why iFs of mixtures released into the indoor environment are usually two to three orders of magnitude higher than the respective values of iF for mixtures emitted into the external environment in urban areas. iF is especially useful in obtaining a quick

overview of the emission-to-intake pathway without returning to detailed environmental fate and exposure modeling. This is of particular interest when exposure modeling aims at identifying the extremes of the exposure probability distribution especially when the latter affects the most socioeconomically disadvantaged population (Marshall and Nazaroff 2007).

Parameters affecting iF include (a) the location where the release occurs (i.e., indoor or outdoor) and the area of interest, (b) the population density and size of the exposed population close to the area associated with the quantity released, (c) dispersion parameters related to the natural or the built environment, (d) the compound-specific environmental fate parameters and (e) based on the different environmental media distribution, the exposure pathway(s) of relevance.

The iF may be an important tool for complex exposure modeling, since it can be applied to groups of pollutants. This pertains to compounds emitted from the same source characterized by similar properties with regard to environmental fate and transport. Thus, by breaking the mixture into several fractions, each of which comprises chemicals with similar environmental fate features, one can be certain that the compounds within the same group will have similar iFs, irrespective of the overall mixture chemical composition and mass emission rates.

4.2.2 Environmental Fate Modeling of Real Life Chemical Mixtures

To properly address complex chemical exposures, it is essential to develop modeling tools that cover a wide chemical space including a large number of industrial chemicals and metals characterized by significantly diverse physicochemical properties. These affect the distribution of mixture components in different environmental media (air, soil, water, sediment), their persistence (regulating processes such as biodegradation and photo degradation) and the respective bioaccumulation and biomagnification potentials.

4.2.2.1 Environmental Fate and Exposure Models for Complex Mixtures

Environmental fate models describe the interactions between different environmental scales and media (air, soil, water, sediment) using physicochemical properties, such as the octanol water partition coefficient (K_{ow}) and octanol-air partition coefficient (K_{oa}), to describe transfer, partitioning, and degradation (Mackay et al. 1992, 2001). The main inputs of multimedia models relate to environmental releases and mode of entry in the environment, properties of the environment or landscape receiving the contaminants (e.g., organic content of soil, distribution of land cover) and compound specific physicochemical properties.

The European Union System for the Evaluation of Substances (EUSES) (Lijzen and Rikken 2004) provides a comprehensive framework for evaluating human and ecosystem exposures and health risks from new and existing chemicals in the European Union. EUSES directly links the overall uptake to probable health endpoints through exposure/response relations without taking into account the toxicokinetics/toxicodynamics and the related internal dose (Fryer et al. 2004). The Calendex™ model system is currently used by the U.S. EPA to evaluate aggregate and cumulative human exposures to pesticides. Calendex™ is similar in both scope and approach to the LifeLine™ (Hampshire 2002) and CARES: Cumulative and Aggregate Risk Evaluation System (CropLife 2002) models. Out of these three models, Calendex™ is generally the least complex in terms of the methodology and techniques it adopts to conduct exposure assessments. However, the proprietary nature of the model and its dependence on expert judgement would appear to limit its potential for widespread adoption (Fryer et al. 2004). The CARES model is fundamentally similar in scope and approach to both the LifeLine™ and Calendex™ models. All three models focus on predicting risks to the U.S. population from dietary, drinking water and residential pesticide exposures. However, the exact methodology adopted in CARES is different from that used by the other models, particularly with regard to the use by CARES of a reference population. The source code of the model has been published and is freely available (Farrier and Pandian 2002). This means that although the model has been developed for use in the USA, it could be updated and adapted to be representative of situations in the EU and other world regions. The LifeLine™ model provides a comprehensive, in-depth tool for assessing human exposures to pesticides and subsequent health risks. LifeLine™ focuses on intra-individual variability in exposure levels in more detail than both Calendex and CARES. The ConsExpo model (Vermeire et al. 1993) provides a framework for evaluating exposures to chemicals in consumer products. The inclusion of models of varying degrees of complexity means that ConsExpo provides a useful tool for assessing consumer product exposures at all tiers of the risk assessment framework, from screening level to specific exposure situations. Validation studies have assessed the performance of some of the individual ConsExpo models against measured datasets (Van Veen et al. 1999; Wilschut et al. 1995) and calculated exposure estimates were generally found to be within an order of magnitude of the measured values. A multimedia modeling approach focusing on spatially explicit modeling of chemical fate and transport processes has been proposed by Pistocchi et al. (2010). The basic idea of this approach is to replace the numerical solution to the advection–dispersion equation with a series of local analytical solutions. Such simplified models comprise the box model, or "continuous stirred tank reactor" (CSTR), the plug flow (PF) and Gaussian plume (GP) models. This set of models judiciously combined may represent most of the typical environmental distributions. E-FAST (Exposure and Fate Assessment Screening Tool) is a model developed by U.S. EPA aimed at providing screening-level estimates of the concentrations of chemicals released to air, surface water, landfills, and from consumer products (Egeghy et al. 2011). E-FAST intentionally provides reasonable overestimations

(90% confidence limit of the upper bound of the estimate) of exposures (ERG 2001), for use in screening level assessment.

For more elaborate calculations, the Stochastic Human Exposure Dose Simulation for multimedia, multipathway chemicals (SHEDS-Multimedia) system is available for download from the U.S. EPA website (https://www.epa.gov/chemical-research/stochastic-human-exposure-and-dose-simulation-sheds-estimate-human-exposure). The residential scenarios included in the SHEDS-Pesticides model focus on organophosphate pesticides (Hore et al. 2006). The SHEDS models do however allow detailed assessments of specific exposure scenarios to be made and are non-proprietary in nature, also providing some links to biomonitoring data (Zartarian et al. 2002). To further improve the exposure assessment approach, the methodology first developed for the SHEDS model was enhanced and incorporated through new, generalized code into the Modeling ENvironment for TOtal Risk studies (MENTOR) (Georgopoulos et al. 2005; Georgopoulos and Lioy 2006; Georgopoulos et al. 2006; Lioy et al. 2007; Georgopoulos et al. 2008c), which was designed to analyze not only exposures to individual contaminants but to assess physiologically based target tissue doses of Multiple co-occurring contaminants via Multimedia, Multipathway, Multiroute exposures (4 M) for specific individuals or for study-specific populations. The GIS extension module of MENTOR, is the Prioritization/Ranking of Toxic Exposures (Royce et al. 2014; Georgopoulos et al. 2014), that utilizes simplified versions of MENTOR components to provide screening level analyses. Similarly to MENTOR, INTEGRA (Sarigiannis et al. 2014a) provides a multimedia environmental model similar to EUSES (Vermeire et al. 1997), following the European Chemicals Agency (ECHA) recommendations, a detailed micro environmental multi-zone model (Sarigiannis et al. 2012a, b), and addresses in detail multi-route exposure and internal dosimetry. Among all the above models, MENTOR-4 M and INTEGRA provide the most complete methodological framework for assessing aggregate exposure from environmental and consumer sources. In addition, INTEGRA integrates a large database for industrial chemicals and additional QSAR models, enabling environmental modeling for multiple chemicals.

4.2.2.2 Specific Considerations for Addressing Complex Exposures

Despite the limitations of the above models, many of them are able to address the issue of complex exposures as a substance by substance problem. One significant issue that relates to complex exposure is the effect of biotransformation in the environment and consequently the fate of transformation products. This results in exposure to additional compounds than the one that was initially released in the environment. The next step is the incorporation of interactions between compounds released in the environment. At present, these types of interactions are limited to consideration in atmospheric (Morris et al. 2004) and indoor air chemistry, where these types of transformation play a significant role.

4.2.3 *Addressing Multi-pathway and Multi-route Complex Exposures with the Compound-by-Compound Approach*

To properly account for the contribution of different pathways and routes of exposure, the key parameters affecting the major exposure mechanisms have to be briefly described. It is important to understand that differences in behavioral patterns are also relevant to exposure, e.g., infants and children are more likely to be exposed to compounds found in settled dust than adults due to significantly more frequent hand to mouth behavior, or people performing intensive exercise close to busy roads are more likely to be exposed to ambient air mixtures.

4.2.3.1 Inhalation

Inhalation is a major route for numerous outdoor (e.g., CO, NOx, SO_2, PM, PAHs) and indoor (e.g., aldehydes, phthalates, and benzene, toluene, ethylbenzene, and xylenes (BTEX)) air pollutants. Personal exposure is equal to the average concentration of a pollutant that a person is exposed to over a given period of time. If over the given period of time, T, the person passes through n locations, spending a fraction f_n of the period T in location n where the concentration of the pollutant under consideration is C_n, then the personal exposure for this period T, represented by the concentration C_T, is given by Eq. 4.4:

$$C_T = \sum_n f_n \cdot C_n \tag{4.4}$$

Inhalation uptake is estimated by the area under the curve of exposure E multiplied by the inhalation rate inh, divided by the bodyweight BW and for the desired simulation time.

$$\text{Uptake}_{\text{inh}} = \frac{\sum_n E_n \cdot \text{inh}_n}{\text{BW}} \tag{4.5}$$

where inh_n is the inhalation rate which is age and activity dependent (ICRP 2002) for each type of microenvironment n encountered.

To properly estimate inhalation exposure, age, gender and activity intensity differences have to be taken into account. There are databases that categorize the majority of daily activities based on their intensity; intensity of activity is associated with age- and gender-dependent inhalation rates (Sarigiannis et al. 2012a, b). In the absence of data, default daily activity patterns can be used. Intensity of activity can also be measured using personal wearable sensors such as Fitbit or Actigraph. This significantly alters the outcome of actual exposure and intake, either between

different individuals encountering the same locations (Sarigiannis et al. 2012a, b), or the intra-day variability for a given individual (Sarigiannis et al. 2014b).

4.2.3.2 Dietary Ingestion

Dietary exposure sources include water and food, and may occur through environmental contamination and bioaccumulation (e.g. pesticides, mercury) or leaching from food contact materials (e.g. bisphenol A from can lining).

To estimate human exposure through diet, contaminant concentrations in foods are multiplied by the corresponding intake rates. The sum of these individual food contaminant intake values is corrected for bodyweight to obtain the daily contaminant exposure via the diet (Lambe 2002):

$$E_{\text{diet}} = \sum_{x=1}^{n} \frac{\left(C_{\text{food}_x} \cdot q_{\text{food}_x}\right)}{\text{BW}} \tag{4.6}$$

E_{diet}: daily contaminant exposure through diet (mg/kg/day)
$C_{\text{food}x}$: contaminant concentration in food item x (mg/kg)
$q_{\text{food}x}$: food item x consumption (kg/day)
BW: body weight (kg)

To properly account for dietary exposure, detailed food consumption databases have to be used that take into account ethnicity, age, gender and socioeconomic status differences (EFSA 2011). Food residues are estimated as the sum of the contribution of the contamination transferred through the food web and migration from food contact materials.

4.2.3.3 Non-dietary Ingestion

4.2.3.3.1 Dust and Soil Ingestion

Scenarios simulating the ingestion of dust and soil combine amounts of dust and soil ingested daily with concentrations of chemicals in these media. The amount of soil and dust ingested daily might be estimated either from daily determinations of trace elements in food intake and fecal output (Stanek and Calabrese 1995, 2000), or by predicting (modeling) soil and dust ingestion by pathway, source type, population group, geographic location, and other factors (Ozkaynak et al. 2011). These exposure pathways are particularly relevant for infants and toddlers who are known to incidentally ingest small amounts of dust and soil daily. Such quantities are higher than the ones for adults by one to two orders of magnitude.

Average daily dose from non-dietary ingestion of chemicals from dust is estimated by the following formula (Wormuth et al. 2006):

$$E_{\text{dust\_ing}} = \frac{C_{\text{dust}} \cdot q_{\text{dust\_ing}}}{\text{BW}} \cdot r_{\text{uptake}} \tag{4.7}$$

where,

$E_{\text{dust\_ing}}$: the internal exposure to chemical (μg/kg/day);
C_{dust}: Concentration of the chemical in dust (μg/mg)
$q_{\text{dust\_ing}}$: Amount of dust ingested (mg/day)
r_{uptake}: absorbed fraction from the ingested quantity
BW: body weight (kg)

Similarly, for soil ingestion, the following formula is used (Wormuth et al. 2006)

$$E_{\text{soil\_ing}} = \frac{C_{\text{soil}} \cdot q_{\text{soil\_ing}}}{\text{BW}} \cdot r_{\text{uptake}} \tag{4.8}$$

where,

$E_{\text{soil\_ing}}$: the internal exposure to chemical (μg/kg/day);
C_{soil}: Concentration of the chemical in soil (μg/mg)
$q_{\text{soil\_ing}}$: Amount of soil ingested (mg/day)
r_{uptake}: absorbed fraction from the ingested quantity
BW: body weight (kg)

4.2.3.3.2 Object-to-Mouth

Several literature sources that describe non-dietary ingestion exposure to chemical residues in objects contacted via object-to-mouth activity can be found. One of them is the U.S. EPA's EXPOsure toolBOX (EPA-Expo-Box[1]), a toolbox created to assist individuals from government, industry, academia, and the general public with assessing exposure (USEPA 2013). To estimate the average daily potential dose from ingestion of surface residues from object-to-mouth contact, the U.S. EPA proposes the following algorithm:

$$\text{ADD} = C_{\text{surface residue}} \cdot \text{CR} \cdot \text{EV} \cdot \text{ET} \cdot \text{EF} \cdot \frac{\text{ED}}{\text{BW} \cdot \text{AT}} \tag{4.9}$$

where,

ADD: Average daily potential dose (mg/kg/day)
$C_{\text{surface residue}}$: Concentration of contaminant on the surface of the hands or objects that are mouthed (mg/cm^2)
CR: Contact rate with contaminated surface (cm^2/event)
EV: Event frequency (events/h)

[1] http://www.epa.gov/risk/expobox/index.htm.

ET: Exposure time (h/day)
EF: Exposure frequency (days/year)
ED: Exposure duration (years)
BW: body weight (kg)
AT: Averaging time (days)

This could be further refined if more detailed information is available for the microactivity (i.e., a specific mouthing event in a specific microenvironment) resulting in indirect ingestion over a given exposure period. In this case, total indirect ingestion exposure is estimated in two steps (Tulve et al. 2002): (a) individually for each microactivity, and/or (b) summed for all activities for an exposure duration of interest (i.e., 24-h).

For each microactivity resulting in indirect ingestion, exposure over a 24-h period can be defined as:

$$E_{\mathrm{nd}} = C_X \cdot \mathrm{TE}_X \cdot \mathrm{SA}_X \cdot \mathrm{EF} \tag{4.10}$$

where,

X: body, hand, surface, toy, or any other object that is mouthed
E_{nd}: indirect ingestion exposure from a specific mouthing event over a 24-h period (μa_x) (μg/day)
C_x: total contaminant loading on object x (μg/cm^2)
TEx: transfer efficiency, fraction transferred from object x to mouth
SA$_x$: surface area of object x that is mouthed (cm^2/event)
EF: frequency of mouthing events over a 24-h period (event/day)

The total indirect ingestion exposure over a 24-h period can be estimated by summing exposures for all microactivities. For any particular microenvironment being modeled, the potential exposure is the sum of all exposures for all microactivities conducted in that microenvironment (e.g., indoors, at home, on carpet).

4.2.3.3.3 Unintentional Swallowing of a Substance in a Product During Normal Use

Here, it is assumed that consumers incidentally ingest small amounts of a chemical substance in a consumer product. The best known application of this exposure scenario is the unintentional ingestion of personal care products (PCPs). Scenarios for ingestion of PCPs use information on amounts of products ingested daily and on chemical concentrations in such products. Usually detailed information on how much PCPs are ingested daily is not available; thus, a worst-case assumption could be used here: infants, toddlers, children, and female teenagers and adults ingest 50 mg product per day; male teenagers and adults ingest 25 mg product per day (Wormuth et al. 2006). The higher amounts ingested should reflect the more

careless use of PCPs by infants, toddlers and children and the more frequent use of PCPs by female consumers.

The mathematical formulation is

$$E_{\text{prod\_ing}} = \frac{C_{\text{prod}} \cdot q_{\text{prod\_ing}}}{\text{BW}} \cdot r_{\text{uptake}} \tag{4.11}$$

where,

$E_{\text{prod\_ing}}$: the internal exposure to chemical (μg/kg/day);
C_{prod}: Concentration of the chemical in the product (μg/mg)
$q_{\text{prod\_ing}}$: Amount of product ingested (mg/day)
r_{uptake}: absorbed fraction from the ingested quantity
BW: body weight (kg)

4.2.3.4 Dermal Exposure

Dermal exposure is determined by the processes involved in contact between the skin and the product or article. Since processes and exposure determinants differ largely between articles and products, different approaches are needed. Also within the category of products, a number of processes play an important role in dermal exposure, depending on the type of product and its use. For example, dermal exposure to substances in personal care products can be approximated by the applied dose and the application surface, whereas dermal exposure to substances in household products is not only affected by the amount of product used, but also depends on duration and type of contact between the product and skin during the application phase, and by the contact between skin and the surface on which the product is applied (post-application phase). Therefore, mathematical description of dermal exposure is split up for different types of products, and articles as a separate category.

Exposure through skin includes several mechanisms that relate to the different uses of industrial chemicals. Major mechanisms of dermal exposure include (Delmaar et al. 2005):

- *Instant application:* The instant application mode assumes that all compounds in the product are directly applied to the skin. This is the situation for personal care products, but can also be used as a first tier worst-case approach or if details on how the skin is exposed to the compound are not known.
- *Constant rate of application to the skin:* This mode of dermal loading describes a situation in which a compound is loaded onto the skin during a certain time, with a constant rate (e.g., when skin comes into contact with a clothing).
- *Rubbing off mechanism:* Contrary to the previous dermal exposure modes, the rubbing off mode describes a secondary exposure situation. Instead of direct application of a product to the skin, the rubbing off mode describes a situation

in which a surface (e.g., table top, floor) is treated with a product and dermal exposure arises from contact with the treated surface.
- *Exposure during showering or swimming pool*
- *Deposition of particles onto the skin*

4.3 Individual and Population Exposure Modeling

Exposure to chemicals is rarely characterized by regular, uniform events; thus exposure assessment needs to account for the frequency, duration and level (magnitude) of exposure (Nieuwenhuijsen 2003). Since the degree of exposure often varies with time, the period during which an exposure estimate is based can have a large influence on the result (Benford and Tennant 1997). Thus, exposure assessment may target either an individual, or the population at large; the latter is usually the target group of regulatory bodies.

4.3.1 Deterministic Exposure Modeling

Theoretically, there is no single risk for a particular exposure circumstance; rather, there are as many different risk values as there are individuals (Harper 2004). To overcome the problem of addressing variability in exposure and risk assessment, regulatory authorities have traditionally characterized the risks to individuals in a population who are likely to encounter the greatest exposure. The approach they have used, frequently referred to as a 'point-estimate' or 'deterministic' approach, uses single values to represent each exposure variable and produces a single risk estimate. In chemical risk assessments, initial screening of potential human health risks from chemicals of concern is often carried out by calculating 'worst-case', (a.k.a. 'high-end' or 'upper bound') point estimates of exposure using maximum or upper percentile values for exposure variables. In risk characterization, these point estimates of exposure are then combined with an appropriate toxicological end-point to determine whether a hypothetical 'worst' case individual exceeds the regulatory threshold of concern (or other calculated margins of safety). Where worst-case exposure estimates exceed regulatory thresholds, refined point-estimate exposure estimates (or 'best-case' estimates), are sometimes derived using average, mean or median values for exposure variables to provide a more realistic estimate of exposure.

The main advantages of using deterministic approaches for modeling exposure are that these are generally simple, quick and inexpensive and can be used as a screening tool for assessing chemical health risks. These approaches have, however, a number of disadvantages, which can undermine their use in regulatory decision-making. Deterministic approaches provide little information on the extent to which exposure or risk varies within a population or subgroup under investigation; certain

models are inflexible and do not allow different assumptions or scenarios to be considered and they can provide conservative or unrealistic exposure estimates. For example, it is not possible to determine from a 'worst-case' point estimate whether this represents an exposure likely to be encountered by the 95th, 99th or 99.999th percentile individual in a given population or is so extreme that it is unlikely ever to take place. If a high-end point estimate significantly exceeds the maximum (100th percentile) exposure likely to be encountered by a real population, it is likely to be highly unrealistic and provide an extremely conservative basis upon which to regulate safety to chemicals.

4.3.2 Probabilistic Exposure Modeling

To expand exposure assessment from the single individual to the wider population groups, probabilistic modeling techniques can be implemented (Bogen et al. 2009; Mutshinda et al. 2008; Zidek et al. 2005). Probabilistic analysis is an alternative approach used in exposure modeling which addresses the shortcomings of deterministic, point-estimate methods in terms of variability and uncertainty and produces more accurate and realistic estimates of exposure across the populations under investigation (Harper 2004). Depending on the availability and quality of data, distributions for any exposure variable relevant to a given exposure assessment scenario can be used in a probabilistic exposure model. In probabilistic modeling, distributions of exposure variables are combined in such a way as to give an exposure distribution. Exposure variables are also sometimes combined with toxicological endpoint levels to give risk distributions. Although there are several ways to combine exposure input distributions, the most common approach involves the use of a mathematical sampling technique called Monte Carlo simulation. The Monte Carlo technique, as applied to exposure assessment, involves combining the results of hundreds or thousands of random samplings of values from input distributions to produce an output distribution, which reflects the expected range and frequency of exposures.

Monte Carlo analysis is used to determine the probability of occurrence for the point estimates of a deterministic risk assessment and, in this way, deal with the uncertainty associated with these assessments (Hayes 2000). Whilst deterministic risk assessment applies a single value for each of the model's input parameters and calculates a single output value, probability risk assessment assigns a probability distribution to these input parameters, either as a probability density function, which is an analytical continuous function, or as a probability mass function, which is a discretized distribution. For a continuous random variable (*i*.e., a variable that can assume any value within some defined range), the probability density function expresses the likelihood that the value for a random sample will fall within a particular very small interval. Well known probability density functions are: normal, triangular, uniform and lognormal (Wilson et al. 2013).

A key part of developing a comprehensive probabilistic exposure model is to conduct a global sensitivity analysis of the exposure determinants. Sensitivity analysis is a technique that allows determination of the effect on the overall outcome of altering the value of one variable. The relative importance of each variable in determining the values of the output distribution can then be independently assessed.

4.3.2.1 Hierarchical Population Modeling Based on Bayesian Statistics

The Bayesian approach provides a formal way to incorporate prior knowledge on model parameters together with observed data in the modeling process. The analysis starts with the construction of prior probability distributions of the model parameters of interest, usually based on studies available in the literature. These distributions are then evaluated on the basis of their likelihood given observed data to compute posterior distributions of the model parameters. Hierarchical modeling with Bayesian Markov Chain Monte Carlo simulation is suitable for population exposure and internal dose (PBBK) models because the development of these models often involves non-linear processes, small datasets, high uncertainty, and biological variability (Bernillon and Bois 2000).

Markov Chain Monte Carlo methods are a class of algorithms for sampling from generic probability distributions (for more detailed information, see Robert and Casella 2004). A basic concept of the method is that of a Markov chain, i.e., a sequence of random variables:

$$Y_0, Y_1, Y_2, \ldots,$$

for which the distribution of the future state of the process, given the current and the past values, depends only on the immediately preceding state:

$$p(Y_{t+1} \mid Y_0, Y_1, \ldots, Y_t) = p(Y_{t+1} \mid Y_t)$$

Markov Chain Monte Carlo methods are based on the construction of a Markov chain that converges to the desired target distribution p (i.e., the one from which one wants to simulate from, for instance the unknown distribution of a parameter of interest). More formally, we say that p is the stationary distribution of the Markov chain. In most practical cases, after a sufficiently large number of iterations, referred to as "burn-in," the chain will forget the initial state and will converge to a unique stationary distribution, which does not depend on state t or Y_0. Once convergence is reached, it is possible to calculate any required statistic using Monte Carlo integration.

Figure 4.1 shows an intuitive representation of the process of convergence for a Markov Chain. Initially, the values sampled for two chains are dependent on the two different starting points. However, after the burn-in period, they tend to converge to the same distribution (this process is also known as mixing up). The first set of simulated values can then be discarded and the ones after convergence used as a

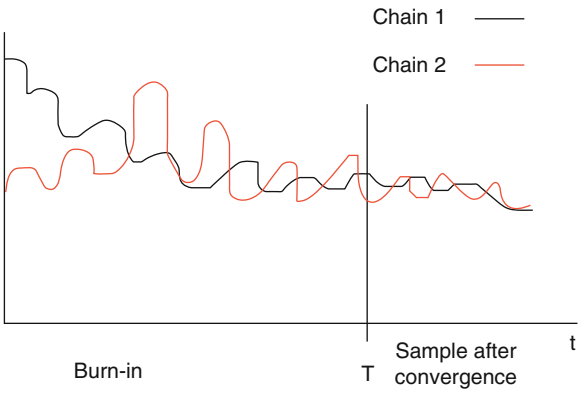

Fig. 4.1 A graphical representation of the process of convergence of Markov Chains: the two chains start from very different points but after the burn-in they converge to the stationary distribution

sample from the target distribution. One of the most popular Markov Chain Monte Carlo methods is Gibbs sampling (Geman and Geman 1984). The steps needed to perform the simulation via Gibbs sampling are schematically described in the following.

1. Define an initial value to be arbitrarily assigned to the parameter of interest. The sampling procedure starts from that value.
2. Perform a set of simulations during which the Markov chain converges to the stationary distribution, *i.e.* the required posterior. It is usually convenient to define more than one chain (two are generally sufficient), starting from distant initial values, to assess the convergence more efficiently (see Fig. 4.1).

Once convergence is reached (this process can be monitored by suitable statistics, such as that proposed by Gelman and Rubin (1996), a sample of values is drawn from the estimated target distribution. Using this sample all the inferences of interest can be performed; for instance, the whole distribution might be analyzed (i.e., by means of graphical methods, such as histograms or kernel density estimations), or point estimations such as the posterior mean or median can be computed.

4.3.2.2 Maximum Likelihood Estimates

Maximum likelihood estimation is a statistical method used for fitting a statistical model to data, and providing estimates for the model parameters. The method of maximum likelihood corresponds to many well-known estimation methods in statistics. Given a sample of some number of exposure attributes, but not the entire population, with knowledge that their values are normally distributed with some unknown mean and variance, the sample mean is the maximum likelihood estimator of the population mean, and the sample variance is a close approximation to the maximum likelihood estimator of the population variance.

For a fixed set of data and underlying probability model, maximum likelihood picks the values of the model parameters that make the data "more likely" than any

other values of the parameters would make them. Maximum likelihood estimation gives a unique and easy way to find a solution in the case of the normal distribution and many other problems, although in very complex problems this may not be possible. If a uniform prior distribution is assumed over the parameters, the maximum likelihood estimate coincides with the most probable values thereof.

4.3.3 Agent-Based Modeling

Using data fusion techniques, health and exposure data derived from fixed monitoring networks may be supplemented by a range of emerging novel techniques and technologies such as agent-based modeling, mobile phone apps, environmental sensor-webs, micro-sensors and satellite remote sensing. The information from the coupled use of agent-based models and sensor webs improves exposure modeling using deterministic and/or probabilistic approaches, and supports the application of new epidemiological and biostatistical methods to relate modeled exposure to health outcomes. The input to agent-based models consists of data relating to an individual's behavior within his/her environment (such as movement data within specific micro-environments) and between individuals exploring interactions around health-related behaviors and key risk determinants such as low socio-economic status. Using these parameters and the evolution of the virtual agents, simulations produce detailed information relating to the overall societal systems and populations considered. The estimated values produced can be used to fill the gaps of traditional datasets. This holistic approach is highly novel, taking the best from existing monitoring and sensor technology, but supplementing it with computational modeling. It is of particular relevance where real-world data are unavailable at the spatial and temporal scales that modeling complex exposures at the individual or population subgroup level requires. Although commonly used elsewhere, agent-based models and fusion methods have not been regularly applied in exposure assessment yet. This array of novel technologies, coupled with state-of-the-art fate modeling of chemicals will provide a complete and dynamic picture of external exposure to environmental chemicals in the near future supporting comprehensive, yet refined exposure and health risk assessment.

4.3.3.1 Improving Assessment of Activity Patterns: Use of Personal Sensors

Technological advances in recent years have produced sophisticated monitoring devices which can be carried or worn by a person during his/her regular daily routine, allowing for personal exposure to be monitored explicitly. Smartphone apps, wireless devices and the downsizing of monitoring technologies and costs make it possible for various environmental stressors and exposure factors to be measured more easily and frequently, thus providing a more reliable "time–

geography of exposure" shifting the current paradigm from population to individual level exposure.

Direct reading monitors help us to identify whether peak exposures are more important than average exposure values, identify specific exposure pathways that dominate in critical time windows over an individual's lifetime, and finally build individual exposure profiles. The advent of multiple sensor classes makes the use of sophisticated data and model fusion schemes necessary if the full potential of remote and personal sensing is to be harvested for improved cumulative exposure assessment. Such algorithmic schemes include the use of advanced statistical models such as random forest optimization, artificial intelligence techniques such as back-propagation artificial neural networks or data clustering techniques such as fuzzy set modeling.

Combining information on individual position with spatially resolved pollution levels allows assignment of pollutant concentrations to persons as they move through different microenvironments. Moreover, information on individual physical activity as tracked by personal sensors supports the estimation of breathing rates during different activities, which, in turn, translate into inhaled dose. The possibility to use personal sensors able to provide real-time data on air pollution exposure (CO_2, CO, NO_2, O_3, PM_x of different size fractions) has been explored by several investigators (Snyder et al. 2013; De Nazelle et al. 2013). If proven to be reliable, these sensors will constitute an added value to the array of remote sensing instrumentation building the sensor web of exposome related studies (Sarigiannis and Gotti 2014; Nieuwenhuijsen et al. 2014).

4.3.3.2 The Development of Space-Time-Exposure Trajectories

Time-Geography provides a coherent ontological framework within which to explore spatio-temporal behavior of individuals and their interaction with the environment. By analyzing and modeling these trajectories an individual's behavior can be determined in terms of time-geography, thus beginning to estimate individual level exposure. In Fig. 4.2 one sees conceptually how an individual can coincide spatially in X,Y and time, either with an environmental hazard prism (left) or vector (right).

In this ontological modeling framework an individual who resides in one place may be represented by a vertical line (a process in the time dimension alone) while horizontal lines show changes of place (processes in the spatial dimension as well). Time periods usually contain innumerable moves in space, which in turn create trajectories. By analyzing and modeling these trajectories, one could determine an individual's behavior in terms of time geography, and thus begin to estimate individual level exposure. With increasing access to individual residential history data, and computational power (e.g., exploring the possibilities offered by cloud-based and distributed computing), the time-geography approach has recently regained popularity in environmental health sciences. An example of this multi-layered data fusion coupled with agent-based modeling for the estimation of exposure to particulate matter through the ambient air is given graphically in Fig. 4.3.

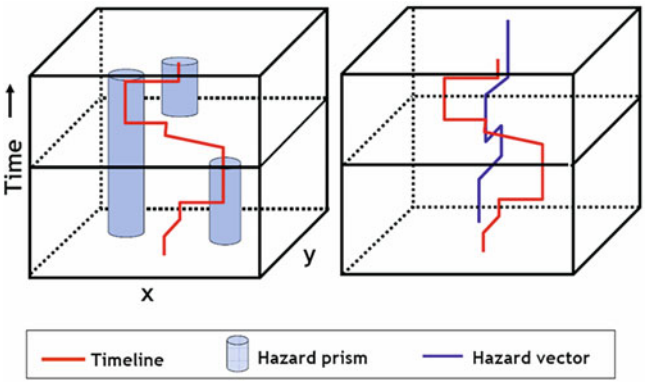

Fig. 4.2 Space-time trajectories through environmental hazard prisms (left) and vectors (right)

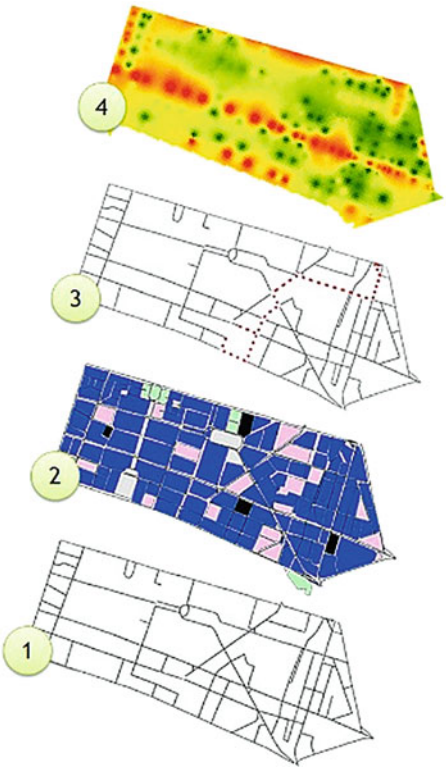

Fig. 4.3 Layer 1: road network, layer 2: buildings network, layer 3: agent's trajectory, layer 4: daily average PM concentration map

Administrative and spatially resolved infrastructure information such as the road and building networks in the area of interest (e.g., city, district) are used as knowledge substrates upon which the agent-based model estimates space-time trajectories of individual agents within the exposure time frame of reference. The emission and

environmental concentration model or the data fusion model that integrates multi-platform environmental monitoring data across the area of interest is then coupled with the space-time trajectories to reckon personal exposure estimates. Allowing the simulation to unfold and running it using a Monte Carlo algorithm to perturb the initial conditions stochastically within specified limits (determined to ensure plausibility of exposure scenarios) results in emerging patterns of behavior and corresponding combined exposure burden to the pollutants of interest. Exposure estimates are differentiated by type of population subgroup modeled, and time-dependent exposure profiles for characteristic individuals can be drawn; the method gives an explicit account of the residual uncertainty and variability in exposure profiles.

4.3.4 Critical Time Windows of Exposure

Vulnerability (defined here as variations in exposure between individuals or groups) and susceptibility (the degree to which individuals or groups may respond to a given exposure) related to complex exposure vary significantly during an individual's lifespan. Thus, it is of great importance to identify the critical periods and the types of complex exposures that require special attention during certain life stages. Within the frame of the HEALS[2] (Health and Environment-wide Associations based on Large population Surveys) project, ten critical periods of exposure were identified (Table 4.1). Critical periods include early developmental stages such as preconception, the three trimesters of pregnancy and the age before and after 3 years of age. Puberty is a period with significant hormonal alterations, and as such, it has been proven to be crucial for asthma, weight and behavioral variations. Middle age lifestyle parameters (e.g., nutrition, exercise, smoking), health status (hypertension, diabetes) and use of drugs are determinant for the onset and the progress of

Table 4.1 Critical periods of exposure

Preconception
1st semester of pregnancy
2nd semester of pregnancy
3rd semester of pregnancy
3rd year of age
Puberty
Middle age
Menopause
Age of 50
Age of 65
Age of 80

[2] www.heals-eu.eu.

neurodegenerative diseases. Change in lifestyle choices after the age of 30 introduces new conditions that increase the risk of metabolic disorders that may eventually lead to obesity and type 2 diabetes. Menopause in women (between 45 and 55 years of age) is a period of significant change in the hormonal system, related to a cascade of effects, asthma, and increased susceptibility to metabolic disorders, as well as to neuroinflammation. At the age of 50 significant changes in gene expression involved in brain-related function seem to be determinant for the onset of neurodegenerative disorders. After 65 years both males and females are more susceptible to environmental insults, due to reduced detoxification capacity, as well as reduced capacity of maintaining homeostasis. After 80 and 85 years, normal ageing is accompanied by pathological ageing.

As a general rule, for stages related to development or significant hormonal changes, assessment of complex exposures should be more focused on endocrine disrupting chemicals (EDCs), including several chemical classes, (e.g. PCBs and dioxins, phthalates, BFRs), each of which includes multiple individual mixture components. To properly account for these compounds, multiple pathways and exposure routes have to be addressed; their relative importance is also age dependent. At later stages, complex air quality mixtures (PAHs, BTEX, CO, NO_x ozone and PM_x) that relate to oxidative stress (and the related cascade of effects) are more important than exposure to EDCs. Thus, modeling efforts of complex exposures should account for the specific needs of the critical windows of exposure that pertain to the individuals or the population at risk.

4.4 Internal Exposure Modeling of Real Life Chemical Mixtures

4.4.1 Overview of Physiology Based BioKinetic (PBBK) Models

PBBK models are continuously gaining ground in regulatory toxicology, describing in quantitative terms the absorption, metabolism, distribution and elimination processes in the human body, with a focus on the effective dose at the expected target site (Bois et al. 2010). This trend is further amplified by the continuously increasing scientific and regulatory interest about aggregate and cumulative exposure; PBBK models translate external exposures from multiple routes (Yang et al. 2010) into internal exposure metrics, addressing the effects of exposure route in the overall bioavailability (Sarigiannis and Karakitsios 2011; Valcke and Krishnan 2011) or the dependence on critical developmental windows of susceptibility, such as pregnancy (Beaudouin et al. 2010), lactation (Verner et al. 2008) and infancy (Edginton and Ritter 2009). With regard to cumulative exposure, PBBK models offer the advantage of calculating the effect of the interactions among the mixture compounds at the level of metabolism, however due to the inherent difficulties arising, the existing

applications are currently limited mainly to VOCs (Haddad et al. 2000; Sarigiannis and Gotti 2008) and metals (Sasso et al. 2010). Recently, efforts have shifted towards the integration of whole-body physiology, disease biology, and molecular reaction networks (Eissing et al. 2011), as well as integration of cellular metabolism into multi-scale whole-body models (Krauss et al. 2012).

The use of internal dose modeling aims at integrating exposure data and modeling output with human biomonitoring data. Its goals are to (a) provide the time history of the exposure profile, focusing on susceptible developmental stages; (b) assimilate the biomonitoring data related to the cohorts to estimate the individual exposome in quantitative terms; and (c) derive reliable biologically effective dose values for the compounds of interest so that they can be associated to observed health outcomes. The key component of the above is the development of a lifetime (including gestation and breastfeeding) generic PBBK model (Sarigiannis and Karakitsios 2012) incorporating mixtures interaction (Sarigiannis and Gotti 2008) and a framework for biomonitoring data assimilation (Georgopoulos et al. 2008b). Aiming to expand the applicability of the generic PBBK model to cover the chemical space as much as possible, parameterization of the model for known and new chemicals with limited information is done through the development of QSAR models. The generic PBBK model will also be used to reconstruct exposure from human biomonitoring data (Andra et al. 2015). A tiered approach will be followed as a function of data availability (periodicity and size of sampling, specimen type) and requirements of the exposure reconstruction analysis (temporal analysis of exposure, contribution from different routes), ranging from Exposure Conversion Factors (Tan et al. 2006), up to Markov Chain Monte Carlo analysis. Inputs involve spatial and temporal information on micro-environmental media concentrations of xenobiotics and corresponding information on human activities, food intake patterns or consumer product use that results in intakes; outputs are the observed biomarkers; and the error metric can be defined in terms of population variation (the latter has to be lower than the intra-individual variation, which may be associated with measurement or other random error source). On the individual level, PBBK will be combined with multimedia models and survey questionnaires to identify exposure sources. PBBK modeling will also be used to estimate the internal doses of xenobiotics that exceed levels associated with biological pathway alterations (Judson et al. 2011) and, eventually, health risk. The latter can involve the use of specific omics results (e.g., metabolomics analysis) and associations of biologically effective doses to early biological responses. In addition, biologically effective doses would be used to quantify the effect of compound-induced extracellular perturbations on metabolic states, so as to directly couple the PBBK model with metabolic regulatory networks. Direct coupling defines a feedback loop that connects clearance and metabolite production rates to metabolism regulation (Eissing et al. 2011) via dynamic flux balance analysis (Krauss et al. 2012).

Considering the opportunities offered by the use of PBBK models in exposure/risk characterization, several research groups are developing generic PBBK models, either as stand-alone models such as PK-Sim (Willmann et al. 2003) and IndusChem (Jongeneelen and Berge 2011), or incorporated within integrated computational platforms for exposure assessment such as INTERA (Sarigiannis et al. 2011)

and MENTOR (Georgopoulos et al. 2008c). The development of generic PBBK models is substantiated by the recent advances in quantitative structure–activity relationships (QSARs) and quantitative structure–property relationships (QSPRs) (Price and Krishnan 2011; Peyret and Krishnan 2011), providing the basis for development of relevant PBBK models for data-poor or new chemicals.

The INTEGRA methodology is advancing the existing state of the art by integrating all of the above elements, with a plan to develop a generic lifetime (including pregnancy) (Sarigiannis and Karakitsios 2012) multi-route PBBK model. The integration of this generic PBBK model into a wider modeling framework will allow forward (internal exposure) or reverse calculations (exposure reconstruction) so as to provide the link among exposure components and biomonitoring data. Additional elements of using physiologically based modeling to understand the kinetics and effects of chemical mixtures are covered in Chap. 12.

4.4.2 Internal Dosimetry Models

PBBK models are tools that describe the mechanisms of absorption, distribution, metabolism and elimination of chemicals in the body resulting from acute and/or chronic exposure regimes. They are independent structural models, comprising the tissues and organs of the body with each perfused by, and connected via, the blood circulatory system. In PBBK models the organism is frequently represented as a network of tissue compartments (e.g., liver, fat, slowly perfused tissues, and richly perfused tissues) interconnected by systemic circulation. A generic PBBK model, reflects the incorporation of basic physiology and anatomy. The compartments actually correspond to anatomic entities such as liver, lung, etc., and the blood circulation conforms to the basic mammalian physiology. The primary means of transport for xenobiotic chemicals that enter the body through one or more of these routes is via blood, the main vehicle for nutrient supply and waste removal from tissues. In the basic PBPK model, transport of chemicals between blood and tissues is assumed to be flow-limited, which implies that the transport barriers between the free molecules of chemical in blood and tissue are negligible, and equilibration between free and bound fractions in blood and tissue is rapid. Concentrations of chemical in venous blood exiting a tissue, and tissue concentrations are assumed to be at equilibrium, and the tissue is assumed to be homogeneous with respect to the concentration of the chemical. The flow-limited assumption is usually appropriate for lipophilic or low molecular weight compounds, which easily partition or diffuse through cell membranes. Every PBBK model requires several parameters that are critical determinants of chemical uptake and disposition. These determinants can be classified into three main categories, namely, anatomical/physiological, physicochemical, and biochemical. A partial list of anatomical/physiological parameters includes cardiac output, tissue blood flow rate, organ and tissue weight and volumes. In addition to physiological/anatomical data, PBBK models require information on the ability of the body to metabolize chemicals – these are known as biochemical

parameters. Typical biochemical parameters include the maximal velocity for metabolism (V_{max}), binding association constant (Kb) and Michaelis affinity constant (K_m). The third type of data required by these models is the solubility of pollutants in the organs and tissues of the body. These are physicochemical data known as partition coefficients (P). Partition coefficients are experimentally determined parameters that give an indication of the distribution of a chemical between two different phases, e.g. air and blood, blood and liver, blood and muscle, blood and fat, etc. The fundamentals of PBBK modeling are to identify the principal organs or tissues involved in the disposition of the chemical of interest and to correlate the chemical absorption, distribution, metabolism, and excretion within and among these organs and tissues in an integrated and biologically plausible manner.

A scheme is usually formed where the normal physiology is followed in a graphical manner. Within the boundary of the identified compartment (e.g., an organ or tissue or a group of organs or tissues), whatever inflows must be accounted for via whatever outflows or whatever is transformed into something else. This mass balance is expressed as a mathematical equation with appropriate parameters carrying biological significance. A generic equation, for any tissue or organ, is:

$$V_i \frac{dC_{ij}}{dt} = Q_i\left(\mathrm{CA}_j - \mathrm{CV}_{ij}\right) - \mathrm{Metab}_{ij} - E\mathrm{lim}_{ij} + \mathrm{Absorp}_{ij} - \mathrm{PrBinding}_{ij} \quad (4.12)$$

where V_i represents the volume of tissue group i, Q_i is the blood flow rate to tissue group i, CA_j is the concentration of chemical j in arterial blood, and C_{ij} and CV_{ij} are the concentrations of chemical j in tissue group i and in the effluent venous blood from tissue i, respectively. Metab_{ij} is the rate of metabolism for chemical j in tissue group i; liver, is the principal organ for metabolism and, with some exceptions, Metab_{ij} is usually equal to zero in other tissue groups. Elim_{ij} represents the rate of elimination from tissue group i (e.g., biliary excretion from the liver), Absorp_{ij} represents uptake of the chemical from dosing (e.g., oral dosing), and $\mathrm{PrBinding}_{ij}$ represents protein binding of the chemical in the tissue. All these terms are zero unless there is definitive knowledge that the particular organ and tissue of interest has such processes.

A series of similar mass balance differential equations representing all of the interlinked compartments are formulated to express a mathematical representation, or model, of the biological system. This model can then be used for computer simulation to predict the time course behavior of any given parameter in the model. See Chap. 12 for more information on PBBK development.

The generic model developed in INTEGRA is designed to describe as closely as possible the actual absorption, distribution, metabolism and elimination processes occurring in the human body, so that it can be easily applicable for a broad variety of chemicals assuming proper parameterization. The model includes the parent compounds and at least three potential metabolites for each of the compounds in the mixture. For each compound/metabolite all major organs are included and the link among the compounds and the metabolites is through the metabolizing tissues. This is mainly the liver, but also other sites of metabolism (e.g., gut, skin) might be

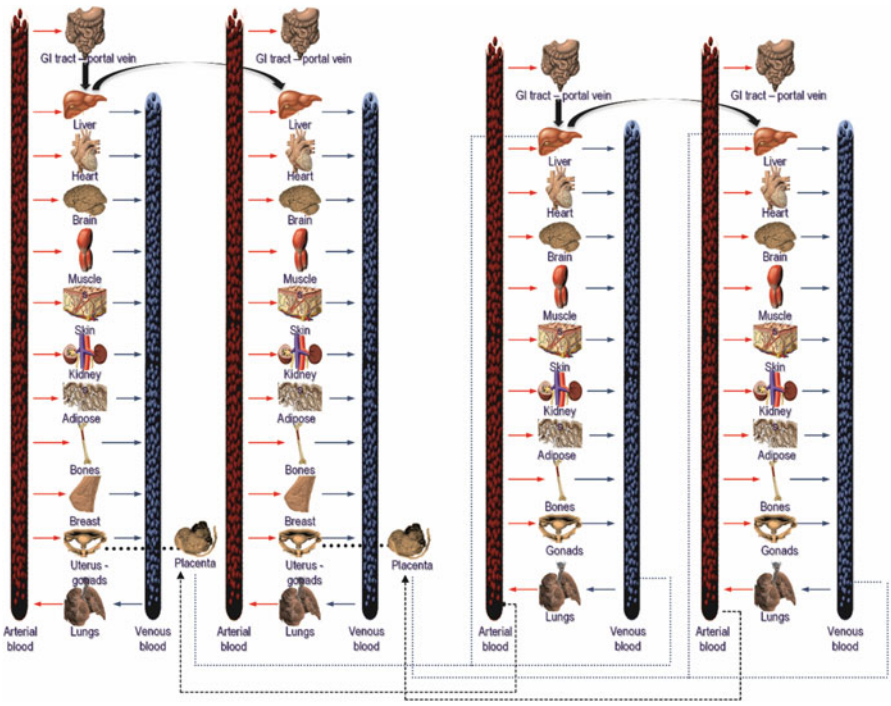

Fig. 4.4 Conceptual representation of the Mother-Fetus PBBK model, including both the parent compound and one metabolite

considered based on the presence of the enzymes involved in the metabolism of the compound of interest. To capture in utero exposure, the model is replicated to describe the functional interaction of the mother and the developing fetus through the placenta (Fig. 4.4). The anthropometric parameters of both the mother and the fetus models are age-dependent, so as to provide a life stage-dependent internal dose assessment.

4.4.3 Expanding the Chemical Space to Assess Internal Dose for Multiple Chemicals

A critical limiting factor in describing ADME processes accurately for a large chemical space is the proper parameterization of PBBK models for "data poor" compounds. Advanced Quantitative Structure-Activity Relationships (QSARs) can be used to predict input parameters for these models allowing PBBK models to cover a large number, and several classes, of chemicals. In silico approaches, including QSARs, are widely used for the estimation of physicochemical and biochemical properties and predicting how they might lead to biological responses (Puzyn et al.

2010). QSARs are described as regression or classification models, which form a relationship between the biological effects and chemistry of each chemical compound (Puzyn et al. 2010). Significant progress in expanding the chemical space for industrial chemicals has been made by the INTEGRA project, where parameterization of essential parameters such as blood:tissue partition coefficients for several tissues, maximum initial velocity of the enzyme catalyzed reaction (V_{max}) and the substrate concentration that gives half maximal velocity of an enzymatic reaction (K_m or Michaelis-Menden constant) has been carried out for a large number of chemicals. The mathematical formulation coupled Abraham's solvation equation with Artificial Neural Networks of variable geometry in order to optimize the performance of the model. Abraham's solvation equation (Linear Free Energy Relationship) describes the process of the transfer of chemicals from the liquid phase to a large number of solvents or other condensed phases, including biophases. The descriptors, which characterize these physicochemical and biochemical phenomena, are combined into Eq. 4.13,

$$\log SP = c + e \cdot E + s \cdot S + a \cdot A + b \cdot B + v \cdot V \tag{4.13}$$

Where SP is a biological property for a set of chemicals in a given system. The independent descriptors are the properties of the examined chemicals, E is the excess molar refractivity of the chemical, S is the chemical's dipolarity/polarizability, A and B are the chemical's effective or summation hydrogen bond acidity and basicity, respectively, and V is the McGowan characteristic volume of the chemical (Abraham 1993; Payne and Kenny 2002). The coefficients c, e, s, a, b and v reflect the properties of chemicals, so e corresponds to the tendency of the chemical to interact with solute π- and n- electrons, s corresponds to the chemical's dipolarity/polarizability, a and b correspond to the chemical's hydrogen bond basicity and acidity, respectively, and v is a measure of the chemical's lipophilicity. Artificial Neural Networks were used to develop a non-linear model based on Abraham's solvation equation.

The calculated values of metabolic constants using the statistical method described above (Abraham's solvation equation coupled with Artificial Neural Networks) were compared to experimental values and the results obtained by Price and Krishnan (2011) in Fig. 4.5. The methodology followed by Price and Krishnan (2011) was based on the group contribution method, implying that each fragment in the molecular structure contributes to the metabolic parameters, depending on its frequency of occurrence in the given molecule (Gao et al. 1992). In previous studies, the parameters used to describe the interactions between chemicals and tissues were mainly related to chemical structure or tissue composition in water, proteins and lipids Price and Krishnan 2011; Zhang 2004). In the present example, Abraham's equation descriptors are not linked directly with tissue composition. They encode specific chemical information regarding the size, polarizability and hydrogen bonding of the examined chemicals and each term can reveal the factors that influence a particular interaction. The modeling results indicate that the molecular descriptors of the equation can be suitable for the estimation of the parameters that characterize

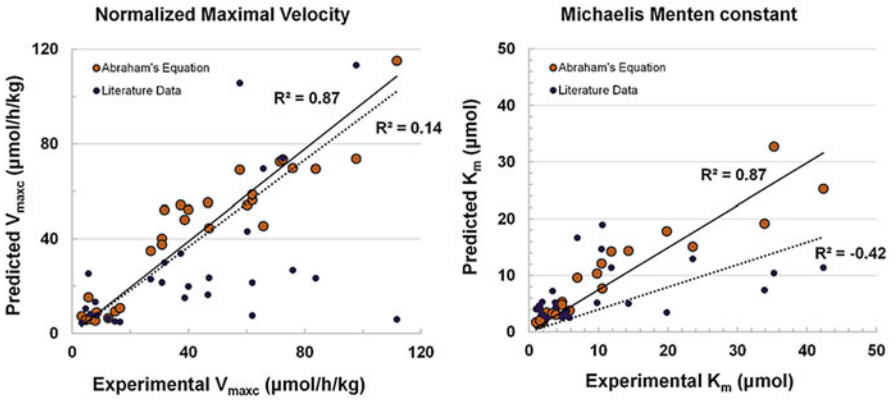

Fig. 4.5 Predicted vs experimental values of normalized maximal velocity and Michaelis – Menten constant under Abraham's equation (orange dots) and a group contribution method (Literature data; blue dots)

relevant physicochemical and biochemical phenomena. The improved performance of Abraham's equation compared to the group contribution method can be attributed to its capacity to represent the complex interactions of the micro-processes of chemicals' distribution and metabolism into several tissues.

4.5 Complex Exposure Modeling Using Human Biomonitoring Data

4.5.1 Overview of Biomonitoring

The main achievement of human biomonitoring is that it provides an integrated overview of the pollutant load to which an individual is exposed, and hence serves as an excellent approximation of aggregate exposure including all pathways, mechanisms and routes of exposure. For additional information on biomonitoring and its utility in measuring exposure to mixtures, see Chap. 2. The internal dose of a chemical, following aggregate exposure has a much greater value for environmental health impact assessment as the internal body concentration is much more relevant to the impact on human health than mere exposure data. However, it needs to be stressed that HBM in itself cannot replace environmental monitoring and modeling data. At the same time, mathematical approaches to describe the pharmacokinetic and toxicokinetic behavior of environmental agents (i.e. PBBK models) offer a more mechanistic insight into the behavior and fate of environmental agents following exposure. As biomarker data also reflect individual ADME characteristics of chemicals, HBM data offer an excellent opportunity to validate PBBK models. Ultimately, coupling both lines of evidence to assess exposure proves to be the

optimal solution towards relating complex exposure to environmental stressors to potential adverse health effects assessment.

There are three approaches for linking biomonitoring data to health outcomes: direct comparison to toxicity values, forward dosimetry, and reverse dosimetry. Biomonitoring data can be directly compared to toxicity values when the relationship of the biomarker to the health effect of concern has been characterized in the human. In forward dosimetry, pharmacokinetic data in the experimental animal can be used to support a direct comparison of internal exposure in humans derived through the application of PBBK models, providing an estimate of the Margin of Safety in humans. It is possible to determine the relationship between biomarker concentration and effects observed in animal studies. An evolution of this concept is the biomonitoring equivalents. Alternatively, reverse dosimetry can be performed to estimate the external exposure that is consistent with the measured biomonitoring data through the backward application of PBBK models. In a more elaborate scheme, the reconstructed exposure could be used to run the PBBK model in forward mode, so as to estimate the biologically effective dose at the target tissue.

4.5.2 Exposure Reconstruction in Practice

Human biomonitoring typically is an integrative measure of different exposure episodes along various routes and over different time scales; thus, it is often difficult to reconstruct the primary exposure routes from human biomonitoring data alone. This uncertainty limits the interpretative value of biomarker data. However, several mathematical approaches have been developed to reconstruct exposures related to population biomonitoring studies, and can be subdivided into a number of different approaches. Exposure reconstruction techniques combined with PBBK models can be divided into Bayesian and non-Bayesian approaches (Georgopoulos et al. 2008a). Moreover, computational inversion techniques (and exposure reconstruction techniques as well), can be classified as deterministic or stochastic (Moles et al. 2003) based on the identification of a global minimum of the error metric, the input parameters and the model setup.

The deterministic methods aim to achieve convergence on a global minimum. The problem is solved using an "objective function" based on biomarkers. Additionally, constraints in the form of bounds, equalities and inequalities are incorporated. Deterministic models have been used in several biological applications using different methods. Muzic Jr and Christian (2006) have applied a regression technique to estimate pharmacokinetic parameters. A gradient method has been used by Isukapalli et al. (2000) to calculate the uncertainty in PBBK models. A maximum likelihood method has been carried out for short- and long-term exposure reconstruction using a PBBK model for chloroform (Roy et al. 1996).

In contrast, stochastic methods aim to provide a reasonable solution, not a mathematically optimal one. A probabilistic framework for the inverse computation problem is the Bayesian approach, which is based on Bayes' theorem. According to

the methodology developed in the frame of the INTEGRA project, the analysis of exposure reconstruction problems based on the Markov Chain Monte Carlo and Differential Evolultion Markove Chain technique is realized according to the following steps:

1. The process starts from exposure related data which are fed into the INTEGRA exposure model;
2. This in turn provides input to the PBBK model, taking into account the duration and the magnitude of exposure from all exposure routes (inhalation, skin and oral route);
3. The result of the PBBK model simulation (also taking into account the distribution of PBBK parameters, e.g., inter-individual variability in clearance), is then evaluated against the human biomonitoring data distributions. Based on the outcome of the comparison, the optimization algorithm changes the exposure model input parameters after each iteration, so as to achieve convergence to biomonitoring data;
4. More detailed information on exposure parameters reduces uncertainty in back-calculating doses from biomarker information, resulting in faster and more efficient convergence;
5. Several iterations are repeated, until the error between the predicted and the actual biomonitored data is minimized.

The Bayesian Markov Chain Monte Carlo technique described above simulates and calculates the investigated exposure conditions. The sampling scheme is set appropriately according to the problem and to the available data for the proposed function. The flowchart of the overall process is shown in Fig. 4.6.

4.6 Case Studies of Complex Mixtures Modeling

4.6.1 Exposure Assessment of Indoor Air Complex Mixtures

Indoor air is one of the most typical examples related to complex exposures. The combination of building materials (e.g., paint, floors, doors and windows), consumer products (e.g., electronic devises, furniture, carpets) and activities (e.g., biomass combustion, smoking, cooking) creates a variable and complex mixture of chemical and biological health stressors (e.g., mold, pollen). The multitude of compounds found in indoor air (Sarigiannis 2014), as well as the respective health risks are graphically illustrated in Fig. 4.7.

Modeling complex exposures in the indoor environment requires a virtual reconstruction of the actual environmental setting of interest. This implies the virtual reconstruction of the indoor environment, including all potential emission sources. After calculating emissions, the next step is to calculate indoor concentrations in the three media of exposure relevance, meaning gaseous phase, particles and settled dust. The latter is of particular interest, since based on the physicochemical

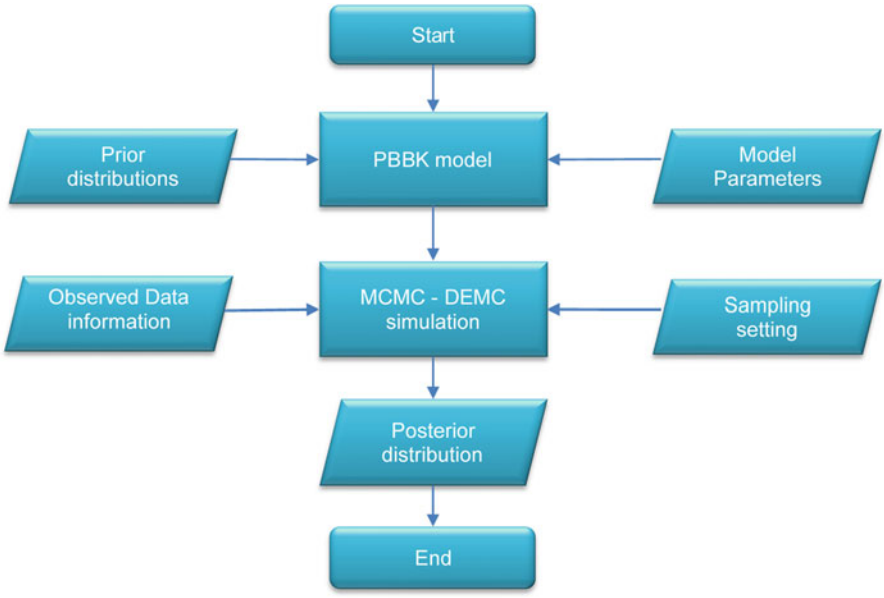

Fig. 4.6 Exposure reconstruction flowchart

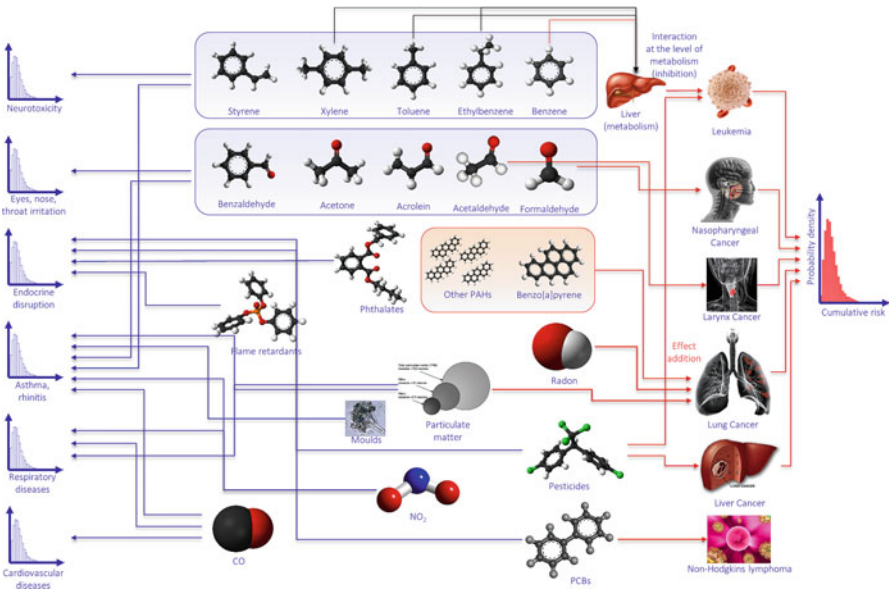

Fig. 4.7 Multiple stressors found in indoor environment and related health endpoints

properties of the compound (e.g., K_{ow} and Henry constant), significant differences in the respective phase distribution are expected; more volatile compounds such as aromatics and aldehydes are found only in the gaseous phase, semi-volatile compounds (e.g., phthalates) tend to distribute in all phases, while heavier and more lipophilic compounds (e.g. PBDEs) are found mostly in dust (Weschler and Nazaroff 2010; Weschler and Nazaroff 2008). In turn, the way compounds are distributed in different phases determines the pathways and routes of exposure involved, e.g., non-dietary ingestion and inhalation respectively. This allows the proper estimation of external exposure, which in turn provides input to the internal exposure model. The full series of calculations, starting from emissions, calculating indoor environmental levels, exposure and internal dose for many chemicals can be performed with the INTERA computational platform[3] (Sarigiannis et al. 2012a), an open access online computational platform running via the world wide web at the Centre for Research and Technology Hellas (CERTH). A special case of complex exposure in the indoor environment is tobacco smoke. During smoking, several compounds are emitted, including particles and organic compounds such as alkenes, nitrosamines, aromatic and heterocyclic hydrocarbons and amines. Some of these compounds are emitted from several other sources as well; thus, it is not always easy to attribute poor indoor air quality to cigarette side stream smoke. However, nicotine, serves as a unique marker of exposure to environmental tobacco smoke. Nicotine is rapidly metabolized to cotinine upon entering the human body, which is excreted through urine. Urinary cotinine serves as an exposure biomarker to environmental tobacco smoke. Using complex exposure modeling the amount of cotinine found in urine could be used as a starting point for reconstructing exposure to nicotine. This would allow identification of exposure levels and in turn the indoor concentration of nicotine that resulted in the observed biomonitored cotinine levels (Sarigiannis et al. 2009). By continuing the reverse calculation, smoking intensity is estimated. At this point, estimated smoking intensity can be used to estimate the emissions and concentration levels for the hundreds of compounds present in environmental tobacco smoke...

Exposure reconstruction of urinary cotinine levels allows us to further identify the exposure and effects of individual carcinogenic compounds e.g., benzene, formaldehyde, Nicotine-derived nitrosamine ketone (NNK), B[a]P and their interactions at different levels among the individual compounds (Sarigiannis et al. 2009):

- Interaction at the level of metabolism (using PBBK modeling) among benzene, toluene, ethylbenzene and xylene
- Effect summation of lung cancer related to NNK and B[a]P
- Independent action in terms of cumulative cancer risk (at different sites) among benzene (leukemia), formaldehyde (nasopharyngeal cancer), NNK and B[a]P (lung cancer)

[3] http://www.intera.cperi.certh.gr/auth/login.

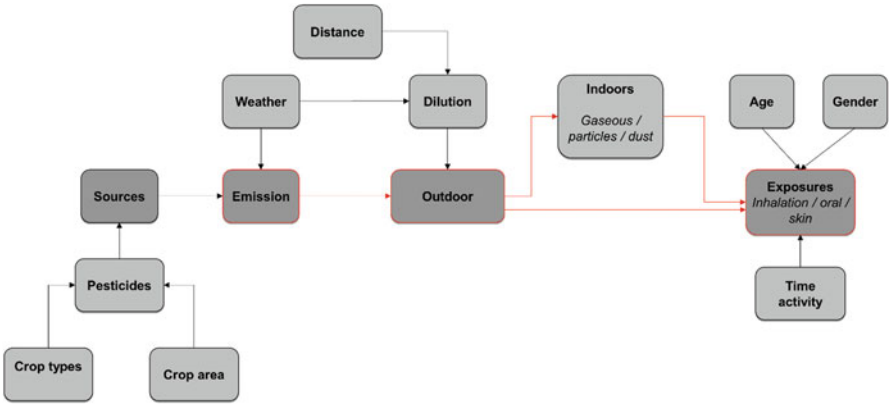

Fig. 4.8 Full chain complex exposure assessment from pesticides

4.6.2 Pesticides: Multi-pathway and Multi-route Exposure by Chemical Class

Complex exposure to pesticides for bystanders has been computed on the European scale at a very high spatial resolution using a multi-compartment model, the information flow of which is depicted in Fig. 4.8. The modeling methodology has three main components: (1) modeling the emission of active substances (AS) (i.e., AS emissions to air per km^2 extracted from the emission inventory), (2) modeling the fate and transport of the AS in the environment to estimate concentrations (expressed in computed AS concentration per hour in 1 year), and (3) modeling population exposure (expressed as intake computed from daily average AS concentrations) differentiated by age and gender for all AS. The overall model is spatially resolved and all estimates are given at a pan-European 0.1 × 0.1 km grid.

The multi-step methodology used included the following steps:

1. Starting from the emission inventory, annual emission data per AS were extracted for 25 EU Member States.
2. A typical emission profile was used in accordance to the local agriculture practices for a time window that coincided with the growing season in each country/region.
3. A pesticide dispersion model was developed to compute concentration into the ambient air at a 1 × 1 km grid using as input the variable emission profile, local meteorological data and AS physicochemical characteristics.
4. Outdoor to indoor penetration modeling was used to estimate the indoor concentration of AS and its partitioning among the different phases (gaseous, particles and settled dust).
5. An exposure model was developed, based on which intake rates per population group differentiated by age and gender (i.e., adult male-female, children 0–4 yr, 5–9 yr and 10–14 yr) was computed comprising all exposure pathways

(inhalation of gaseous and particles, dust ingestion, particles deposition on skin, dust rubbing off) and routes. The effect of changing the daily duration of pesticide application, the total application window as well as uncertainty in the meteorological conditions and variability in the physiological parameters were incorporated in the assessment.

The pesticide release inventory model (Sarigiannis et al. 2013) comprised five crop types (three seasonal and two permanent), the list of pesticides used per crop and their usage quantities at the country level (source: Eurostat 2011), a pesticide disaggregation algorithm to distribute quantities at the grid and the computed annual emission data based on wind drift, volatilization during application and from the crop canopy. This inventory model was based on crop data extracted from the Common Agricultural Policy Regionalised Impact (CAPRI) Modeling System (CAPRI 2012).

According to the methodology followed to create this pesticide inventory (Sarigiannis et al. 2013), usage quantities for each AS per cell and crop were disaggregated from the country level to the 1 × 1 km grid, using an area weighing algorithm and assuming a constant annual 'area reduced dosage' per AS for each cell in the same country. The 'area reduced dosage' is a measure of pesticide use per crop area based on country data, and incorporates estimates of uncertainty in the actual crop area to which a specific AS is applied within the spatial grid. Average annual emissions, ER_{ijk},(in kg/yr), of each AS applied in a field to the air of an AS i applied on crop j for a country k, at short range from the site of application were computed from the sum of spray wind drift D_{ijk}, volatilization during application $E_{\text{app,air},ijk}$ and volatilization from the crop canopy $E_{\text{crop,air},ijk}$ as shown by Eq. 4.14.

$$\text{ER}_{ijk} = D_{ijk} + E_{\text{app, air}, ijk} + E_{\text{crop, air}, ijk} \tag{4.14}$$

The annual emission data generated from Eq. 4.14 per grid cell were fed to the concentration model described in the following sections, assuming typical emission profiles.

The pesticides were prioritized on the basis of a hazard factor that accounts for both toxicity and persistence in the environment based on the methodology of Gunier et al. (2001). According to this methodology, the hazard factor (HF) is multiplied by AS quantities and then the AS with the highest score (HF × quantity) from each group (i.e., herbicides, fungicides, insecticides, other) was selected for more detailed presentation. The top chemicals in each group were glyphosate (herbicide), chlorpyrifos (insecticide), mancozeb (fungicide) and 1,3-dichloropropene (other). 1,3-dichloropropene has one of the largest hazard factors due to high toxicity and high volatilization flux.

In practice, application periods are limited to 1–3 months during the year and correspond to specific crop types, climatic conditions and agricultural practices that differ among countries, even among regions. It is assumed that the applicators of pesticides and farmers use the total quantity of pesticides in a specific time period, regardless of weather conditions to render the assessment conservative. Therefore, the annual quantity of AS i for crop j for a country k (ER_{ijk}) is applied, in the form of

a 'pulse' with a 10-h period for a total window of several months, in accord with the estimated country annual emission estimates and the typical AS uses. Moreover, since application practices in Europe vary, pesticide drift does occur in many cases, differentiated between primary drift, off-site movement of spray at the time of application, and secondary drift associated with pesticide vapor. The effects of pesticide drift were included in this assessment via the AgDrift (Teske et al. 2002) and AgDisp (Bird et al. 2002) models. They were used to evaluate the average deposition fraction (i.e., implicitly drift), under different operational and environmental conditions, focusing in particular, on the droplet size in accordance, to the ASAE S572 standard (very fine <150 μm, fine 150–250 μm, medium 250–350 μm and coarse 350–425 μm), the wind speed, the temperature and the relative humidity. Hence, changes in emissions over time were deduced and used as input to the concentration model.

A critical step for calculating exposure was the estimation of outdoor concentrations. The concentration model employed at each cell was of a box-volume form, described by the differential equation:

$$V \cdot \left(\frac{dC_{ijk}}{dt}\right) = \mathrm{ER}_{ijk} - C_{ijk} \cdot I \cdot V - K_i \cdot C_{ijk} \cdot V \tag{4.15}$$

where C_{ijk} is the concentration of an AS i applied on crop j for a country k, in g/m^3, ER_{ijk} is the average emission rate of an AS i, in g/h during application, I is the air changes per hour in the volume (i.e., $I = u/L$ with u the average wind speed in m/s, L the lateral distance covered in m), V is mixing volume, in m^3 (i.e., $V = L^2 \cdot H$, with L the lateral distance, in m and H the mixing height, in m), t is the time, in h, Ki is the decay rate of an AS i, in h^{-1} (i.e., $Ki = ln2/(\mathrm{HL}_i)$, with HL_i the half life in air of an AS i, in h). The following solution of Eq. 4.15 is obtained for discrete time steps Δt:

$$C_{ijk}(t) = \frac{1}{u/L + \ln 2/\mathrm{HL}_i} \cdot \left(\frac{\mathrm{ER}_{ijk}(t)}{L^2 \cdot H}\right) \cdot \left(1 - \exp\left(-\left(\frac{u}{L} + \frac{\ln 2}{\mathrm{HL}_i}\right) \cdot \Delta t\right)\right) + C_{ijk}(t-1) \cdot \exp\left(-\left(\frac{u}{L} + \frac{\ln 2}{\mathrm{HL}_i}\right) \cdot \Delta t\right) \tag{4.16}$$

In addition, when the application rate is zero, Eq. 4.16 becomes,

$$C_{ijk}(t) = C_{ijk}(t-1) \cdot \exp\left(-\left(\frac{u}{L} + \frac{\ln 2}{\mathrm{HL}_i}\right) \cdot \Delta t\right) \tag{4.17}$$

Pesticides in the particle phase were also estimated. This calculation was based on the partition coefficient K_p between gaseous and particles phase based on the Pankow model (Pankow 1994):

$$K_p = \frac{N_s \cdot \alpha_{\mathrm{TSP}} \cdot T \cdot e^{\frac{(Q_1 - Q_v)}{R \cdot T}}}{1600 \cdot p_L^{\circ}} \tag{4.18}$$

where N_s (cm^{-2}) is the available surface for adsorption, a_{tsp} ($m^2\ g^{-1}$) is the special surface of aerosols, Q_I (kJ mol^{-1}) is the enthalpy of adsorption from the surface, Q_V is the enthalpy of vaporization of the subcooled liquid, R is the ideal gas constant, T *is the temperature* (°K), and p_L° is the vapor pressure at 25 °C. Pesticide concentrations in the particle phase were then estimated by the following relationship:

$$K_p = \frac{F/\mathrm{TSP}}{A} \tag{4.19}$$

where F is the concentration of pesticides in the particles phase, TSP is the total suspended particles (in practice all the amount of pesticides is adsorbed in PM up to 10 μm aerodynamic diameter) and A is the concentration of pesticides in the gaseous phase. This calculation was done for each of the AS using EPISuite v4.11 (EPA 2012)

Concentration estimates were obtained with a time step of 1 h. This ambient air concentration was used as input to the microenvironmental model, allowing the estimation of the concentration in the different exposure relevant indoor environmental media (gaseous, particles and dust phase).

The inhalation exposure model is described by Eq. 4.20, where the daily average intake rate IR_{ijkg} (in mg/kg_bw/day) for each AS at each cell, was computed from the pesticide concentration, both outdoor and indoor (integrated over a year), the exposed group's inhalation rate and body weight and from the total time of exposure. At each time step, the respective outdoor or indoor concentration was estimated based on the activity pattern of the exposed individuals. The exposed population groups considered, included infants, children aged 4–9 years, 10–14 years, adult females and males. For each age, gender and ethnicity group, different inhalation rates (ICRP 2002), amount of dust ingested (Wormuth et al. 2006) and body weights (Sarigiannis et al. 2012b) were used.

$$IR_{ijkg} = \left(\frac{Q_{\mathrm{inh},g} \cdot t_{\mathrm{exp}}}{\mathrm{BW}_g}/365\right) \cdot \left(\int_{t_1}^{t_2} C_{ijk}(t)dt + \int_{t_2}^{t_3} C_{ijk}(t)dt + \ldots + \int_{t_{n-1}}^{t_n} C_{ijk}(t)dt\right) \tag{4.20}$$

where C_{ijk} is the average pesticide concentration in the exposure medium (in mg AS/m^3) over the exposure period (t_{exp}), $Q_{inh,g}$ is the daily inhalation rate per gender category g (in m^3 air/d), BW_g is the body weight (in kg) per gender category g, t_{exp} is 1 day and t_n is the total simulation time (in hours). The same approach was used for the inhaled pesticides adsorbed in particles. In this case, the actual intake (taking into account the deposition fractions based on PM size distribution) was estimated. Similar considerations (in terms of exposure duration and age and gender dependence) were made for the other pathways and routes, which in practice included (a) particles deposition on the skin, (b) dust exposure to skin through rubbing off and (c) dust ingestion due hand to mouth behavior. Overall intake on a daily basis was the sum of the intake rate from all exposure pathways.

4.7 Conclusions

Exposure assessment is the weak link in the chain of calculations required for assessing the risk of chemical mixtures. The current understanding of the need to capture exposures that take place during different key periods of one's life (i.e. the exposome) to properly investigate the link between chemical mixtures and human health warrants the use of complex models. Intake fraction modeling is a good start for screening purpose modeling. However, more detailed insights on exposure drivers and patterns, dynamics in space and time and variation by gender, age, socio-economic status, location and other determinants are needed to properly account for co-exposure to multiple chemicals in real life. Our work has shown that the real integrator is the human body, i.e. that internal exposure should be considered to properly capture the health effects of complex chemical exposure. Indeed, age, physiology, metabolic capacity, pre-existing health condition and exposure history (especially to persistent and biocumulative compounds) affect significantly how uptake dose of chemicals is transformed into biologically effective dose at the relevant target tissue. Integrating external with internal exposure is key to improving health risk assessment of chemical mixtures. Integrated complex exposure modeling facilitates the assimilation of human biomonitoring data in the actual exposure estimation.

Complex exposure modeling helps the assimilation of human biomonitoring data for exposure estimation. It also helps capture and quantify potential interactions between mixture components at realistic / actual exposure doses. Biokinetics and biodynamics of active xenobiotics may be perturbed from co-exposure to chemicals, which compete for the same metabolic receptor sites or induce allosteric effects perturbing metabolic pathways that may be linked to adverse outcome pathways. Being able to mathematically describe such perturbatory mechanisms avoiding the complexity and cost of extensive experimentation helps to tackle mechanistically the effects of co-exposure to multiple compounds and/or elements. Modeling platforms such as INTEGRA and MENTOR provide the necessary computational infrastructure to perform high performance computing so as to reckon the biologically effective dose of xenobiotics in a mixture and their toxic metabolites.

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