Cross-Mediterranean Environment and Health Network

CROME-LIFE

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Technical report on exposure (external and internal) modeling framework applied to the demonstrations sites

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1 Introduction

The objective of CROME-LIFE is to bring together all available information within a coherent methodological framework for assessing the source-to-dose continuum and to provide a comprehensive methodology for exposure assessment, translating external exposure into internal dosimetry (and vice versa). This provides two very significant opportunities for advancing risk assessment towards exposure based assessments:

- Forward estimation of exposure will allow us to translate external exposure data to Biologically Effective Dose (BED) at the tissue dose; this will allow the interpretation to mechanism based hazard assessment based metrics such as the Biological Pathway Altering Dose (BPAD) (Judson, Houck et al. 2010, Judson, Kavlock et al. 2011). The quantity that will be calculated, the biological pathway altering dose (BPAD), is analogous to current risk assessment metrics in that it combines dose–response data with analysis of uncertainty and population variability so as to derive exposure limits. The analogy is closest when perturbation of a pathway is a key event in the mode of action (MOA) leading to a specified adverse outcome. BPADs are derived from relatively inexpensive, high-throughput screening (HTS) in vitro data. Use of as detailed as possible Physiology Based Biokinetic (PBBK) modeling is the key component so as to estimate the in vivo doses required to achieve the BPAD in the target tissue. Uncertainty and variability will be incorporated in both the BPAD and the PBBK parameters and then combined to yield a probability distribution for the dose required to perturb the critical pathway. Thus, the more confident and explicit we are about the biokinetic behavior of the compound, the less conservative we have to be when translating BPAD into external exposure metrics. Information about BPAD for several chemicals can be easily obtained from the publicly available ToxCastDB (USEPA).
- Assimilation of the biomonitoring data collected in the frame of existing cohorts in the Mediterranean countries (i.e. Italy, Spain, Slovenia, Croatia and Greece). Assimilation of biomonitoring data is greatly facilitated by exposure reconstruction (or reverse dosimetry) techniques, allowing us to estimate the actual external dose corresponding to the observed biomonitored levels. This will also allow us the possible identification of route contribution, and consequently to exposure pathways contribution identification. Also, reconstructing exposure will allow us to re-run forward our assessment, and to estimate BED, starting actually from biomonitoring data.
- Refine overall assessment of exposure, as well as assimilation of biomonitoring data, taking strongly into account the differences in physiology among different age groups, capturing the differences in BED for similar exposure situations, as well as to attribute the appropriate variance of exposure, to significantly varying biomonitoring data.

The report describes the above methodological approach and to this aim is organized in a series of chapters addressing the different steps along the source-to-dose continuum starting from multimedia modelling needed to estimate the environmental contamination levels, proceeding with indoor microenvironment modelling and with exposure modeling for different exposure routes and mechanisms needed to derive external exposure profiles to finally internal exposure modeling needed to estimate internal doses of contaminants in human tissues.

2 Multimedia environmental modeling

2.1 General considerations

For the development of the multimedia environmental modelling framework for CROME-LIFE, we followed the ECHA guidance on information requirements and chemical safety assessment (ECHA 2012). All different spatial scales, media exchange and processes used in EUSES (Lijzen 2004) were taken into account. A description of the main adapted concepts is described in the following sub-chapters.

2.2 Environmental releases module

2.2.1 General considerations

Releases of chemicals can occur to air, surface fresh and marine water, wastewater and soil and are estimated separately for every environmental compartment and each relevant stage of the life cycle. Release estimation is the process whereby releases to the environment are quantified during the life cycle stages and uses of a substance, taking into account the different release pathways, receiving environmental compartments and the spatial scale of the releases. Therefore, the aim of the release estimation is to calculate the following parameters (ECHA 2012):

- Release rates (expressed in kg/day) to wastewater, surface water, air and soil for each relevant life cycle stage and use at the local scale.
- Release rates (expressed in kg/day) to wastewater, surface water, air and soil at the regional scale.

2.2.2 Information needed for release estimation

Proper release estimation can only start after the definition of the life cycle stages for the substance and the identification of the uses for each of them.

The information that needs to be considered for the release estimation is (ECHA 2012):

- Life cycle stages of a substance
- Supplied tonnage for the use, or group of uses, for each life cycle stage of a substance
- Information on Operational Conditions (OC) and Risk Management Measures (RMM)
- Release factors (expressed in kg/kg or %) depending on the type of use, the stage in the life cycle and the OC and RMM

2.2.3 Life cycle stages of a substance

The generalized life cycle stages of a substance are given in Figure 1. The release pattern and the estimated release factor are closely related to the life cycle stages of a substance. The release estimate in a registration should in principle be seen from the perspective of the entire life cycle of a substance, as described here.

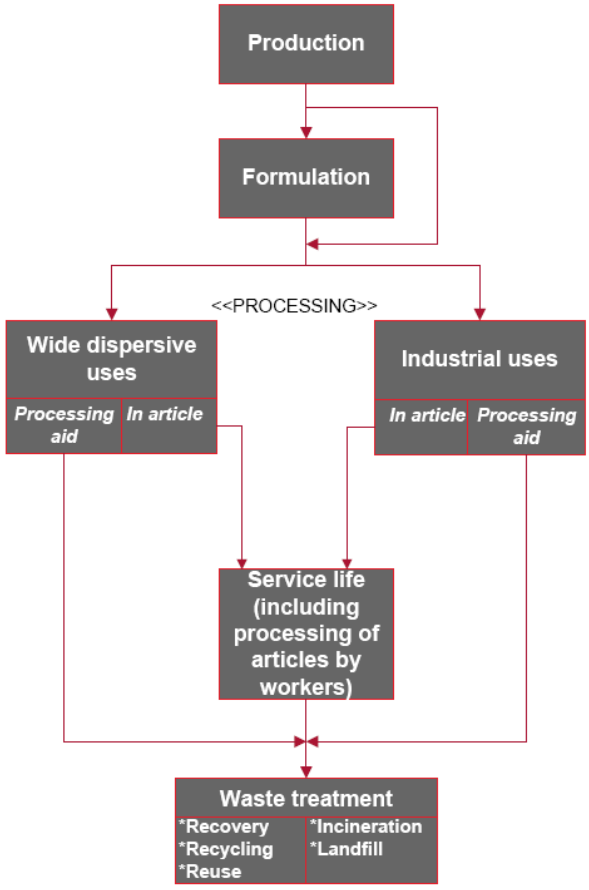

Figure 1: Life cycle stages of substances (ECHA 2012)

Manufacture (production): Chemical synthesis of the substance. Manufacture is the stage where the substance is manufactured, i.e. formed by chemical reaction(s), isolated, purified, drummed or bagged, etc. Different types of intermediates (substances used to make other substances) can be manufactured and distinguished.

Formulation: Mixing and blending into a mixture. Formulation is the stage where substances are combined in a process of blending and mixing to obtain a mixture. This may be a formulation such as a paint, or a mixture on a carrier material, such as a photographic film. Formulations are applied or used at the next stages of the life-cycle (industrial/professional use, private use).

Industrial use: Use of the substance as such or in a mixture, in an industrial process with the purpose of incorporating the substance into an article, or technically supporting the production process but not intentionally becoming part of the product (processing aid). One example of a processing aid is a developer used in a photographic bath that is disposed of after use large outside industrial installations. They consist in professional and consumer uses:

a) Professional use may include the use of substances as such or in mixtures, in order to deliver services to business or private customers. This may include sophisticated equipment and specialized, trained personnel.

b) Consumer use includes the use of substances as such or in mixtures. It is assumed that the user is not trained. Use can take place in closed systems (lubricants for vehicles or hydraulic systems) or open systems (lubricants for bicycles). It may also include processing of material.

Service life: "Use"5 of articles or the polymer matrix of a mixture (paints, adhesives) containing the substance over a period > 1 year. Such activities include for example wearing and maintenance of textiles, housing, using and maintenance of vehicles, use and maintenance of sport articles, etc.

Waste treatment: Final stage where substances, mixtures or articles are disposed of after their service life, such as for example used lubricants or solvents, old tires or home appliances. Unintended losses of mixtures may also enter into the waste life stage, like e.g. overspray from coating, surplus of dyes, inks or residues from cleaning of machinery. Treatment includes incineration, landfilling, or recovery of the basis material or substance.

At each of the life-cycle stages a larger or smaller fraction of the substance is lost via releases and will therefore not enter the next life cycle stage. In case a refinement of the release is needed, this aspect could be taken into account. Between the various life cycle stages transport, storage, and handling may occur. Releases due to storage, handling, repacking and filling, including local transfer, are assumed to be included within the relevant life cycle stage. Transport is not considered further under REACH.

2.2.4 Releases based on production data (tonnage)

The starting point for release estimation is the tonnage of substance manufactured/imported by the registrant and the tonnage associated with each use (or group of uses) during the life cycle of the substance for which exposure scenarios need to be developed.

The manufacturer's annual production or the importer's annual import of a substance will be distributed in the EU market, and flows down the supply chains. The registrant usually knows his own production/import tonnage and the markets to which he sells the substance. However, often he has little information on the annual or daily tonnage used by the downstream users (including formulators and industrial users).

If the registrant is able to get information of the tonnage used by his downstream users and if he has enough market data, he can assign a tonnage to every life cycle step (formulation, industrial use, consumer and professional use, service life articles) and uses identified in the use mapping section. For each downstream use, it is possible to consider as a worst case assumption, the tonnage used by the largest customer. It is assumed that for each use, the evaluation performed using this tonnage ensures control of risk for all smaller customers.

If specific and reliable data are not available, conservative assumptions (like the use of the total manufactured volume for every identified use) need to be made by the registrant to assign the tonnage to identified uses for releases estimation. In doing so, the calculated exposure estimates and corresponding RCR will help the registrant to set priorities for collection of more specific information.

A major element for the use of tonnage is the incorporation of release factors. Release factors express the fraction (either kg/kg or %) of the used amount being released to the environmental compartment under consideration. The release of a substance from a certain use (e.g. technical processes in installations or vehicles, application of mixtures in private households) depends on the operational conditions (like e.g. temperature, pressure, level of containment of machinery, level of internal regeneration of processing fluids, dry or wet process, dipping or spraying) and risk management practices. ERCs are based on the life cycle stage, level of containment, type of use and technical fate of a substance, dispersion of release sources,indoor or outdoor use and release potential during service life and waste stage.

Table 1. Release factors based on the respective Environmental Release Categories (ERCs)

No	ERC	Default worst case release factors resulting from the conditions of use described in the ERCs. Grey cells indicate release factors which are used for the regional release estimation only (and not for the local one).		
		to air	to water (before STP)	to soil
1	Manufacture of chemicals	5%	6%	0.01%
2	Formulation of mixtures	2.5%	2%	0.01%
2	Formulation in materials	30%	0.2%	0.1%
4	Industrial use of processing aids	100%	100%	5%
5	Industrial inclusion into or onto a matrix	50%	50%	1%.
6A	Industrial use of intermediates	5%	2%	0.1%
6B	Industrial use of reactive processing aids	0.10%	5%	0.025%
6C	Industrial use of monomers for polymerisation	5%	5%	0%
6D	Industrial use of auxiliaries for polymerisation	35%	0.005%	0.025%
7	Industrial use of substances in closed systems	5%	5%	5%
8A	Wide dispersive indoor use of processing aids, open	100%	100%	n.a.
8B	Wide dispersive indoor use of reactive substances , open	0.10%	2%	n.a.
8C	Wide dispersive indoor use, inclusion into or onto a matrix	15%	1%	n.a.
8D	Wide dispersive outdoor use of processing aids, open	100%	100%	20%
8E	Wide dispersive outdoor use of reactive substances, open	0.10%	2%	1%
8F	Wide dispersive outdoor use, inclusion in matrix	15%	1%	0.5%
9A	Wide dispersive indoor use in closed systems	5%	5%	n.a.
9B	Wide dispersive outdoor use in closed systems	5%	5%	5%
10A	Wide dispersive outdoor use of long-life articles, low release	0.05%	3.2.%	3.2%
10B	Wide dispersive outdoor use of long-life articles, high or intended release	100%	100%	100%

11A	Wide dispersive indoor use of long-life articles, low release	0.05%	0.05%	n.a.
11B	Wide dispersive indoor use of long-life articles, high or intended release	100%	100%	n.a.
12A	Industrial processing of articles with abrasive techniques	2.5%	2.5%	2.5%
12B	Industrial processing of articles with abrasive techniques	20%	20%	20%

2.2.5 Emissions based on actual environmental releases

When more specific information is available on the release sources, this information can be used to deviate from these default parameters and refine the assessment. If, for example, the manufacture or use of a certain substance is confined within a specific country, parameters which are relevant for that country can be used. In the case that actual environmental releases data are available, these data are considered as more representative as an input for the multimedia model. In such case, the predicted environmental concentrations will be much closer to the actual environmental values. In any case, these data have to fulfill requirements such as:

- adequate measured data should be selected by evaluation of the sampling and analytical methods employed
- the data should be assigned to local or regional scenarios by taking into account the sources of release and the environmental fate of the substance
- the measured data should be compared to the corresponding calculated PEC. For naturally occurring substances background concentrations have to be taken into account. For risk characterization, a representative PEC should be decided upon based on comparison of measured data and a calculated PEC.

2.3 Environmental contamination - Interaction among different environmental media at different scales

2.3.1 General considerations

The exposure to the environment is in principle assessed on two spatial scales: locally in the vicinity of point sources of release to the environment, and regionally for a larger area which includes all point sources and wide dispersive sources in that area. Releases at the continental scale are considered to provide inflow concentrations for the regional environment. The end results of the exposure estimation are concentrations or Predicted Environmental Concentrations (PECs) in the environmental compartments air, surface water (fresh and marine), soil, sediment, and biota (e.g. earthworms and fishes for secondary poisoning) and human daily intake of the substance via the environment for both local and regional scale.

2.3.1.1 Local assessment

The concentrations of substances released from a single point source are assessed for a generic local environment. This is not an actual site, but a hypothetical site with predefined characteristics, defined by a 'standard environment' and a standard town of 10,000 inhabitants. The exposure targets are assumed to be exposed in, or at the border of, the site. In general, concentrations during a release episode are calculated. This means that local concentrations are calculated on the basis of a daily release rate, regardless of whether the discharge is intermittent or continuous. They represent the concentrations expected at a certain distance from the source on a day when the release occurs. For the exposure assessment of terrestrial organisms, of predators and of man indirectly exposed via the environment a

longer term average is used instead of daily release rates. This is because exposure is assumed not to be influenced by temporal fluctuation in release rates. In principle, degradation and distribution processes should be taken into consideration at the local scale. However, because of the relatively short time between release and exposure, concentrations at local scales are entirely controlled by initial mixing (dilution into environmental compartment) and adsorption on suspended matter. No other process is considered in the calculation of local PEC. A fixed dilution factor is applied to the effluent concentration of an STP (by default assumed to be present). The actual dilution factor after complete mixing can be calculated from the flow rate of the river and the effluent discharge rate of the STP. This approach is used for rivers only and not for estuaries or lakes. In other cases, the calculation of the PEC_{local} can be carried out using actual environmental conditions around the point source. Figure 2 shows the relationship between the local release routes and the subsequent distribution process modelled for the environmental compartments.

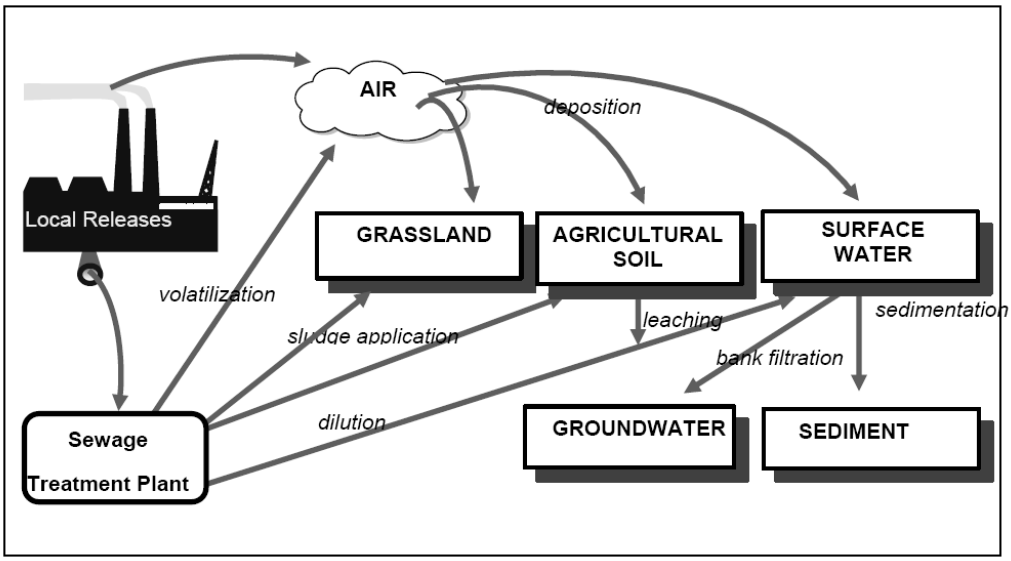

Figure 2: Local distribution calculation (for an industrial setting scenario) (Mackay 2001)

2.3.1.2 Regional assessment

The concentrations of substances released from point and wide dispersive sources in a larger area are assessed for a generic regional environment. The fate of substances at the regional scale differs from the fate at the local scale in the sense that more time is available for transport and transformation processes. At longer distances from point sources or when releases are wide dispersive and not collected into a single point source, the further distribution and fate of the substance are taken into account. It can be assumed that inter-media transport and degradation become relatively more important. For calculating the regional PEC, a multi-media fate-modelling approach is used (e.g. the SimpleBox model).

All releases to each environmental compartment for each use, assumed to constitute a constant and continuous flux, are summed and averaged over the year, and steady-state concentrations in the environmental compartments are calculated. The regional concentrations are used as background concentrations in the calculation of the local concentrations.

Figure 3 gives a general overview of the distribution processes in the regional model. A standard region is represented by a typical densely populated EU-area located in Western Europe (~ 20 million inhabitants, 200 X 200 km^2). Regardless of the assumptions made at local scale, regional releases to water are

based on a scenario where 80% (representing the EU average) of the wastewater is treated in a biological STP and the remaining 20% is released directly into surface waters.

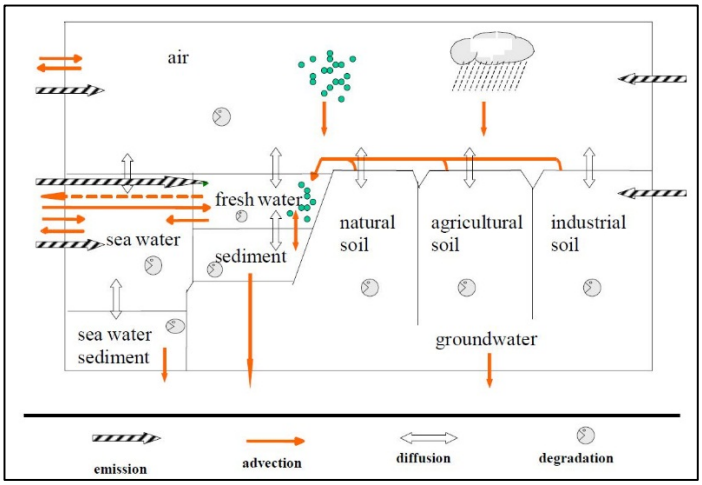

Figure 3. Schematic representation of the model for calculating the regional PECs (ECHA 2012)

2.3.1.3 Continental assessment

Concentrations in air and water are also estimated at a continental scale (Europe) to account for the chemical flux - due to passive transport of the substance with air and water into the regional area. Both continental and regional concentrations are calculated using a multimedia fate model. Figure 4 illustrates the relationships between continental, regional and local scale.

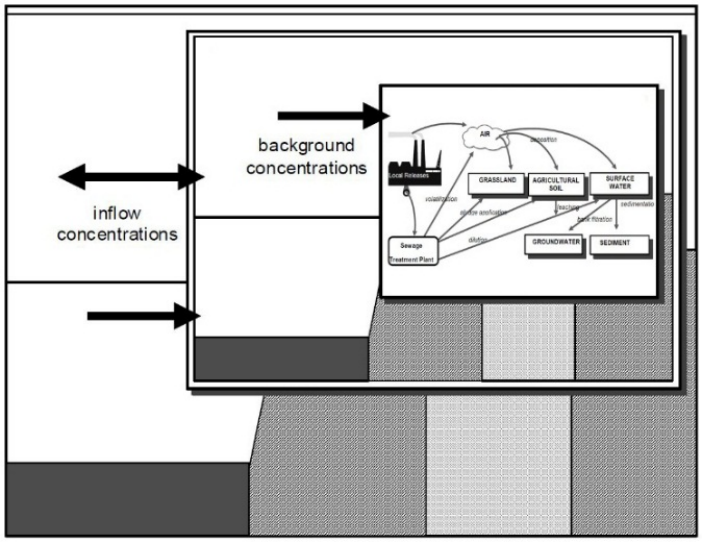

Figure 4. The relationship between the continental, regional, and local scale (ECHA 2012)

2.3.2 Input to PEC estimation calculations

Input data needed to estimate the PEC at different spatial scales are:

- Substance properties. The following minimum information are required: molecular weight, water solubility, vapour pressure, octanol-water partition coefficient and information on ready

biodegradability for the substance. For an inorganic substance, it is also advised to provide information on the abiotic degradation, and solid-water partition coefficients and the water-biota partition coefficients.

- Release rates as described in paragraph 2.1.
- Removals and distribution in waste treatment systems.
- Partition coefficients and degradation rates.

Two types of PEC-values are derived to be used in the further exposure and risk assessment: the regional concentration (PECregional) and the local concentration (PEClocal). In addition, continental PEC values are derived, but they are not used in the risk assessment. The continental PEC values are used to account for the chemical exchange - due passive transport of the substance with air and water - with the surrounding area of the regional area. These three types of concentrations differ in temporal and spatial scale.

The regional concentration mainly serves as estimates for background levels, and the estimate of these are so-called steady-state concentration, i.e. the concentration obtained at releases and fate processes taking place over infinite time. The estimated values are thus considered worst-case estimates. How conservative the estimate is depends on the rate of the fate processes, being most conservative for substances where the fate processes take place very slowly. The size of the regional scale is a default set at 10% of the size of the EU.

The local concentration is calculated for each identified local point source. The temporal scale is in days, i.e. for discharges with varying magnitude over the day, the daily average concentration is typically used in the further assessment. Also a "standard" environment for the local scale has been defined, e.g. operating with a default dilution of 10 in fresh water systems. This does not exclude that for specific industrial point sources the calculation of PEClocal can be carried out using actual environmental conditions around the source.

Three spatial scales are used in the distribution calculations: continental, regional and local. The local scale receives the background concentration from the regional scale; the regional scale receives the inflowing air and water from the continental scale.Figure 4 illustrates the relationships between the three scales.It should be noted that the use of regional data as background for the local situation may not always be appropriate.

2.3.2.1 Local environmental distribution

Distribution on the local scale is assessed in the vicinity of point sources. Each application of the substance and each stage of the life cycle are assumed to occur at different point sources. Therefore, in principle, a local assessment has to be performed for each relevant application and each relevant life-cycle step (which can be summed if several steps occur on the same location). A generic standard environment is defined to allow for a risk assessment on the European level. As it is impossible to characterise an 'average European environment', default parameter values are chosen which reflect typical, or reasonable worst-case, settings. Dedicated modelling approaches are used to calculate the concentrations in air, surface water and soil. The sediment and groundwater concentrations are estimated from the surface water and soil concentration respectively. The mathematical formulation used to derive PEC local follows the one reported in the ECHA report (ECHA 2012).

2.3.2.2 Regional distribution

For calculating the regional PEC, the multi-media fate-model SimpleBox is used. The basic characteristics of this model are shown in Figure 3.

The model have been described by Mackay et al. (1992), Van de Meent (1993) and Brandes et al. (1996) (SimpleBox). The model is consisting of a number of compartments which are considered homogeneous and well mixed. A substance released into the model scenario is distributed between the compartments according to the properties of both the substance and the model environment. Several types of fate processes are distinguished in the regional assessment, as drawn in Figure 3:

- release, direct and indirect (via STP) to the compartments air, water, industrial soil, and agricultural soil;
- degradation, biotic and abiotic degradation processes in all compartments;
- diffusive transport, as e.g. gas absorption and volatilisation. Diffusive mass transfer between two compartments goes both ways, the net flow may be either way, depending on the concentration in both compartments;
- advective transport, as e.g. deposition, run-off, erosion. In the case of advective transport, a substance is carried from one compartment into another by a carrier that physically flows from one compartment into the other. Therefore, advective transport is strictly one-way.

The results from the model are steady-state concentrations, which can be regarded as estimates of long-term average exposure levels. The fact that a steady state between the compartments is calculated, does not imply that the compartment to which the release takes place is of no importance.

In the multi-media model used, the environmental media are represented by the following homogeneous and well-mixed compartment 'boxes':

- Atmosphere;
- Surface water (freshwater and marine environment);
- Sediment (freshwater and marine environment);
- Soil.

For all the above compartments the mathematical formulation used to derive PEC regional are based on SimpleBox model as detailed in the ECHA guidance report (ECHA 2012).

2.3.2.3 Continental distribution

Concentrations in air and water are also estimated at a continental scale (Europe) to provide inflow concentrations for the regional environment (Figure 4). These concentrations are also derived using the SimpleBox model as detailed in the ECHA guidance report (ECHA 2012).

2.3.3 Environmental processes occurring at different scales

2.3.3.1 General considerations

It is well established that certain chemicals, when discharged to the environment, can persist for a sufficiently long period of time (months and years), can travel considerable distances (1000s of km) and can migrate between the available media of air, fresh and marine waters, soils, sediments, vegetation and other biota, including humans (Mackay 2001). The environment is complex in nature and is continually changing, thus chemical fate is correspondingly complex. It is impossible to describe, or even know, the fate of chemicals accurately, but it is believed that the broad features of chemical fate can be understood and even predicted, provided that sufficient information is available on certain key chemical and environmental properties. Notable among these properties are partitioning properties, which control how the chemical is distributed at equilibrium between media, such as air and water and reactive properties, that govern how fast the chemical reacts or degrades (usually expressed for convenience as a half-life in each environmental medium) (Mackay 2001). An essential point is that these properties vary enormously in magnitude from chemical to chemical, i.e. by a factor of a million or more, thus chemical behavior is correspondingly different by such a factor. Environmental conditions such as temperature, sunlight intensity, rainfall and soil and vegetation types also vary greatly.

Certain attributes of chemicals in the environment can be measured directly, notably concentrations. Other attributes cannot be measured directly, notably fluxes such as evaporation rates, persistence and distance travelled. They can only be estimated by using models. We thus need the assistance of a calculating tool that will accept the available input data, process them and give relevant output. This is the role of the MMM. Its predictions are not likely to be highly accurate (i.e. rarely better than a factor of two

in accuracy), but they can be consistent, repeatable, transparent and they can be validated to some extent by comparing predictions with observations (Mackay 2001).

Modellers set up their equations in several formats depending on the objective. Most common in this context are compartment, box or Eulerian models in which the environment is divided or segmented into a number of volumes or boxes, which are fixed in space and are usually treated as being homogeneous, i.e. well-mixed, in chemical composition. This has the advantage that only one concentration need be defined per box. Another (Lagrangian) approach that is widely used in atmospheric and river modelling, is to define a parcel of air or water and follow it, and the chemical in it, in time as the parcel moves from place to place. There are also situations where there is marked heterogeneity in concentration, and it is preferable to set up diffusion/advection/reaction differential equations and solve them either numerically or analytically. This is often done when describing chemical migration in sediments and soils, but it can also be applied to atmospheric dispersion, aquatic and oceanic systems (Mackay 2001). In principle, all approaches should give the same, or similar results. Here we focus primarily on compartmental models because it is likely that they will be most commonly applied in the regulatory context. For some purposes Lagrangian models may be used when evaluating Long Range Transport in air or water. Diffusion models can be valuable when seeking a general picture of chemical fate in the global atmosphere or oceans, or when estimating the near-source dispersion of emitted chemicals.

When there are data on chemical properties, inputs and concentrations in a well-identified region, it is possible to set up a model describing this site-specific situation. Models are routinely applied in rivers, lakes, soils, biota and air pollution studies (Mackay 2001). Validation is possible by comparing the model output with observations. MMMs have been set up for regions, nations, continents and even the global system. These can be referred to as models of "real" systems. Another family of models is the "evaluative" models in which the environment is fictitious i.e. it does not correspond to a particular area, but it is realistic. The fate of a variety of chemicals can be evaluated in such models. The same equations are used in real and evaluative models; only the environmental parameters are different. This approach is particularly attractive for international regulation purposes because the assessment is not in a specific region; it is general. Examples are the EQC model of Mackay (1996), CalTox (McKone 1993), the SimpleBox model included in the European Union System for the Evaluation of Substances (EUSES) model used in the European Union (Lijzen 2004) and the Mentor 4M (Georgopoulos, Wang et al. 2008).

2.3.3.2 The single compartmental mass balance

The first step in model development is to divide the environment into a number of compartments of defined volume that are fixed in space. Considering first a single compartment, it is possible to set out the input and output processes. Included can be discharge or emission, advective inflow in air or water (and the corresponding advective outflow), diffusion to and from adjacent compartments, formation from other chemical species and degrading reactions to form other chemical species. A simple mass balance foresees that the rate of inventory change of the mass of chemical in the volume must equal the total rate of chemical input minus the total rate of output. Mathematically this is expressed by the differential equation:

$$\frac{dm}{dt} = \mathrm{T}_{IN} + E + F_{IN} + S - \left(T_{OUT} + F_{OUT} + R\right)$$

(with units such as g/h) where ***m*** is mass in the compartment (e.g. g), ***V*** is volume (e.g. m^3) and ***C*** is concentration (e.g. g/m^3). A particularly useful and simple version applies when the inventory is fairly constant with time, and thus the derivative on the left side is small or zero. Input rates then equal output rates under steady-state conditions. The advantage of making this steady-state assumption is that the mathematics becomes algebra rather than calculus.

The next task is to predict the various output process rates as a function of the chemical concentration. If the input rates are known and all output rates can be expressed as a function of concentration, then the

mass balance equation can be used to calculate the chemical concentration and hence the mass of chemical in the box and the rates of the various loss processes.

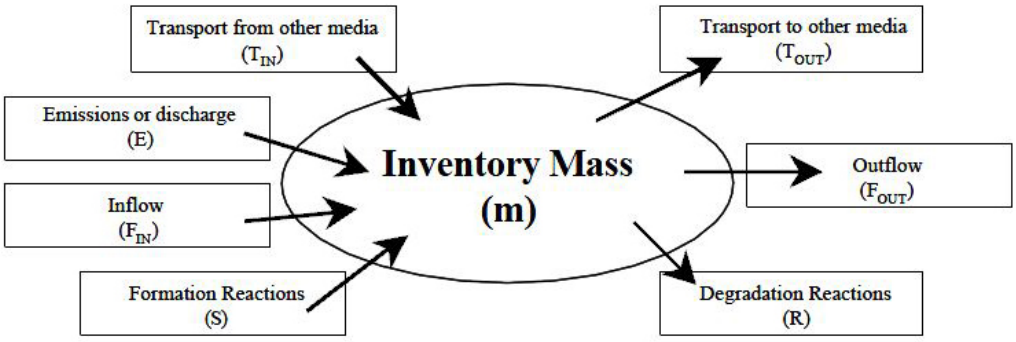

Figure 5 Derivation of expressions for compartmental concentrations at steady-state using rate constants (Mackay 2001)

An important quantity is the persistence of the chemical. This can be expressed as the residence time of the chemical in the box, which is best calculated at steady-state. This is the mass of chemical in the box divided by the total rate of output (or input when steady-state applies). Under non steady-state, or dynamic conditions, a characteristic time can be calculated similarly as the mass divided by the output rate. This is the average time that the chemical spends in the single compartment or box and is a first indication of persistence. It is possible to calculate a residence time attributable to reaction and other loss processes such as outflow both individually and collectively. Here we use the word persistence as generally expressing the longevity of the chemical in the environment. Residence time, characteristic time and half-life have specific mathematical definitions.

When calculating persistence, not all loss processes are relevant. Outflow by advection is not a permanent environmental loss process. It only transports a chemical from one environmental location to another. On the other hand, reaction eliminates a chemical from the environment permanently and completely. If the only loss is by reaction with a half-life $t_{1/2}$, then the rate of reaction is VCk_R where k_R is $0.693/t_{1/2}$ and is the rate constant. The residence time t is then $[VC]/[VCk_R]$ or $1/k_R$ and equals $t_{1/2}/0.693$. In this case $t_{1/2}$ is 69% of t. Some models consider loss processes other than reactions as irreversible losses, e.g. sediment burial or transport to the stratosphere.

2.3.3.3 Extension to multiple compartments

If the model consists of two connected boxes, the same approach can be applied twice, once to each box, and to the combination of the two boxes, i.e. the system as a whole. The residence time in each box or in the system of two boxes is a simple extension of the single box approach. Overall persistence in a multimedia system can be expressed using the residence time in the system without considering advective losses:

$$\frac{dm}{dt} = \mathrm{T}_{IN} + E + F_{IN} + S - \left(T_{OUT} + F_{OUT} + R\right)$$

where $f_i = m_i/m_{tot}$=mass fraction in compartment i and R_{total} is the total rate of reaction.

Other compartments can be added. Klecka et al. (2000) have suggested a minimum of three compartments but there is a general consensus that four (air, water and soil plus sediment) are required to adequately represent the environmental fate of a chemical and by extension, its overall persistence.

A key conclusion is that persistence is best expressed as a residence time attributable to reaction only. For a single compartment this is the half-life divided by 0.693. For multiple compartments the overall

residence time is a weighted average of the individual residence times, and the weighting depends on the mode-of-entry and the partitioning characteristics of the chemical (Mackay 2001).

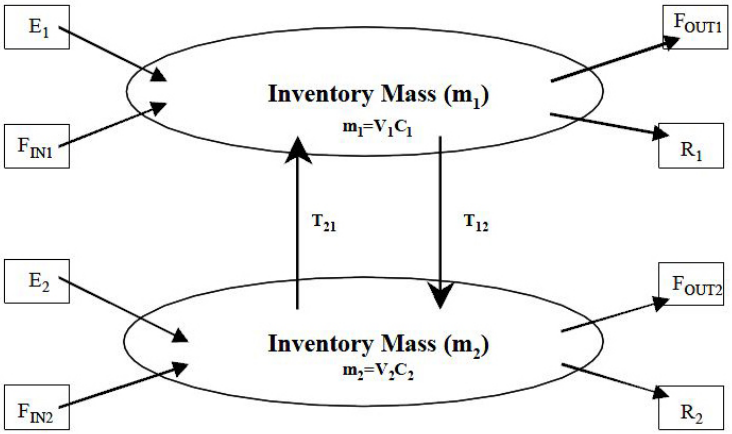

Figure 6. A multiple compartment system using steady state rate constants (Mackay 2001)

2.3.3.4 Humans exposed indirectly via the environment

Indirect exposure of humans via the environment may occur by consumption of food (fish, crops, meat and milk) and drinking water, inhalation of air and ingestion of soil. The different routes of exposure are illustrated in Figure 7.

The indirect exposure is assessed by estimating the total daily intake of a substance based on the predicted environmental concentrations for (surface) water, groundwater, soil and air.

The concentration of a substance in food is related to its concentration in water, soil and air and to its potential for bioaccumulation and its biotransfer behaviour. The models for the estimation of daily intake allow the use of local or regional environmental concentrations, as appropriate. The methods require the use of a limited number of input parameters and can, if required, be adapted for specific human populations for which it may be necessary to assess the exposure separately.

Indirect exposure is principally assessed on two spatial scales: locally near a point source of the substance, and regionally using averaged concentrations over a larger area. In the local assessment, all food products are derived from the vicinity of one point source, in the regional assessment, all food products are taken from the regional model environment. It should be noted that the local and regional environments are not actual sites or regions, but standardised environments as defined in paragraphs 2.3.1.1 and 2.3.1.2. Clearly, the local scale represents a worst-case situation. People do not consume 100% of their food products from the immediate vicinity of a point source. Therefore, the local assessment represents a situation which does not exist in reality. However usually, one or two routes dominate the total exposure and local exposure through these routes may not be unrealistic. In contrast, the regional assessment represents a highly averaged exposure situation which cannot insure protection of individuals who consume food products from the vicinity of point sources. A regional assessment gives an indication of potential average exposure of the inhabitants of the region. In light of the above considerations the assessment should be seen as a helpful tool for decision making and not as a prediction of human exposure actually occurring at some place or time.

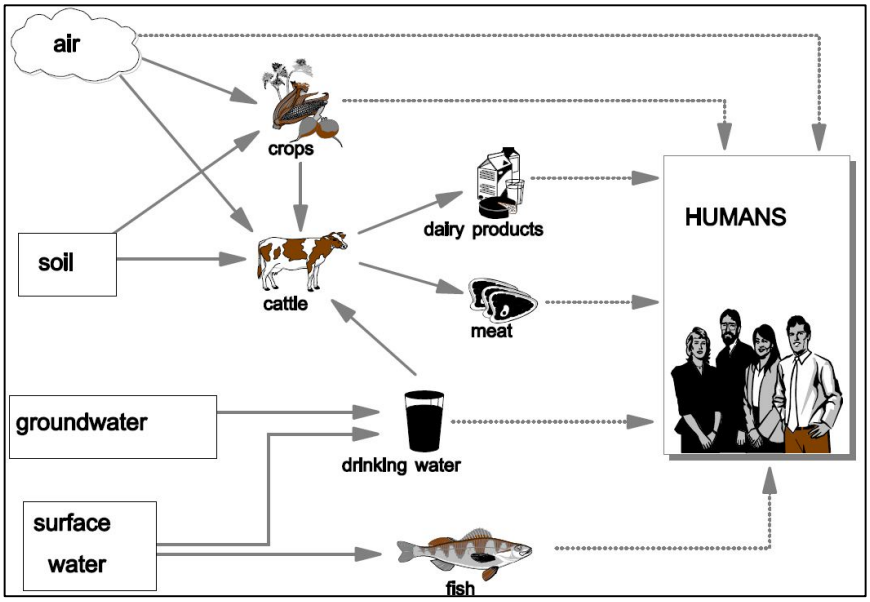

Figure 7. Schematic representation of the exposure routes considered in human exposure

In a case where the regional assessment indicates reason for concern, there is a clear need for refinement of the assessment. In cases where the local assessment does not indicate a potential risk, there is no reason for concern. The situation is less clear in the grey area where a regional assessment does not give reason for concern, but the local assessment does. It should be noted that there is no testing strategy triggered by the indirect exposure estimation. Instead, when there is reason for concern in the local assessment only, a further analysis of the major exposure routes is required to investigate the realism of the local exposure scenario. As the most important routes are indicated by the assessment, this provides a clear starting point for refinement.

The data needed for the calculations are PEC-values derived in the local and regional scales assessment and are summarized in Table 2.

Table 2. Environmental concentrations used as input for indirect exposure calculations

Compartment	Local assessment	Regional assessment
surface water	annual average concentration after complete mixing of STP-effluent	steady-state concentration in surface water
air	annual average concentration at 100 m from source or STP (maximum)	steady-state concentration in air
agricultural soil	concentration averaged over 180 days after 10 years of sludge application and aerial deposition	steady-state concentration in agricultural soil
Porewater	concentration in porewater of agricultural soil as defined above	steady-state concentration in porewater of agricultural soil
groundwater	concentration in porewater of agricultural soil as defined	steady-state concentration in porewater of agricultural soil

Compartment	Local assessment	Regional assessment
	above	

In addition to the data required for the environmental exposure estimation, the bioconcentration factor (BCF), soil accumulation factors (BSAFs).

According with REACH regulation assessment of indirect exposure is generally only conducted if:

- the tonnage >1,000 t/y or
- the tonnage >100 t/Y and the substance is classified
  - as "Toxic" with a risk phrase "R48";
  - or as a carcinogen or mutagen (of any category);
  - or as toxic to reproduction (category 1 or 2).

Currently, the scenario for indirect human exposure cannot take into account exposure from aquatic organisms apart from fish, because to date an internationally validated bioaccumulation standard testis only available for fish and consumption data on aquatic organisms other than fish are scarce.

Assessing concentrations in food products (in this context fish, leaf crops, root crops, meat and dairy products) in initial or intermediate screening stages usually involves calculation of bioconcentration (BCF) or biotransfer factors (BTF). These are defined as the external exposure (as a concentration or a dose) divided by the internal concentration in the organisms. The use of fixed factors implies that these factors describe a steady-state situation in which the exposure period is assumed long enough to reach a steady-state.

2.4 Food contamination

2.4.1 Food contamination through the food chain

2.4.1.1 Plants uptake

Plant products form a major part of the food products for humans and cattle. Contamination of plants will therefore have significant influence on the exposure of humans. When trying to predict concentrations in plant tissues, one will immediately encounter several important conceptual problems:

- there are hundreds of different plant species forming the heterogenous group of food crops.
- Furthermore, varietal differences can also account for large differences;
- different tissues from plants are consumed (roots, tubers, fruit, leaves);
- crops differ in contaminant exposure, many crops are for instance grown in greenhouses;
- crops can be exposed through uptake from the soil, but also through gas uptake and aerial deposition.

From the above it may be clear that a modelling approach can only give a rough approximation of the concentrations in plants. To account for the predicted variety in plant products, it is proposed to distinguish between tuberous plants and leaf crops. Furthermore, the exposure of plants should include the soil route, as well as the air route. Uptake from soil is, in general, a passive process governed by the transpiration stream of the plant (in case of accumulation in leaves) or physical sorption (in case of roots). Uptake into the leaves from the gaseous phase can be viewed as a passive process, in which the leaves components (air, water, lipids) equilibrate with the air concentration. A general form of steady state partitioning coefficient between these compartments is given by Riederer (1990). Kow and Kaw (the air-water partitioning coefficient) are used to assess the distribution between the air and the plant. It is proposed to use the modelling approach of Trapp and Matthies (1995) to estimate levels in leaves and roots due to uptake from soil and air.

2.4.1.2 Meat and milk

Lipophilic substances are known to accumulate in meat, and can be subsequently transferred to milk. Cattle can be exposed to substances in grass (or other feed) with adhering soil, drinking water, and through inhalation of air. Biotransfer factors can be defined as the steady-state concentration in meat, divided by the daily intake of the substance. Travis and Arms (1988) calculated biotransfer factors for cow's meat and milk by log-linear regression on a number of substances (28 for milk and 36 for beef). Even though the theoretical background is limited, these factors provide a useful tool in risk assessment. It is proposed to use the same exposure estimates for air and crops which have been derived for human exposure for cattle,

2.4.1.3 Fish

Fish, residing in contaminated surface water, are able to take up appreciable amounts of (especially lipophilic) substances through the gills or through their food. The concentration in fish may be orders of magnitude greater than the concentration in water. The bioconcentration factor in fish is found to be well correlated with the octanol-water partitioning coefficient (Kow), indicating that lipid or fat is the main dissolving medium (ECHA 2012).

2.4.2 Food contamination through migration from food contact materials

2.4.2.1 Migration of Plastic Packaging

Migration of compounds from plastic packages into foodstuffs depends on many factors, but for a given migrant-polymer system and under controlled/fixed time/temperature conditions, migration greatly depends on the physicochemical characteristics of food, especially the fat content. To assess migration of additives and contaminants from food-packaging films, mathematical modeling based on Fick's second law is used.

In the case of negligible mass transfer resistance on the side of the food, which is the case for a well-mixed food or a Bi number greater than 100, the migration process is controlled by the diffusion of the migrant through the packaging material and the migrant is well distributed in the food (Poças, Oliveira et al. 2008). The process is described with the equation below:

$$\frac{M_A^F}{M_A^F(\infty)} = 1 - \sum_{n=1}^{\infty} \frac{2 \cdot a \cdot (1+a)}{1 + a + a^2 \cdot q_n^2} \exp\left(-D_A^P \cdot t \cdot \frac{q_n^2}{L^2}\right)$$

where M_A^F: the mass of the migrating species A present in the food, $M_A^F(\infty)$: the mass of A present in the food at equilibrium, D_A^P: the diffusivity of A in packaging material, L: the thickness of the packaging material, q_n: the positive roots of the transcendent equation:

$$\tan q_n = -a \cdot q_n$$

The volumes V_P (cm^3) and V_F (cm^3) of packaging material and food are used to calculate α

$$a = \frac{V_F}{V_P \cdot K_P}$$

where K_P: the partition coefficient of *A* in the system packaging material/food, which can be assumed as constant for low concentrations and is calculated using the equation below:

$$K_P = \frac{c_A^P(\infty)}{c_A^F(\infty)}$$

where $c_A^P(\infty), c_A^F(\infty)$: the concentration of A in the packaging material and food at equilibrium, respectively.

If α>>1 because $V_F >> V_P$ and/or $K_P < 1$, then a simplified solution:

$$\frac{M_A^F}{M_A^F(\infty)} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left(-D_A^P \cdot t \cdot \frac{(2n+1)^2\pi^2}{L^2}\right)$$

And for low migration times:

$$\frac{M_A^F}{M_A^P(0)} = \frac{2}{L\sqrt{\pi}}\sqrt{D_A^P t}$$

If α<<1 because $V_F \approx V_P$ and/or $K_P >> 1$, then a simplified solution:

$$\frac{M_A^F}{M_A^F(\infty)} = 1 - \exp\left(Z^2\right) erfc(Z)$$

Where

$$Z = \frac{K_P}{a}\sqrt{D_A^P t}$$

$$a = \frac{V_F}{A}$$

In the case where the mass transfer resistance on the side of the food is not negligible, but can be approximated by a convective process, with a gradient in the boundary layer, and the convective mass transfer coefficient (h) is not infinite, the equations below are used (Poças, Oliveira et al. 2008):

$$\frac{M_A^F}{M_A^F(\infty)} = 1 - \sum_{n=1}^{\infty}\frac{2 \cdot Bi}{\left(q_n^2 + Bi^2 + Bi\right) \cdot q_n^2}\exp\left(-D_A^P \cdot t \cdot \frac{q_n^2}{L^2}\right)$$

$$q_n \tan q_n = Bi$$

Bi represents the Biot number:

$$Bi = \frac{L \cdot h}{D_A^P}$$

If Bi>100:

$$\frac{M_A^F}{M_A^F(\infty)} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left(-D_A^P \cdot t \cdot \frac{(2n+1)^2\pi^2}{L^2}\right)$$

The determination of model coefficients can be as time consuming as the actual migration experiments. For this reason, an empirical relationship between the diffusion coefficient and the molecular

weight of the migrant and the temperature was established for LDPE, HDPE, and PP based in published results (Poças, Oliveira et al. 2008):

$$D_A^P = 10^4 \exp\left(A_P - C_1 \cdot M_W^{2/3} + C_2 M_W - \frac{C_3}{T} \right)$$

where T: the temperature in °K, C1, C2, and C3: constants, A_P: a polymer dependent constant, according to:

$$A_P = A_P^{'} - \frac{\tau}{\mathrm{T}}$$

This equation is applicable to migrants of molecular weight Mw in a wide range from 100–2000, and represents an Arrhenius like relationship, with the parameter τ and the constant C_3, both with the dimension of temperature, contributing to the diffusion activation energy. The parameter C_1 results from a correlation between the molar volumes and masses of the series of n-alkanes. C_2 accounts for the decreasing impact of molecular weight on the diffusion coefficient at increasing molecular weights (Poças, Oliveira et al. 2008).

Sanches Silva et al. (2009) transformed the Poças et al. (2008) equation, in order to provide a general description of migration of an additive or contaminant from an amorphous polymeric packaging film:

$$\frac{m_{F,t}}{A} = c_{P,0} \cdot \rho_P \cdot d_P \left(\frac{a}{1+a} \right) \times \left[1 - \sum_{n=1}^{a} \frac{2 \cdot a \cdot (1+a)}{1+a+a^2 \cdot q_n^2} \exp\left(-D_P \cdot t \cdot \frac{q_n^2}{d_P^2} \right) \right]$$

where $m_{F,t}/A$: the amount of the migrated compound after the contact time t (s) of food with the packaging material (mg/cm²), A: the contact area (cm²), $c_{P,0}$: the initial concentration of the migrant in packaging material (ppm), ρ_P and ρ_F: the densities of packaging material and food (g/cm³), respectively, and d_P: the thickness of packaging material (cm).

The partition coefficient ($K_{P/F}$) is calculated from the α value and polymer and food volumes. The V_P for all assays is 0.439 cm³ and the V_F is 7.912 cm³. It is assumed that at the beginning of the mass transfer the migrant is homogeneously distributed in packaging material and that there is no boundary resistance for the transfer between packaging material and food. The migrant is homogeneously distributed in food, and the total amount of the migrant in packaging material and food remains constant during the migration process (Sanches Silva, Cruz Freire et al. 2009).

2.4.2.2 Migration of Paper Packaging

Castle (2004) applied the classical diffusion models based on Fick's 2nd law for the case where migration is controlled by diffusion in the paper packaging material. The diffusion coefficient was estimated as already described. The A_P parameter ranged from 7 to 12 for the migration from different papers into Tenax® (Castle 2004).

However, it has been suggested that assuming a one-dimensional diffusion process has limitations in describing transfer in papers with low porosities and low thicknesses. Therefore, the diffusion model should not be applied to greaseproof and glassine papers that have typically low porosity and low thickness (Hellén, Ketoja et al. 2002).

A kinetic model based on the Weibull distribution function was previously applied to describe migration from plastics additives in comparison with the models based on Fick's 2nd law.

$$\frac{C(t)}{C(\infty)} = 1 - \exp\left[-\left(\frac{t}{\tau}\right)^{\beta}\right]$$

where t: the system time constant or the scale parameter, associated to the process rate and related to the diffusion coefficient and the material thickness, and β the shape parameter, related to the initial rate of the process, quantifying the pattern of curvature observed (Poças, Oliveira et al. 2012).

This model was found to be simple and flexible in describing several mass transfer processes within food engineering area and in food packaging systems, in cases where diffusion theories could not fully explain the mass transfer process. The values obtained for the τ and β parameters of the Weibull model and for the relative migration were analysed in relation to the size of the alkyl chain of the phthalates (CH_n) and the respective boiling point (Pocas, Oliveira et al. 2011). The values of parameter β ranged from 0.5 to 1.5. It increased with the size of the phthalate alkyl chain (or with the boiling point). The increase of β with the number n of methyl groups can be described by the following exponential function:

$$\beta = \alpha\left[1 - \exp\left(-b \cdot n\right)\right]$$

where α and b: constants.

The parameter τ describes the rate of mass transfer and indicates that the higher the n (or the boiling point), the lower the rate of migrant transfer from the paper to the Tenax® is. The behaviour can be described by a function of the type:

$$\tau = \alpha \cdot \exp(b \cdot n)$$

3 Indoor micro-environmental modelling

3.1 General considerations

A two-zone model gives a more realistic depiction of the dispersion process instead of assuming all the room as a homogenous space. Moreover, the lower zone is considered to be the breathing zone for infants and young children. All the calculations used below are carried out taken this differentiation into account. To model these processes, the following formulation (eq. 2 and 3 of a two zone model was used (Pepper 2009):

$$V\frac{dC_1}{dt} = Q\cdot\left(C_a - C_1\right) + E_1 - k\cdot C_1\cdot V_1 + Q_{1-2}\cdot\left(C_2 - C_1\right)$$

$$V\frac{dC_2}{dt} = Q\cdot\left(C_a - C_2\right) + E_2 - k\cdot C_2\cdot V_2 + Q_{1-2}\cdot\left(C_1 - C_2\right)$$

Where:

E₁, E₂ : *the strength of the emission sources (mass/time)*

V₁, V₂ : *the volumes of the respective zones*

Cₐ : *the outdoor concentration*

C : *the indoor concentration of the respective zone*

Q : *the indoor-outdoor air exchange rate*

Q₁₋₂ : ***the air exchange rate between the zones***

k : *the decay rate of the substance in the examined microenvironment*

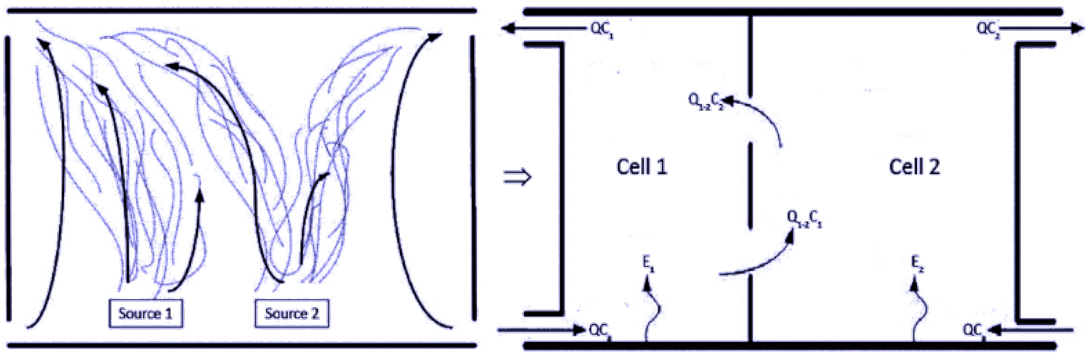

Figure 8. A dual-zone microenvironmental model

3.2 Exchange among gaseous, particles and settled dust phase

The initial component of the modelling framework is the environmental media module. The aim of this module is to describe the interaction of gaseous, particles and dust phase within an indoor location, characterized by DEHP emissions.

According to Weschler et al. (2008), the equilibrium between the gas phase and the surface of the airborne particles is described by an equilibrium constant referred to as the particle–gas partition

coefficient, *Kp*, which describes the fraction of the compound that is adsorbed on the particles (per mass of particles).

According to Weschler et al. (2008), *Kp* is well described by the following empirical formula, reported by Naumova et al. (2003), based on over 1800 partition coefficients for PAHs measured outdoors and indoors in three US cities:

$$\log Kp = -0.860 \cdot \log p_L^0 - 4.67$$

Where P_L^0 is the vapour pressure of the liquid sub-cooled

The $K_{\_dust}$ coefficient which describes the partitioning of DEHP between the airborne phase (including gaseous phase and particles) and the settled dust is calculated empirically based on actual measurements data. More in detail, the fraction between airborne and dust phase of DEHP and other phthalates from actual environmental samples (Fromme, Lahrz et al. 2004) were correlated to their vapour pressure. These data were fitted by non-linear regression (R^2=0.9759), and the $K_{\_dust}$ is estimated by the following formula:

$$K_{\_dust} = 0.0063 \cdot \left(p_L^0\right)^{-0.561}$$

Based on the above considerations and by modifying a generic mass balance model (Pepper 2009), the exchange between gas, particles and dust is described by the following mas-balance differential formulas.

$$V\frac{dC_{chem\_gas}}{dt} = E_{chem\_gas} - Q_{ind\_out}\cdot\left(C_{chem\_gas} - C_{chem\_gas\_out}\right)\cdot V$$
$$-k\cdot C_{chem\_gas}\cdot V - \left(C_{chem\_gas} - \frac{C_{chem\_PM}}{K_p\cdot C_{PM}}\right)\cdot V$$
$$-\left(\left(C_{chem\_gas} + C_{chem\_PM}\right)\cdot V - \frac{C_{chem\_dust}\cdot m_{\_dust}}{K_{\_dust}}\right)$$

$$V\frac{dC_{chem\_PM}}{dt} = \left(C_{chem\_gas} - \frac{C_{chem\_PM}}{K_p\cdot C_{PM}}\right)\cdot V$$
$$-Q_{ind\_out}\cdot\left(C_{PM} - C_{PM\_out}\right)\cdot\frac{C_{chem\_PM}}{C_{PM}}\cdot V$$

$$V\frac{dC_{chem\_dust}}{dt} = \left(\left(C_{chem\_gas} + C_{chem\_PM}\right)\cdot V - \frac{C_{chem\_dust}\cdot m_{\_dust}}{K_{\_dust}}\right)$$

Where,

E_{chem_gas} : chemical emission rate

Q_{int_out} : Indoor/outdoor air exchange rate

K : chemical decay coefficient

K_P : gas/particles partition coefficient

K_{dust} : gas/dust partitioning coefficient

V : location volume

C_{PM} : PM concentration indoors

C_{PM_out} : PM concentration outdoors

C_{DEHP_gas} : chemical concentration in gas phase

C_{DEHP_PM} : chemical concentration in PM phase

C_{DEHP_dusts} : chemical concentration in dust phase

$m_{\_dust}$: mass of dust in the location

4 Exposure modelling

4.1 Route specific exposure analysis

4.1.1 Inhalation exposure mechanisms

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 Ott (1982):

$$C_T = \sum_n f_n \cdot C_n$$

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

$$Uptake_{inh} = \frac{\sum_n E_n \cdot inh_n}{BW}$$

where inh_n is the inhalation rate which is age and activity based depended (ICPR 2002) for each type of microenvironment ***n*** encountered.

4.1.2 Oral exposure mechanisms

4.1.2.1 Dietary ingestion

Dietary exposure sources include water and food contamination that may occur either by a sequence of events through environmental contamination and bioaccumulation (e.g. pesticides, As), either by food contact materials (e.g. Bisphenol A from plastic cups and baby bottles).

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

$$E_{diet} = \sum_{x=1}^{n} \frac{(C_{food_x} \cdot q_{food_x})}{BW}$$

E_{diet}: daily contaminant exposure via the diet (mg/kg·bw·day)

C_{foodx}: contaminant concentration in food item x (mg/kg)

q_{foodx}: food item x consumption (kg/day)

BW : body weight (kg)

4.1.2.2 Non-dietary ingestion

4.1.2.2.1 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. These exposure pathways are addressing particularly infants and toddlers who are known to incidentally ingest small amounts of dust and soil daily.

For estimating the average daily dose from non-dietary ingestion of chemicals from dust we propose to follow the Wormuth et al. (2006) algorithm:

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

where,

E_{dust_ing} : the internal exposure to chemical (mg/kg·bw·day);

C_{dust} : Concentration of the chemical in dust (ug/mg)

q_{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, Scheringer et al. 2006)

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

where,

E_{soil_ing} : the internal exposure to chemical (mg/kg·bw·day);

C_{soil} : Concentration of the chemical in soil (ug/mg)

q_{soil_ing} : Amount of soil ingested (mg/day)

R_{uptake} : absorbed fraction from the ingested quantity

BW : body weight (kg)

4.1.2.2.2 Object-to-mouth

In literature, several sources can be found that describe non-dietary ingestion exposure to chemical residues on objects that are contacted via object-to-mouth activity. One of them is the EPA's EXPOsure toolBOX (EPA-Expo-Box1) which is a toolbox created to assist individuals from within government, industry, academia, and the general public with assessing exposure (USEPA 2013). For estimating the average daily potential dose from ingestion of surface residues from object-to-mouth contact, EPA proposes to use the following algorithm:

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

where,

ADD: Average daily potential dose (mg/kg·bw·day)

$C_{surface\ residue}$: 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/hour)

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

ET : Exposure time (hours/day)

EF : Exposure frequency (days/year)

ED : Exposure duration (years)

BW : body weight (kg)

AT : Averaging time (days)

Temporal parameters in the dose equation include:

- Exposure frequency (EF) refers to the frequency with which the exposure occurs and might be provided in days per year or events per day.
- Exposure duration (ED) is the amount of time that an individual or population is exposed to the contaminant being evaluated and is typically provided in years.
- Averaging time (AT) is the amount of time over which exposure is averaged and is equal to ED for assessing non-cancer risks. For chronic assessments (e.g., cancer), potential lifetime average daily dose (LADD) is calculated in which lifetime (LT, in days) is substituted for AT.

For non-dietary ingestion exposures to chemical residues on surfaces or objects that are contacted via hand-to-mouth or object-to-mouth activity, parameters related to contact frequency and timeframe of exposure are incorporated into the ADD equation.

- Contact rate (CR) represents either the surface area of the hand or the surface area of the object being mouthed.
- Event frequency (EV) represents the number of times the hand or object is mouthed over a specified period of time (e.g., events /hour).
- Exposure time (ET) is added to represent the timeframe over which the transfer of residues from the surface or object to the receptor occurs.

Tulve et al. (2002) describes the total indirect ingestion exposure in two steps: (1) individually for each microactivity, and/or (2) 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:

$$E_{nd} = C_X \cdot TE_X \cdot SA_X \cdot EF$$

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 (μax). For any particular microenvironment (μe) being modeled, the potential exposure is the sum of all exposures for all microactivities conducted in that microenvironment (i.e., indoors at home on carpet). The total indirect ingestion exposure can be described with the following equation:

$$E_{nd/total} = \sum_{\mu e} \sum_{\mu a_x} E_{nd}$$

where,

End/total : total indirect ingestion exposures over a 24-h period (μg/day)

μe : microenvironment

μa_x : microactivity, specific mouthing event in a specific microenvironment

The main differences between the algorithms of Tulve et al. (2002) and EPA (2013) are that Tulve et al. (2002) takes into account transfer efficiency and calculates the dose over a 24-h period and EPA (2013) calculates the average daily dose and doesn't take the transfer efficiency into account. Both algorithms use the frequency of mouthing events.

According to Gorman et al. (2012) equations estimating inadvertent ingestion are of the general form as shown below:

$$E_i = Ld \cdot SA \cdot TE \cdot N$$

where,

E_i : exposure by inadvertent ingestion exposure (mg).

Ld : loading of substances on hand or object (mg/cm^2).

SA : surface area of hand or object that comes in contact with the mouth (cm^2).

TE : transfer efficiency of substance from hands or object to the mouth (proportion).

N : number of hand or object-to-mouth contacts.

Based on the equations above, we propose to calculate the average daily potential dose from ingestion of surface residues from object-to-mouth contact using the following algorithm:

$$E_{nd} = \frac{C_x \cdot TE_x \cdot SA_x \cdot EV \cdot ET \cdot ED \cdot EY}{BW \cdot AT}$$

where,

End : daily potential dose through non-dietary ingestion via object-mouth contact (mg/kg·bw·day)

C_x: concentration of contaminant on object x (μg/cm^2)

TE_x: transfer efficiency, fraction transferred from object x to mouth

SA_x : surface area of x that is mouthed (cm^2/event)

EV : event frequency (events/hour)

ET : exposure time (hours/day)

ED : exposure frequency (days/year)

EY: exposure duration (year)

BW : body weight (kg)

AT :averaging time (days)[2]

4.1.2.2.3 Unintentional swallowing of a substance in a product during normal use

[2] The amount of time over which exposure is averaged and is equal to EY for assessing non-cancer risks. For chronic assessments (e.g. cancer) potential lifetime average daily dose is calculated in which lifetime is substituted for AT

Here, it is assumed that consumers incidentally ingest small amounts of a chemical substance in a consumer product. The most known application of this exposure scenario is relevant to 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. Because no detailed information is available on how much PCPs are ingested daily, 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. The higher amounts ingested should reflect the more careless use of PCPs by infant consumers and the more frequent use of PCPs by female consumers.

The mathematical formulation

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

where,

E_{prod_ing} : the internal exposure to chemical (mg/kg·bw·day);

C_{prod} : Concentration of the chemical in prod (ug/mg)

q_{prod_ing} : Amount of prod ingested (mg/day)

R_{uptake} : absorbed fraction from the ingested quantity

BW : body weight (kg)

4.1.3 Dermal exposure mechanisms

Dermal exposure it determined by the processes involved in contact between the skin and the product or article. Since processes and exposure determinants largely differ between articles and products, different approaches are needed. Also within the category of products, there is a variety of processes playing a role in dermal exposure, depending on the type of the product and its use. For example, dermal exposure to substances in personal care products can be approximated by the applied dose and the surface of the skin of application, whereas dermal exposure to e.g. a substances in household products is not only affected by the dose of used product, 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.

4.1.3.1 Dermal contact with consumer products

Dermal exposure to substances in products is based on model building blocks from ConsExpo (Delmaar, Park et al. 2005). ConsExpo was developed by RIVM and allows users to estimate exposure to chemicals contained in consumer products. It is designed to assess exposure to non-professional indoor uses of chemicals by the inhalation, dermal and ingestion pathway. ConsExpo is a set of mechanistic models, with, in it is most recent version (v5), options for deterministic and probabilistic modelling. In this set of models, there are algorithms for estimating the transfer from products or articles to the skin, followed by the uptake via the skin.

It is considered as a higher model, covering both direct contact, and post-application. The mathematical equations for external exposure are described in the next paragraphs.

4.1.3.1.1 Instant application

The instant application mode assumes that all compound in the product is directly applied to the skin. This is the situation for e.g. 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.

The dermal load (L_{derm} : amount of substance per surface area of skin: mg/cm²) is:

$$L_{derm} = \frac{A_{prod} \cdot Wf}{S_{\exp}}$$

And the external dose (D): amount of substance per kg of bodyweight (mg/kg) is:

$$D = \frac{A_{prod} \cdot wf}{BW}$$

where,

A_{prod}: amount of product applied to the skin [kg]

wf : the weight fraction of the compound in the product [fraction]

S_{exp}: the surface area of the exposed skin [m2]

BW : the bodyweight of the exposed person [kg]

4.1.3.1.2 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.

$$L_{derm} = \frac{R \cdot t \cdot wf}{S_{exp}}$$

and the external dose as:

$$D = \frac{R \cdot t \cdot wf}{BW}$$

where,

R : the rate at which the product is applied to the skin [kg/s]

t : the loading time [s]

wf : the weight fraction of the compound in the product [fraction]

S_{exp} : the surface area of the exposed skin [m3]

BW : the body weight of the exposed person [kg]

4.1.3.1.3 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 (table top, floor) is treated with a product and dermal exposure arises from contact with the treated surface.

$$L_{derm} = \frac{S_{area} \cdot F_{dislodge} \cdot wf}{S_{exp}}$$

and the external dose as:

$$D = \frac{S_{area} \cdot F_{dislodge} \cdot wf}{BW}$$

$$S_{area} = \max(R_{trans} \cdot t, S_{\max})$$

where,

S_{area}: the total area rubbed during exposure, determined by the transfer coefficient R_{trans}, limited by S_{max}, the total treated surface [m^2]

S_{max}: the maximal area that can be rubbed during exposure, for instance the surface area of the entire floor of the room where exposure takes place [m^2]

wf : the weight fraction of the compound in the product [fraction]

$F_{dislodge}$: the dislodgeable amount: amount of product or used formulation that can be rubbed off per unit surface area [kg/ m^2]

S_{exp}: the surface area of the exposed skin [m^2]

BW: the body weight of the exposed person [kg]

R_{trans} : area rubbed per unit time [m^2/s]

4.1.3.1.4 Spray model

In 2006 the German Fraunhofer Institute developed SprayExpo (Koch, Berger-Preiß et al. 2004). The SprayExpo tool is a mechanistic, deterministic model to predict the inhalation and dermal exposure during spray application of biocidal products. SprayExpo calculates the airborne concentration of the respirable, thoracic and the inhalable spray aerosol, or any other size fraction of aerosols containing biocides in indoor environments. The model is based on a simulation of the motion of released droplets taking into account gravitational settling, turbulent mixing with the surrounding air, and droplet evaporation. The dermal exposure assessment is based on an assessment of deposition to the body by sedimentation (assumed that 10% of the body surface is horizontal) and by turbulent diffusion using the calculated air concentration.

The model defines one scenario, namely spray application of biocidal products when spraying along a line, treating a certain area of the walls, ceiling or floor, or treating the room volume.

Notwithstanding that the model has been primarily developed for workplace exposure, it is actually process-oriented (spraying) and therefore considered relevant for the consumer domain as well (similar processes and conditions). The model does not contain workplace or worker specific parameters or protection measures and neither it contains biocides-specific input or parameters, and can thus be applied to beyond the field of biocides, for other chemicals.

Parameters used in this model that are considered relevant for consumer exposure are:

- Room size
- Ventilation
- Release patterns
- Droplet spectrum

- Substance data
- Spray nozzle characteristics

The full mathematical description of SprayExpo can be found at the website of the *Bundesanstalt für Arbeitsschutz und Arbeitsmedizin*[3].

4.1.3.2 Substances in articles – direct contact

Concepts from the CEFIC LRI project DRESS (B9) are used as building blocks for the CROME-LIFE module dermal exposure to substances in articles.

Dermal load to substances released from articles to the skin is determined by 1) the movement from of substances within the article to the surface (diffusion driven process), by 2) the solubility of substances at the article-skin interface, and 3) the mechanical retention of substances by the skin (adherence). These factors are driven by physico-chemical properties of articles and substances, contact pressure, duration,...

Depending on data availability, several options are available to calculate the dermal load and external exposure

The dermal load can be expressed as:

$$L_{derm} = ASRA \cdot t \cdot adherence\ factor \cdot skin\ contact\ factor$$

where,

t : duration of contact

Skin contact factor: a factor that can be used to account for the fact that the material is only partially in contact with the skin (expressed as %)

Adherence factor: a factor that can be used to account for the fact that the amount of substances in contact with skin is only partly retained by the skin (dimensionless)

ASRA : Amount of Substance Released from Article per unit area per unit of time (mg/cm²/h)

ASRA can be determined in several ways; depending on data availability (see paragraphs 4.1.3.2.1, 4.1.3.2.2, 4.1.3.2.3)

4.1.3.2.1 Leachable amounts or fraction

Based on experimental results of artificial sweat extraction (incorporating both diffusion and solubility aspects), ASRA can be set equal to "amount of substance released from artificial sweat per area during duration t".

In case the leachable amount is expressed as a fraction of the total concentration of substance in the article leachable to the artificial sweat ($fraction_{leachable}$):

$$ASRA = fraction_{leachable} \cdot C_{area}$$

where,

*ASRA : a*mount of Substance Released from Article per unit area per unit of time (mg/cm²/h)

[3] http://www.baua.de/en/Topics-from-A-to-Z/Hazardous-Substances/SprayExpo.html

$fraction_{leachable}$: fraction of substance released to artificial sweat within time t (% per unit of time)

C_{area} : amount of substance in article per unit area (mg/cm²)

C_{area} can be calculated as:

$$C_{area} = C \cdot Th$$

where,

C: substance concentration in article (mg/cm³)

Th: article thickness (cm)

4.1.3.2.2 Diffusion model

The mathematical equation of the diffusion model, based on the Fick's 2nd law, can be expressed as the following one-dimensional diffusion equation (Delmaar, Bokkers et al. 2013):

$$\frac{dA}{dt} = S \times D \times \left.\frac{\partial C}{\partial x}\right|_{surface}$$

Where,

A = amount of substance released per article (g)

C = concentration of the substance in the article (g/m³)

S = surface area of the article (m²)

D = diffusion coefficient (m²/s)

c = position in the material (m)

t = time (s)

To approximate the diffusion in a material a simple diffusion layer model may be applied, based on a finite difference approach instead of the complete diffusion model. This simplified diffusion model considers an average distance 'l' ($= \sqrt{2 \times D \times t}$) over which a diffusing molecule will travel, and therefore the original diffusion model can be simplified to the following analytical expression for slab-like articles (Delmaar, Bokkers et al. 2013):

$$ASRA(t) = C_0 \cdot S \cdot \sqrt{2 \cdot D \cdot t}$$

Or expressed per unit of surface area of the article (ASRA):

$$ASRA(t) = C_0 \cdot \sqrt{2 \cdot D \cdot t}$$

where D is the diffusion coefficient.

Diffusion coefficient is article and substance specific. Diffusion coefficients can be determined experimentally, or can be estimated from empirical models for prediction of diffusion coefficients. Holmgren et al. (2012) published the following empirical equation, developed for predicting diffusion rates of additives from food packaging to food (Begley, Castle et al. 2005, Piringer and Banner 2008):

$$D = \exp\left(A_p - 0.1351(Mw)^{2/3} + 0.003Mw - \frac{10450}{T} \right)$$

Where,

D : diffusion coefficient

A_p : material specific coefficient

Mw : molecular weight

T : time

4.1.3.2.3 Mass balance approach

By lack of information on diffusion coefficients or leachable amounts or fractions, one may also apply – as a very worst case approach - the mass balance principle:

$$ASRA = C \cdot Th$$

Where,

Th : thickness of the article (in cm)

C : concentration of the substance in the article (g/cm^3)

5 Internal exposure modeling framework

5.1 Conceptual description

The model is designed to describe as much as possible the actual ADME processes occurring in human body, so as to be easily applicable for a broad variety of chemicals under proper parameterization. The model will include the parent compound and a number of three potential metabolites. For each compound/metabolite all major organs will be included (Figure 9) and the link among the compounds and the metabolites will be through the metabolizing tissues. This is mainly the liver, but also other sites of metabolism might be considered based on the presence or not of the enzymes involved in the metabolism of the compound of interest.

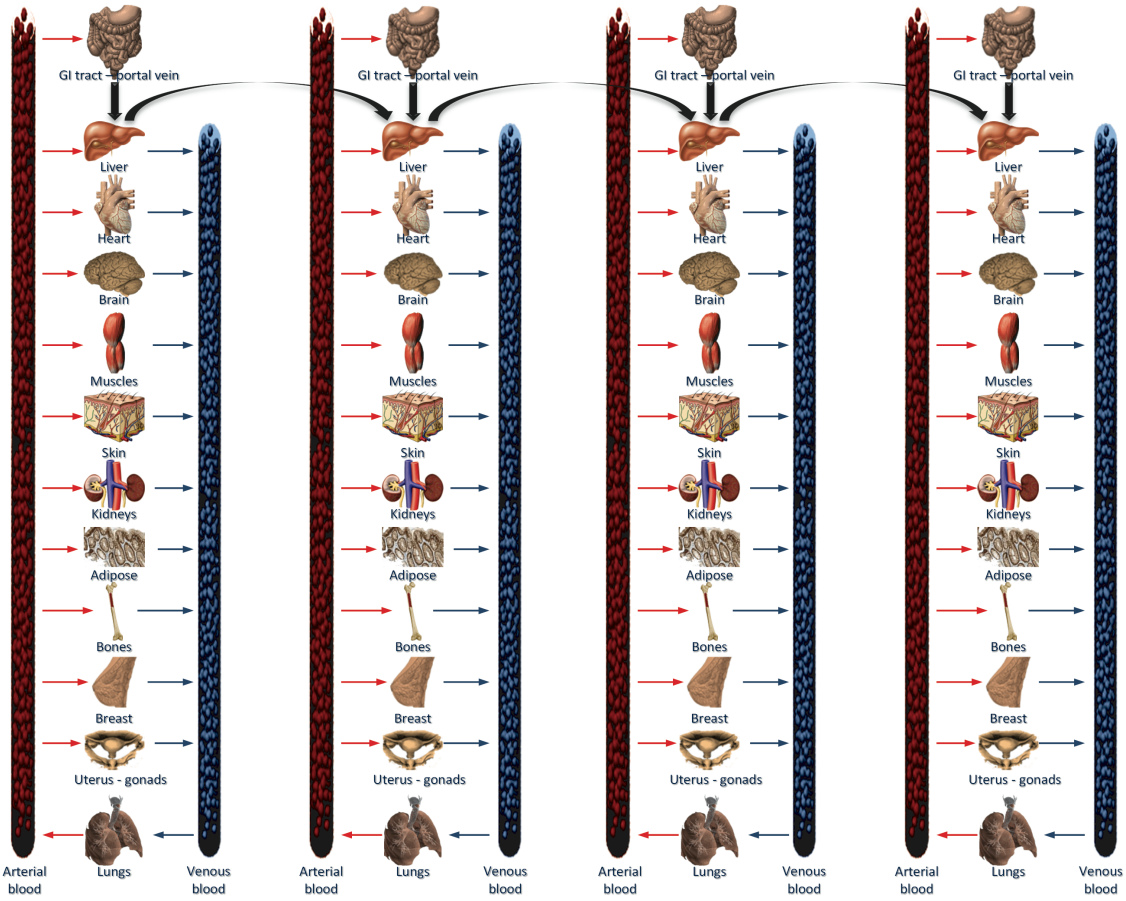

Figure 9. The generic PBTK model (parent compound and 3 metabolites)

In order to capture the in-utero exposure, the model is also replicated in order to describe the functional interaction of the mother and the developing fetus through the placenta. The anthropometric parameters of the models are age dependent, so as to provide a lifetime internal dose assessment. Details on the mathematical framework so as to describe the structure of the generic-lifetime PBTK model are given in the following sections.

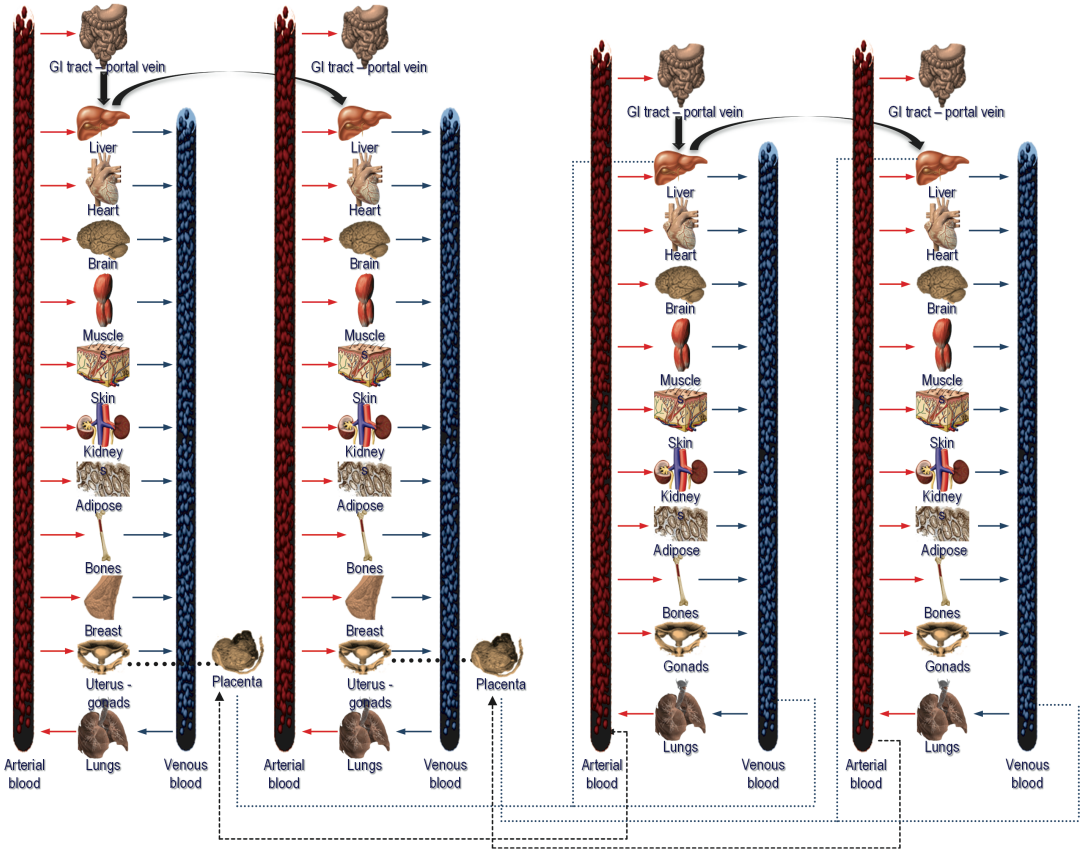

Figure 10. Conceptual representation of the Mother-Fetus PBTK model

5.2 Components of the generic life-time CROME PBTK model

5.2.1 Formulation describing the generic multi-route PBTK model

The following formulas are based on the formulation scheme proposed by Edginton and Ritter:

For non-eliminating organs:

Red blood cells

$$V_{rbc\_org}\frac{dC_{rbc\_org}}{dt} = Q_{org}\cdot HCT\cdot\left(C_{rbc\_art} - C_{rbc\_org}\right)$$

$$+PS_{rbc\_org}\cdot f_u\cdot\left(C_{\text{int}\_org} - \frac{C_{rbc\_org}}{K_{rbc}}\right)$$

Where $V_{rbc\_org} = f_{vas\_org}\cdot V_{org}\cdot HCT$

Plasma +interstitial

$$V_{\text{int\_org}}\frac{dC_{\text{int\_org}}}{dt}=Q_{org}\cdot\left(1-HCT\right)\cdot\left(C_{pls\_art}-C_{\text{int\_org}}\right)$$
$$-PS_{rbc\_org}\cdot f_u\cdot\left(C_{\text{int\_org}}-\frac{C_{rbc\_org}}{K_{rbc}}\right)$$
$$-PS_{cell\_org}\cdot f_u\cdot\left(C_{\text{int\_org}}-\frac{C_{cell\_org}}{K_{org}}\right)$$

Where $V_{\text{int\_org}}=V_{org}\cdot\left[f_{\text{int\_org}}+f_{vas\_org}\cdot\left(1-HCT\right)\right]$

Cellular

$$V_{cell\_org}\frac{dC_{cell\_org}}{dt}=PS_{cell\_org}\cdot f_u\cdot\left(C_{\text{int\_org}}-\frac{C_{cell\_org}}{K_{org}}\right)$$

Where $V_{cell\_org}=f_{cell\_org}\cdot V_{org}$

Kidney

For kidney renal plasma clearance (CLpls_kid) is incorporated:

Plasma + Interstitial

$$V_{\text{int\_kid}}\frac{dC_{\text{int\_kid}}}{dt}=Q_{kid}\cdot\left(1-HCT\right)\cdot\left(C_{pls\_art}-C_{\text{int\_kid}}\right)-PS_{rbc\_kid}\cdot f_u\cdot\left(C_{\text{int\_kid}}-\frac{C_{rbc\_liv}}{K_{rbc}}\right)$$
$$-PS_{cell\_kid}\cdot f_u\cdot\left(C_{\text{int\_kid}}-\frac{C_{cell\_kid}}{K_{kid}}\right)-\frac{CL_{pls\_kid}\cdot Q_{kid}\cdot\left(1-HCT\right)\cdot C_{\text{int\_kid}}}{\left[Q_{kid}\cdot\left(1-HCT\right)-CL_{pls\_kid}\right]}$$

Portal vein

Red blood cells

$$HCT\cdot V_{pv}\frac{dC_{rbc\_pv}}{dt}=Q_{GI\_tract}\cdot HCT\cdot\left(C_{rbc\_GI\_tract}-C_{rbc\_pv}\right)$$
$$+PS_{rbc\_pv}\cdot f_u\cdot\left(C_{pls\_pv}-\frac{C_{rbc\_org}}{K_{rbc}}\right)$$

Plasma + interstitial

$$\left(1-HCT\right)\cdot V_{pv}\cdot\frac{dC_{pls\_pv}}{dt}=Q_{GI\_tract}\cdot\left(1-HCT\right)\cdot\left(C_{\text{int\_GI\_tract}}-C_{pv}\right)$$
$$-PS_{rbc\_pv}\cdot f_u\cdot\left(C_{pls\_pv}-\frac{C_{rbc\_pv}}{K_{rbc}}\right)$$

For venous blood

Red blood cells

$$HCT \cdot V_{ven} \cdot \frac{dC_{rbc\_ven}}{dt} = \sum_{org} Q_{org} \cdot HCT \cdot C_{rbc\_org} + PS_{rbc\_ven} \cdot f_u \cdot \left(C_{pls\_ven} - \frac{C_{rbc\_ven}}{K_{rbc}} \right) - Q_{lung} \cdot HCT \cdot C_{rbc\_ven}$$

Plasma +interstitial

$$(1 - HCT) \cdot V_{ven} \frac{dC_{pls\_ven}}{dt} = \sum_{org} Q_{org} \cdot (1 - HCT) \cdot C_{\text{int}\_org} - PS_{rbc\_ven} \cdot f_u \cdot \left(C_{pls\_ven} - \frac{C_{rbc\_ven}}{K_{rbc}} \right) - Q_{lung} \cdot (1 - HCT) \cdot C_{pls\_ven}$$

For arterial blood

Red blood cells

$$HCT \cdot V_{art} \cdot \frac{dC_{rbc\_art}}{dt} = -\sum_{org} Q_{org} \cdot HCT \cdot C_{rbc\_art} + PS_{rbc\_art} \cdot f_u \cdot \left(C_{pls\_art} - \frac{C_{rbc\_art}}{K_{rbc}} \right) + Q_{lung} \cdot HCT \cdot C_{rbc\_art}$$

Plasma +interstitial

$$(1 - HCT) \cdot V_{art} \frac{dC_{pls\_art}}{dt} = -\sum_{org} Q_{org} \cdot (1 - HCT) \cdot C_{\text{int}\_art} - PS_{rbc\_art} \cdot f_u \cdot \left(C_{pls\_art} - \frac{C_{rbc\_art}}{K_{rbc}} \right) + Q_{lung} \cdot (1 - HCT) \cdot C_{pls\_art}$$

For liver:

Red blood cells

$$V_{rbc\_liv}\frac{dC_{rbc\_liv}}{dt} = HCT\cdot\left(Q_{liv}\cdot C_{rbc\_art} + Q_{pv}\cdot C_{rbc\_pv}\right) - \left(Q_{liv}\cdot C_{rbc\_art} + Q_{pv}\cdot C_{rbc\_pv}\right) + PS_{rbc\_org}\cdot f_u\cdot\left(C_{\text{int}\_org} - \frac{C_{rbc\_org}}{K_{rbc}}\right)$$

Plasma + Interstitial

$$V_{\text{int}\_liv}\frac{dC_{\text{int}\_liv}}{dt} = \left(1-HCT\right)\cdot\left(Q_{liv}\cdot C_{pls\_art} + Q_{pv}\cdot C_{pls\_pv}\right) - \left(Q_{liv}+Q_{pv}\right)\cdot\left(1-HCT\right)\cdot C_{\text{int}\_liv} - PS_{rbc\_liv}\cdot f_u\cdot\left(C_{\text{int}\_liv} - \frac{C_{rbc\_liv}}{K_{rbc}}\right) - PS_{cell\_liv}\cdot f_u\cdot\left(C_{\text{int}\_liv} - \frac{C_{cell\_liv}}{K_{liv}}\right)$$

Cellular

$$V_{cell\_liv}\frac{dC_{cell\_liv}}{dt} = PS_{cell\_liv}\cdot f_u\cdot\left(C_{\text{int}\_liv} - \frac{C_{cell\_liv}}{K_{li}}\right) - \frac{CL_{liv\_met}\cdot C_{cell\_liv}\cdot f_u}{K_{liv}}$$

where CL_{liv} is the intrinsic clearance and is calculated from the plasma clearance using the well stirred liver model.

For lungs:

$$V_{\text{int}\_lung}\cdot\frac{dC_{\text{int}\_lung}}{dt} = Q_{lung}\cdot\left(1-HCT\right)\cdot\left(C_{pls\_ven} - C_{\text{int}\_lung}\right) - PS_{rbc\_lung}\cdot f_u\cdot\left(C_{\text{int}\_lung} - \frac{C_{rbc\_lung}}{K_{rbc}}\right) - PS_{cell\_lung}\cdot f_u\cdot\left(C_{\text{int}\_lung} - \frac{C_{cell\_lung}}{K_{rbc}}\right) + Q_{vent}\cdot C_{amb\_air}\cdot P_{air} - Q_{vent}\cdot\left(\frac{C_{\text{int}\_lung}}{P_{air}}\cdot\left(1-V_{ds}\right) + C_{amb\_air}\cdot V_{ds}\right)$$

5.2.2 Mother-fetus interaction

For uterus:

Plasma +interstitial

$$V_{\text{int}\_uterus}\frac{dC_{\text{int}\_uterus}}{dt} = Q_{uterus}\cdot\left(1-HCT\right)\cdot\left(C_{pls\_art} - C_{\text{int}\_uterus}\right) - K_{d\_uterus\_pla}\cdot\left(C_{placenta} - C_{uterus\_M}\right) - PS_{rbc\_uterus}\cdot f_u\cdot\left(C_{\text{int}\_uterus} - \frac{C_{rbc\_uterus}}{K_{rbc}}\right) - PS_{cell\_uterus}\cdot f_u\cdot\left(C_{\text{int}\_uterus} - \frac{C_{cell\_uterus}}{K_{rbc}}\right)$$

beside the assumption of equal diffusion flow from uterus to placenta and vice-versa during pregnancy, uterus behaves like other organs.

For placenta:

$$V_{\text{int\_placenta}}\frac{dC_{\text{int\_placenta}}}{dt} = K_{d\_uterus\_pla}\cdot\left(C_{\text{int\_placenta}} - C_{\text{int\_uterus}}\right) - K_{d\_pla\_amniot}\cdot\left(C_{\text{int\_placenta}} - C_{amniot}\frac{P_{placenta}}{P_{amniot}}\right)$$

$$+Q_{placenta\_fetus}\cdot\left(C_{\text{int\_art\_fetus}} - \frac{C_{\text{int\_placenta}}}{P_{placenta}}\right) + K_{glu\_deconj}\cdot Q_{placenta\_fetus}\cdot C_{\text{int\_placenta\_glu}}$$

$$-PS_{rbc\_placenta}\cdot f_u\cdot\left(C_{\text{int\_placenta}} - \frac{C_{rbc\_placenta}}{K_{rbc}}\right) - PS_{cell\_placenta}\cdot f_u\cdot\left(C_{\text{int\_placenta}} - \frac{C_{cell\_placenta}}{K_{rbc}}\right)$$

For breast:

$$V\frac{dC_{\_breast}}{dt} = PS_{\_cell\_breast}\cdot f_u\cdot\left(C_{\_\text{int\_breast}} - \frac{C_{\_breast}}{K_{\_breast}}\right) - L_{excr}$$

and the related excretion via lactation

$$L_{excr} = Q_{\_milk}\cdot\frac{C_{\_breast}}{K_{\_breast}}\cdot P_{\_milk/blood}$$

$$P_{\_milk/blood} = \frac{K_{ow}\cdot Fl_{\_tissue} + Fw_{\_tissue}}{K_{ow}\cdot Fl_{\_blood} + Fw_{\_blood}}$$

5.2.3 Lifetime scaling

The parameters related to organ volumes (***V***) and blood flows (***Q***) were taken from the ICRP (ICPR 2002) report and fitted to time (***t in hours***) in order to derive continuous time depended non lineal polynomial formulas in the form of:

$V = a\cdot t^b + c\cdot t^d + e\cdot t + f$ for organ volumes

$Q = a\cdot t^b + c\cdot t + d$ for organ flows

Table 3. Regression coefficients for lifetime scaling (from conception to adulthood)

	organ volumes (mL)						organ flows (mL/min)			
	a	***b***	***c***	***d***	***e***	***f***	***a***	***b***	***c***	***d***
Portal vein	1.00E-01	1.00E+00	9.80E-02	9.96E-01	0.00E+00	5.70E+01	6.09E-02	-2.61E-02	1.06E+00	1.14E+02
Adipose	2.54E-02	1.00E+00	1.88E+01	5.20E-01	0.00E+00	9.06E+02	1.17E-01	1.00E-01	1.01E+00	3.00E+01

	organ volumes (mL)						organ flows (mL/min)			
	a	*b*	*c*	*d*	*e*	*f*	*a*	*b*	*c*	*d*
Bones	5.97E-02	1.00E+00	1.26E+00	6.10E-01	0.00E+00	4.52E+02	2.00E-02	1.04E-02	1.05E+00	3.00E+01
Brain	-5.03E-02	1.00E+00	9.07E-01	7.69E-01	0.00E+00	3.95E+02	-3.99E-01	7.10E-01	9.52E-01	1.80E+02
Gonads	8.25E-02	1.00E+00	8.31E-02	9.99E-01	0.00E+00	1.10E+00	3.57E-02	-3.56E-02	1.00E+00	3.00E-01
Heart	4.68E-02	1.00E+00	-3.81E-02	1.01E+00	0.00E+00	2.80E+01	4.08E-03	1.81E-05	1.41E+00	2.40E+01
Kidneys	3.17E-02	1.00E+00	1.44E-02	1.06E+00	0.00E+00	3.80E+01	3.86E-02	-7.90E-03	1.11E+00	1.10E+02
Liver	2.79E-03	1.00E+00	1.10E+00	6.03E-01	0.00E+00	1.60E+02	8.54E-03	-3.51E-04	1.24E+00	3.90E+01
GI tract	8.20E-02	1.00E+00	4.41E-02	1.04E+00	0.00E+00	9.00E+01	6.09E-02	-2.61E-02	1.06E+00	1.14E+02
Muscle	1.26E-01	1.00E+00	7.76E-06	1.76E+00	0.00E+00	9.50E+02	1.00E-01	-1.03E-01	9.92E-01	3.10E+01
Skin	2.88E-01	1.00E+00	2.71E-01	9.98E-01	0.00E+00	2.00E+02	1.06E-02	-2.72E-03	1.10E+00	3.00E+01
Lungs	9.74E-02	1.00E+00	6.33E-02	1.03E+00	0.00E+00	8.40E+01	-4.98E-01	9.94E-01	9.48E-01	5.58E+02
Arterial/venous blood	1.26E-01	1.00E+00	-1.25E-01	9.98E-01	0.00E+00	3.80E+01				
Total blood	1.15E-01	1.00E+00	-1.10E-01	9.92E-01	0.00E+00	1.33E+02				
WEIGHT	3.90E-01	1.00E+00	8.40E+01	3.66E-01	0.00E+00	3.21E+03				
HCT	-1.00E-01	9.66E-12	-1.00E-01	1.55E-06	5.50E-07	5.80E-01				

Table 4. Gestation parameters (from conception to birth)

	organ volumes (mL)						organ flows (mL/min)			
	a	*b*	*c*	*d*	*e*	*f*	*a*	*b*	*c*	*d*
Uterus	3.90E-01	1.00E+00	8.40E+01	3.66E-01	0.00E+00	3.21E+03	3.57E-02	-3.56E-02	1.00E+00	3.00E-01
Placenta	3.90E-01	1.00E+00	8.40E+01	3.66E-01	0.00E+00	3.21E+03	3.57E-02	-3.56E-02	1.00E+00	3.00E-01
Amniotic fluid	3.90E-01	1.00E+00	8.40E+01	3.66E-01	0.00E+00	3.21E+03				
Breast										

5.2.4 Description of absorption through multiple routes

5.2.4.1 Inhalation absorption model description

The ability to describe inhalation exposures is an important consideration for modeling inhalation pharmacokinetics. By modeling the lungs as a well-mixed compartment with an average, one-directional airflow in the region of gas exchange (i.e., with air moving through the lungs with a constant flow rate equal to the alveolar ventilation rate, Q_P], and with rapid equilibration between lung air and blood in the lung alveoli, the concentration in the blood exiting the lungs, C_a, can be described as (Ramsey and Andersen 1984, Reddy, Yang et al. 2005):

$$C_\alpha = \frac{Q_P \cdot C_{in} + Q_C \cdot C_{BLV}}{Q_C + Q_P / P_b}$$

where C_{BLV} is the chemical concentration in the venous blood compartment, P_b is the blood:air partition coefficient, Q_C is the cardiac output, and C_{in} is the inhaled concentration of chemical during the exposure and zero after the exposure ends.

To determine the concentration of chemical in exhaled air, C_{ex}, the concentration of chemical in alveolar air, C_{alv} (i.e., C_a/P_b], must be adjusted for the concentration of chemical in the dead space of the lungs (i.e., the volume of the lungs where gas exchange does not occur) as follows:

$$C_{ex} = F_{DS} \cdot C_{in} + \left(1 - F_{DS}\right) \cdot C_{alv}$$

where F_{DS} is the fraction of dead space in the lungs, which is about 0.33 in humans under typical physiological conditions.

The overall amount of air inhaled by an average adult is equal to about 20 m^3. It is clear that from the above formula that the contribution of the activity pattern clearly affects the overall intake and distribution within the tissues, through the ventilation rate Q_P and the cardiac output Q_C. When exercising, a higher amount of the body's blood rushes to the muscles to help fuel the physical activity. This surging of blood often results in a need for more oxygen in the blood than is present, and thus the body's breathing rate increases. Considering that the daily average respiratory rate for an adult ranges between 12-20 breaths per minute, it increases to 35-45 breaths per minute, depending on the burden of effort with a subsequent increase of the overall inhaled air and the corresponding uptake of the contaminants included

5.2.4.2 Skin absorption model description

The multi-compartmental model is described using a unity of block diagram format. Each block represent a different compartment, since that type of programming enforces the clear conceptual divisions of the model's compartments. The skin has been modeled with a two layer structure: Stratum corneum (SC) and viable epidermis (VE). The stratum corneum has been described with a "bricks and mortar" structure (Touitou 2002). The structure is presented in Figure 11. Current understanding of solute binding to keratin and other corneocyte constituents of the SC encompasses mostly equilibrium binding, and macro-scale parameterizations of transient binding for a few solutes. Proposed work leverages the latest and emerging results on homogenization theory and rates of binding to produce a broad mechanistic SC model that quantifies transient solute binding in terms of coexisting free and bound concentration fields, and is parameterized at the microscopic scale. This is critical to realistically describe actual chemical exposure, in which most of the penetration often occurs before reaching steady state.

The geometry of the microstructure has been investigated and described by many authors (Johnson, Blankschtein et al. 1997, Ya-Xian, Suetake et al. 1999, Frasch and Barbero 2003, Wang, Kasting et al. 2006, Mitragotri, Anissimov et al. 2011). The characteristics of the skin are presented in Table 3.

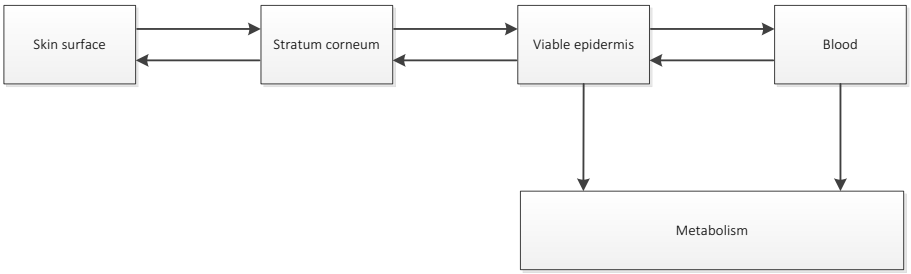

Figure 11. Skin multi-compartmental model

Table 5. Skin characteristics

Description	Symbol	Value	Unit	Reference
Number of layer	N	15	-	(Ya-Xian, Suetake et al. 1999, Bronzino 2000)
Length of corneocyte	d	30	μm	(Wang, Kasting et al. 2006)
Thickness of corneocyte	t	10	μm	(Wang, Kasting et al. 2006)
Length of path 1	d_1	20	μm	(Johnson, Blankschtein et al. 1997)
Length of path 2	d_2	10	μm	(Johnson, Blankschtein et al. 1997)
Vertical gaps	s	0.03	μm	(Johnson, Blankschtein et al. 1997)
Horizontal gaps	g	0.03	μm	(Johnson, Blankschtein et al. 1997)
High of viable epidermis	hv	3	mm	(Bronzino 2000)
corneocyte edge angle	ϕ	90°	degrees	(Rim, Pinsky et al. 2007)
Effective Diffusivity f(ϕ)	D_{ef}	0.002	cm^2/m	(Rim, Pinsky et al. 2007)

The effective tortuosity of the stratum corneum has been calculated according to the method of Johnson et al. (Johnson, Blankschtein et al. 1997):

$$t = 1 + \frac{2g}{h} \cdot \ln\left(\frac{d}{2s}\right) + \frac{N \cdot d \cdot t}{s \cdot h} + \left(\frac{d}{1+\omega}\right)^2 \cdot \frac{\omega \cdot (N-1)}{h \cdot g}$$

Where h is the total thickness of the SC and ω is the ratio between path d1 and d2.

The model uses first order equations for the diffusion. In particular it is assumed that the layer SC retains its form and the viable epidermis is assumed as a homogenous well-mixed layer.

$$\frac{dC_S}{dt} = -D_1 \cdot C_S + D_2 \cdot C_{SC} - evap$$

$$\frac{dC_{SC}}{dt} = D_1 \cdot C_S - D_2 \cdot C_{SC} - D_3 \cdot C_{SC} + D_4 \cdot C_{ve}$$

$$\frac{dC_{ve}}{dt} = D_3 \cdot C_{SC} - D_4 \cdot C_{ve} - M - Q_b \cdot C_{ve}$$

Where C_s is the solute concentration on the skin surface that permeates through a volume of skin, C_{sc} is the solute concentration in the SC and depends on its lipid volume, lipid volume is the layer between the corneocytes and it depends on the geometry of SC, evap is the rate of the evaporation to atmosphere, C_{vs} is the permeant concentration in the viable skin, M is the rate of metabolism and Q is the blood flow rate. Moreover, D_1 and D_2 are fractions of permeate, related to the partitioning coefficient between solvent and SC, the permeant diffusivity, effective diffusivity and the geometry of SC (Nitsche, Wang et al. 2006). D_1 and D_4 are functions of the solvent/stratum partitioning coefficient, the permeant diffusivity in the SC lipid and the thickness of SC and the implementation area. D_3 and D_4 are the partition coefficient between water and SC ($K_{sc/w}$). $K_{sc/w}$ is given by the volume average (Nitsche, Wang et al. 2006):

$$K_{SC/W} = \varphi_{lip} \cdot K_{lip/w} + \varphi_{cor} \cdot K_{cor/w} \;\&\; \varphi_{lip} = 1 - \varphi_{cor}$$

Where φ_{lip} and φ_{cor} denotes the volume fractions of the lipid and corneocyte phases, respectively, with φ_{lip}=0.11 for partially hydrated SC (Nitsche, Wang et al. 2006).

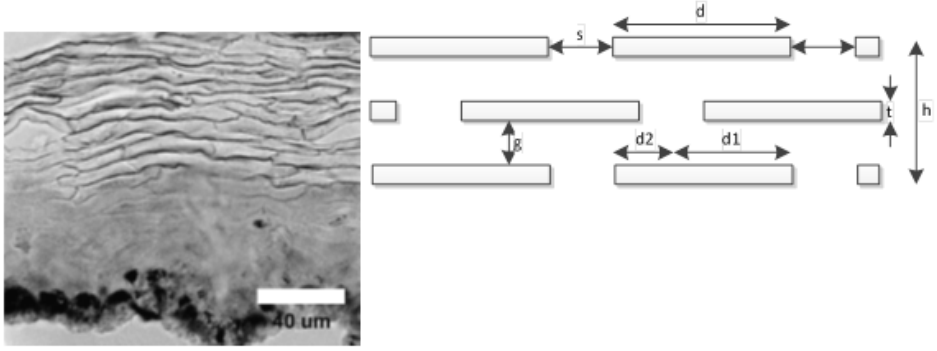

Figure 12. a) Micrograph of human stratum corneum expanded in alkaline buffer showing the stacking of corneocytes layers and the surrounding lipid matrix (Talreja, Kasting et al. 2001), b) SC brick and mortal geometrical structure representation.

Also, $K_{cor/w}$ is given by volume exclusion from the fraction of the corneocyte phase occupied by keratin microfibrils. Under the assumption that there is no solute adsorption to the protein, then $K_{o/w}$=1-φ_f, where φ_f=0.1928, representing the keratin microfibrils occupied fraction (Nitsche, Wang et al. 2006).

The $K_{lip/w}$ has been calculated according to Nitsche et al. (2006) who have proposed an approximation for the predicting permeability taking into account the $K_{o/w}$:

$$K_{lip/w} = 0.43 \cdot K_{o/w}^{0.81}$$

The approximation for the predicting permeability is based on equation of Johnson et al. (1997):

$$\log k_p = 0.71 \cdot \log k_{oct} - 0.0061 \cdot MW - 6.3$$

Also, the effective lag time for the SC is given by the following relationship (Cleek and Bunge 1993, Bunge and Cleek 1995, Wang, Kasting et al. 2006):

$$\tau_{lag} = \frac{(h_{sc})^2}{6D_{ef}}$$

Where D_{ef} is the effective diffusivity and h_{sc} is the thickness of the layer according to the geometry path length proposed by Talreja et al. (Talreja, Kasting et al. 2001):

$$h_{sc} = \left(\frac{d_2}{\frac{N}{N-1} t + g} + 1 \right) \cdot h$$

where h is the total length of layer according the geometrical assumptions of the skin model.

The flow blood rate (Q) to skin layers is similar to that of a 73-kg person and it is 85.8 l/h (Valentin 2002, Abraham, Mielke et al. 2005).

Metabolism rate is given by (Abraham, Mielke et al. 2005):

$$M = \left(\frac{V_{\max} C_{ve}}{K_m + C_{ve}} \right)$$

Where V_{max} is the maximum rate of elimination μg/min and K_m is the permeant concentration (in venous blood) at 50% of V_{max}.

Evaporation has been calculated based on the REACH technical guidance and is given by the following equations:

$$Evaporation\ Rate = \frac{\beta \cdot MW \cdot V_p}{R \cdot T \cdot 10}$$

$$\beta = \frac{0.0111 \cdot V^{0.96} \cdot D_g^{0.19}}{v^{0.15} \cdot X^{0.04}}$$

where MW is the molecular weight of the substance, V_p is the vapor pressure of the liquid at skin temperature (in Pascal), R is the gas constant (in J/Mol/K), T is the skin temperature (in ^{0}K), β is the coefficient of mass transfer in the vapour phase (in m/h), V is the velocity of air (assumed to 0.3 m/s), D_g is the diffusivity of the liquid in the gas phase (0.05 m^2/h), v is the kinematic viscosity of air (0.054 m^2/h) and X is the length of the area of evaporation in the direction of the air stream.

5.2.4.3 Oral absorption model description

The gastrointestinal tract is modelled with four compartments: gut, stomach and their respective lumens. The parameter $Ka_{stomach}$ and Ka_{gut} govern the diffusion of chemicals in the stomach and in the gut, respectively, and then in the systemic circulation. Absorption takes place in the stomach lumen ($Rate_{Ing}$ is the ingestion rate). In gut, chemicals can be metabolized ($QMet_{gut}$):

$$\frac{dQ_{stom_{lumen}}}{dt} = RateIng - \left(F_{stom_{lumen}} + Ka_{stomach}\right) \cdot C_{stom_{lumen}}$$

$$\frac{dQ_{stomach}}{dt} = Ka_{stomach} \cdot C_{stom_{lumen}} + F_{stomach} \cdot \left(C_{art} + \frac{C_{stomach}}{PC_{stomach}}\right)$$

$$\frac{dQ_{gut_{lumen}}}{dt} = F_{stom_{lumen}} \cdot C_{stom_{lumen}} + Ke_{bile} \cdot C_{liver} - \left(Ka_{gut} + F_{gut_{lumen}}\right) \cdot C_{gut_{lumen}}$$

$$\frac{dQ_{gut}}{dt} = Ka_{gut} \cdot C_{gut_{lumen}} + F_{gut} \cdot \left(C_{art} - \frac{C_{gut}}{PC_{gut}}\right) - QMet_{gut}$$

Outputs of spleen, pancreas, stomach and gut feed liver, as well as an arterial entry. In liver, chemicals can be eliminated via bile (Ke_{bile}) or metabolized ($QMet_{liver}$).

$$\frac{\partial Q_{liver}}{\partial t} = F_{liver_{art}} \cdot C_{art} + F_{spleen} \cdot \frac{C_{spleen}}{PC_{spleen}} + F_{pancreas} \cdot \frac{C_{pancreas}}{PC_{pancreas}} + F_{gut} \cdot \frac{C_{gut}}{PC_{gut}}$$
$$+ F_{stomach} \cdot \frac{C_{stomach}}{PC_{stomach}} - F_{liver} \cdot \frac{C_{liver}}{PC_{liver}} - Ke_{bile} \cdot C_{liver} - QMet_{liver}$$

The sorting blood flow in liver is then given by:

$$F_{liver} = F_{liver_{art}} + F_{gut} + F_{pancreas} + F_{spleen} + F_{stomach}$$

6 Applications

6.1 Methodological framework

The main process is based on an integrated framework aiming at determining internal doses of xenobiotics, based on a realistic exposure scenario for different life stages. The applied methodology relies upon the approach developed and described by Sarigiannis et al. (Sarigiannis, Karakitsios et al. 2014). To this aim the INTEGRA platform was adapted to the computation and methodological needs making use of advanced QSARs models to properly parametrize it for the chemicals under investigation.

Due to the lack of suitable and complete exposure data the CROME-LIFE methodological framework was applied to first derive, through reverse dosimetry modelling, exposure probability distributions which were consistent with the human biomonitoring data collected from existing cohorts in the Mediterranean region. Then, these exposure estimates were used to feed the PBTK model which was executed in forward-mode to derive internal doses of chemicals in target tissues.

The CROME-LIFE methodological framework was applied to the chemicals presented in **Error! Not a valid bookmark self-reference.** Results obtained for BDE-47, PCB153, p,p'-DDT, HCB, Arsenic and Methylmercury are reported in the following chapters. For the other chemicals results are reported in Annex 1.

Table 6. Chemical substances were tested by exposure framework

a/a	IUPAC Name	Chemical name	CAS-number	Chemical classification
1	2,4,4'-Trichlorobiphenyl	PCB 28	7012-37-5	Non-dioxin-like polychlorinated biphenyls
2	2,2',5,5'-Tetrachlorobiphenyl	PCB 52	35693-99-3	Non-dioxin-like polychlorinated biphenyls
3	2,2',4,4',5-Pentachlorobiphenyl	PCB 99	38380-01-7	Non-dioxin-like polychlorinated biphenyls
4	2,2',4,5,5'-Pentachlorobiphenyl	PCB 101	37680-73-2	Non-dioxin-like polychlorinated biphenyls
5	2,2',4,4',5,5'-Hexachlorobiphenyl	PCB 153	35065-27-1	Non-dioxin-like polychlorinated biphenyls
6	2,2',3,4,4',5,5'-Heptachlorobiphenyl	PCB 180	35065-29-3	Non-dioxin-like polychlorinated biphenyls

a/a	IUPAC Name	Chemical name	CAS-number	Chemical classification
7	2,4,4'-Tribromodiphenyl ether	BDE 28	41318-75-6	Polybrominated Diphenyl Ethers
8	2,2',4,4'-Tetrabromodiphenyl ether	BDE 47	5436-43-1	Polybrominated Diphenyl Ethers
9	2,2',4,4',5,5'-Hexabromodiphenyl ether	BDE 153	68631-49-2	Polybrominated Diphenyl Ethers
10	2,2',4,4',5,6'-Hexabromodiphenyl ether	BDE 154	207122-15-4	Polybrominated Diphenyl Ethers
11	Hexachlorobenzene	HCB	118-74-1	Organochlorine Pesticide
12	Dichlorodiphenyltrichloroethanes	p,p'-DDT	50-29-3	Organochlorine Pesticide
13	Arsenic	As	7440-38-2	Metal
14	Methylmercury	$HgCH_3$	016056-34-1	Organometalic

6.2 Scenario 1 (infant-children)

The first exposure scenario consisted of one exposure event for newborn until the 4th year of his/her life. The main assumption for the newborns until the first six months of age was that they were fed exclusively with breast milk. Then, it was assumed that from 6th month until the 18th month the daily diet consisted of 6 different meals (each one every 2.5 hours) and from the age of 18 months to 4 years it consisted of differentiated meals every 3 hours. The starting time of the first meal was set to 7:00 AM. It was also assumed that the contribution of each meal to the daily intake dose is the same. This assumption was based to the fact that the modelled chemicals have long half-life time, and consequently they are accumulated for years in the human body.

6.3 Scenario 2 (adult)

This exposure scenario consisted of one exposure event lasting 30 years assuming a daily food consumption based on the actual dietary schedule of the generic European adult population. In this case the main assumption was that the generic population consumes 3 daily meals. The time of these three basic dietary has been set at 7:00 AM for the breakfast, 2:00 PM for the lunch and 7:00 PM for the dinner. It was assumed also that the contribution of the three meals in the daily exposure is respectively 30%, 50% and 20%. The exposure scenario of the simulation was assumed to start at the age of 15 years.

6.4 Polybrominated Diphenyl Ethers

BDE-47 is Polybrominated diphenyl ethers (PBDEs). PBDEs are a class of synthetic chemicals used for the production of padding, textiles or plastics to retard combustion. PBDEs are generally persistent in the environment and have been measured in aquatic sediments as well as in aquatic and terrestrial animals and fishes. The main human exposure is occurring thought diet and mother's milk.

BDEs have been associated with the neurodevelopment effects (Eriksson, Jakobsson et al. 2001) that could potentially enhance on the neurological disruptions. However, the International Agency for Research on Cancer (IARC) and the National Toxicology Program (NTP) indicate that PBDEs are not considered genotoxic with respect to human carcinogenicity (Nhanes 2009).

A PBTK simulation for BDE-47 was performed based on scenario 2 for the Valencia population[4]. Results show that in steady state condition, at the age of 30 year, the of BDE-47 concentration in uterus has a median value of 0.2 (0.1 - 0.8) ug/L.

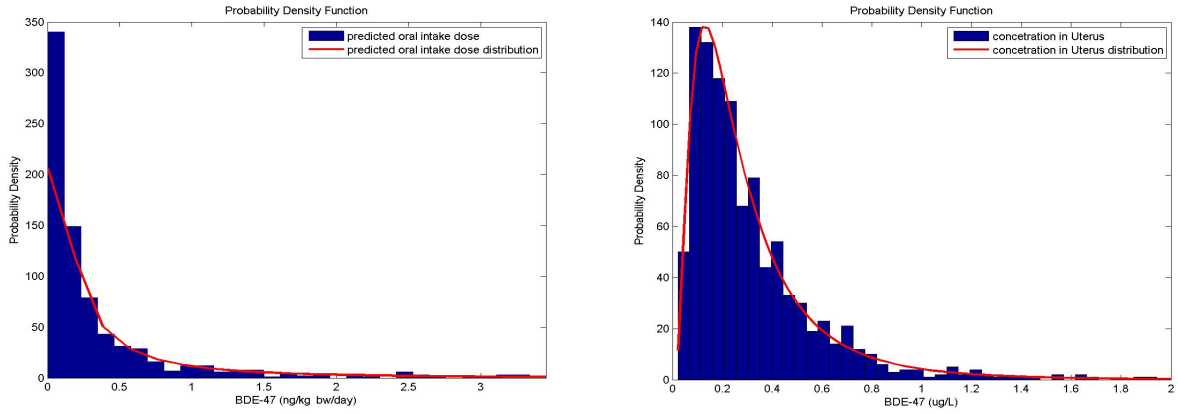

Figure 13. BDE-47 –Valencia (Spain) population – left: predicted oral intake dose. Right: predicted concentration in uterus

6.5 Non-dioxin-like polychlorinated biphenyls

PCBs are a group of organochlorine compounds that are synthesized by catalyzed chlorination of biphenyl. The different position of ring and the position of the chlorine atoms (1-10) can give 209 individual PCB congeners. PCBs accumulate in the food chain and are stored in fatty tissues because of their lipophilicity. The main issue is that the environmental fate of the congeners may varies because of the various transportations and partial eliminations linked to biological metabolisms (Beyer and Biziuk 2009).

[4] More details for the population are presented in Deliverable 5.1.

PCBs are classified as probable human carcinogens by IARC and are classified by NTP as reasonably anticipated to be carcinogens (Nhanes 2009). Early studies associated workplace PCB exposures with increased deaths from cancer of the liver, gastrointestinal tract and brain (Knerr and Schrenk 2006).

Therefore, PBTK simulations for PCB-153 were performed based on scenario 1 for the Valencia population[5]. Results of the simulation show that in steady state condition and at the age of 4 years, the PCB-153 concentration in the liver and in the brain are similar with a median value respectively of 1.3 (0.4 – 4.2) ug/L and 1.5 (0.6 – 3.8) ug/L while the concentration levels in gastrointestinal tract are one order of magnitude lower (median 0.1 (0.01 – 0.7) ug/L).

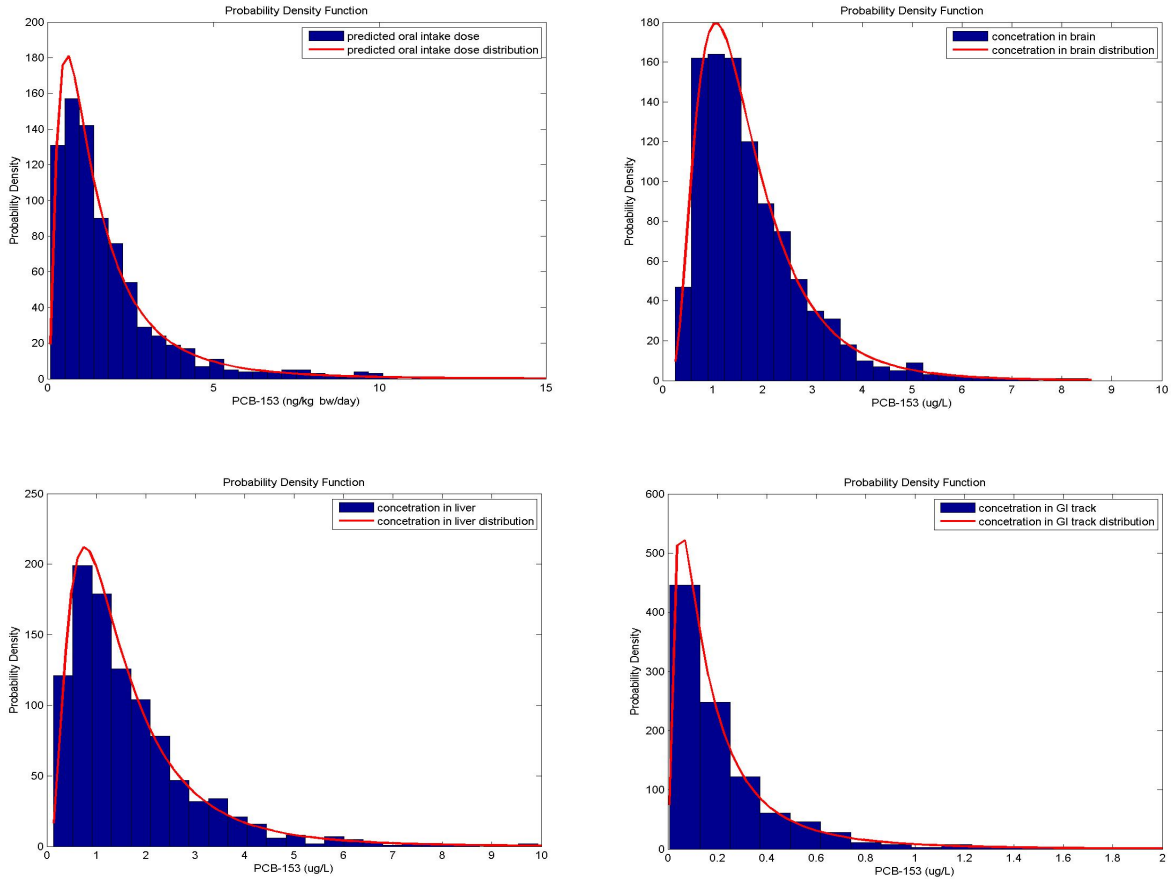

Figure 14. PCB-153 – Valencia (Spain) population – left above: predicted oral intake dose, right above: predicted concentration in brain, left below: predicted concentration in liver, right below: predicted concentration in gastrointestinal.

[5] More details about the population are presented in Deliverable 5.1.

6.6 Organochlorine Pesticides

Hexachlorobenzene (HCB) is an organochlorine pesticides. Organochlorine pesticides, an older class of pesticides, are effective against a variety of insects. They enter to the environment after pesticide applications, disposal of contaminated wastes into landfills, and releases from manufacturing plants that produce these chemicals. Usage restrictions have been associated with a general decrease in serum organochlorine levels in the U.S. population and other developed countries (Hagmar, Wallin et al. 2006). For the general population the oral exposure is the main exposure route, primarily through the ingestion of fatty foods such as dairy products and fish (Nhanes 2009). Infants are exposed through breast milk, and fetuses can be exposed in utero through the placenta. Workers can be exposed to organochlorines pesticides in the manufacture, formulation, or application of these chemicals

Chronic feeding studies in animals have demonstrated kidney injury, immunologic abnormalities, reproductive and developmental toxicities, and liver and thyroid cancers (ATSDR 2002). In humans, very high, acute doses produce central nervous system depression and seizures.

Therefore, PBTK simulations for HCB were performed based on scenario 2 for Valencia population. Results of the simulations show that in steady state condition and at the age of 30 years, the HCB concentration in brain has a median value of 2.1(0.6 - 7.7) ug/L (Figure 15).

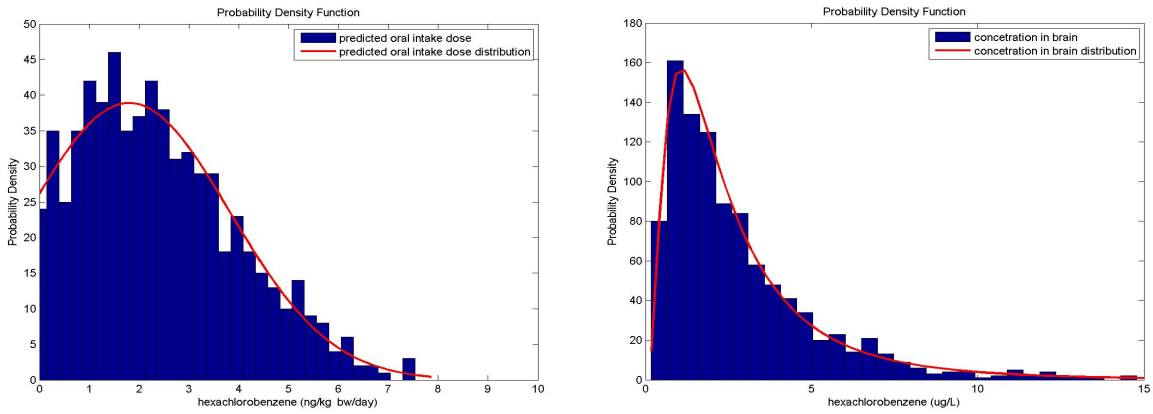

Figure 15. HCB – Valencia (Spain) population – left: predicted oral intake dose. Right: Predicted concentration in brain

Dichlorodiphenyltrichloroethane (pp'-DDT) is an organochlorine pesticides. It has been used widely as a broad spectrum insecticide in agriculture and for control of vector-borne diseases.

DDT probably contributes to the increment of risks for cancers at various sites and its possible role as an endocrine disruptor (Turusov, Rakitsky et al. 2002). Particularly, DDE and DDT has been strongly associated with the Cancer of breast (Wolff, Toniolo et al. 1993). Moreover, the DDT has been reported as toxicants the induce to neurotoxic effects (Eriksson 1992, Eriksson, Ahlbom et al. 1992) and it has been related with disruptions on brain functions.

Therefore PBTK simulations for pp'-DDT were carried out based on scenario 1 for the Valencia and for the Menorca population[6]. Results show that in steady state condition and at the age of 30 years, the concentration of pp'-DDT in brain and in uterus are about 1.5 - 2 times higher for the population of Menorca than in Valencia (Figure 16).

[6] More details about the population are presented in Deliverable 5.1.

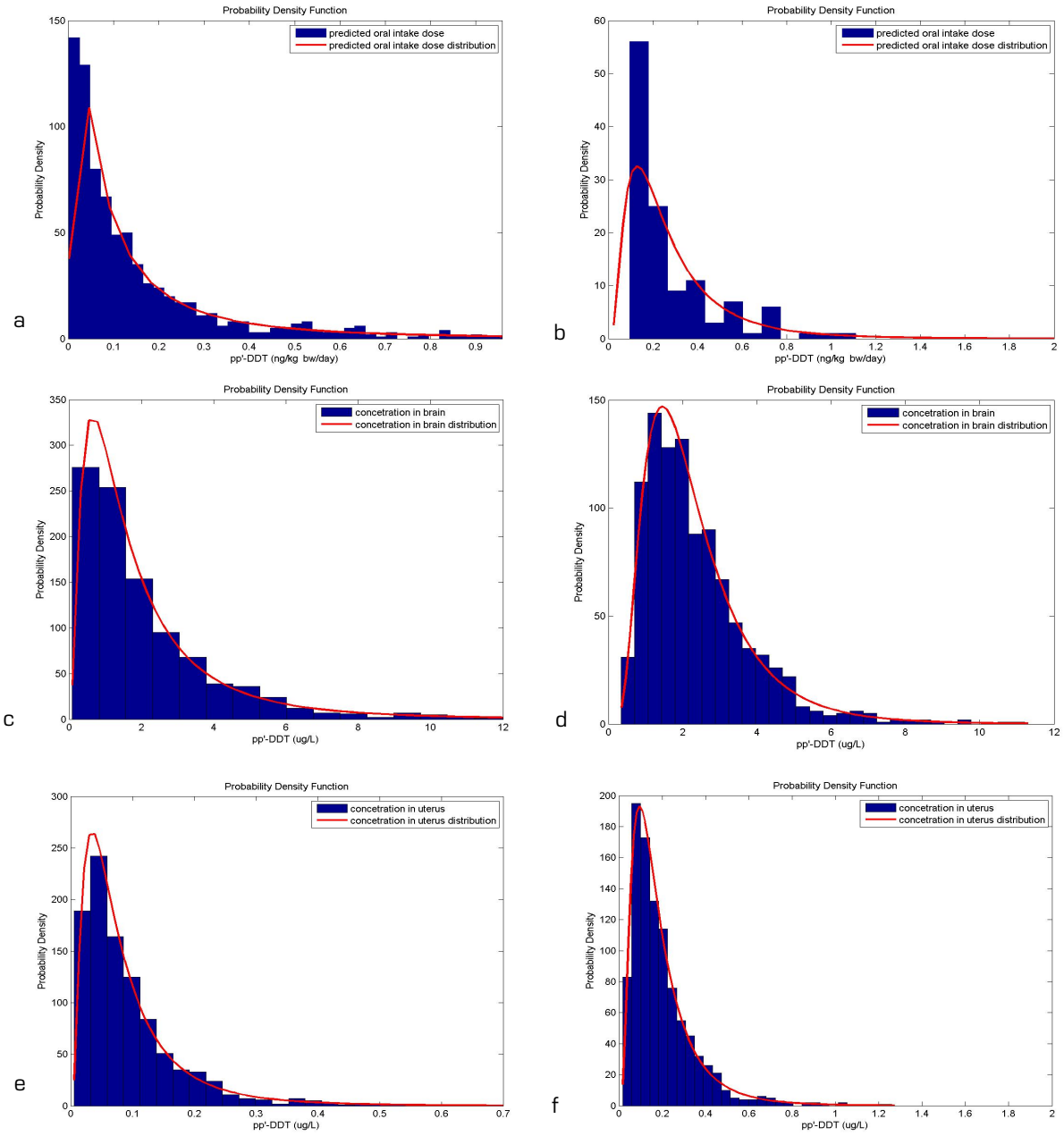

Figure 16. pp'-DDT – a) predicted oral intake dose for Valencia (Spain) population, b) predicted oral intake dose for Menorca (Spain) population, c) predicted concentration in brain for Valencia (Spain) population, d) predicted concentration in brain for Menorca (Spain) population, e) predicted concentration in uterus for Valencia (Spain) population and f) predicted concentration in uterus for Menorca (Spain) population

6.7 Mercury and Methylmercury

Mercury is a naturally occurring metal which has several forms. The metallic mercury is a shiny, silver-white, odorless liquid. If heated, it is a colorless, odorless gas. Mercury combines with other elements, such as chlorine, sulfur, or oxygen, to form inorganic mercury compounds or "salts," which are usually white powders or crystals. Mercury also combines with carbon to make organic mercury compounds. The most common one, methylmercury, is produced mainly by microscopic organisms in the water and soil. More mercury in the environment can increase the amounts of methylmercury that these small organisms make.

Metallic mercury is used to produce chlorine gas and caustic soda, and is also used in thermometers, dental fillings, and batteries. Mercury salts are sometimes used in skin lightening creams and as antiseptic creams and ointments.

Methylmercury is the form most readily incorporated into biological tissues and most toxic to humans The conversion of inorganic mercury to methylmercury is important for two reasons: (1) methylmercury is much more toxic than inorganic mercury, and (2) organisms require considerably longer to eliminate methylmercury.

The health effects of mercury are depend on the form of the mercury, the dose as well as duration that a person is exposed. Lower levels of prenatal exposure due to maternal seafood consumption have been associated with an increased risk for abnormal neurocognitive test results in children (Goyer, Aposhian et al. 2000, Rice 2004). It has to be underlined that the during gestation, the fetus is more vulnerable to neurochemical disruption from methylmercury exposure via the mother and because of the susceptibility of the developing brain, this exposure has high risk (Organization 1990).

PBTK simulations for methylmercury were based on scenario 2 for Slovenian and Croatian population. Simulation results show that the concentration of MeHg in brain and in gastrointestinal tract are 3-4 times higher for the population of Croatia than for the one of Slovenia (Figure 17).

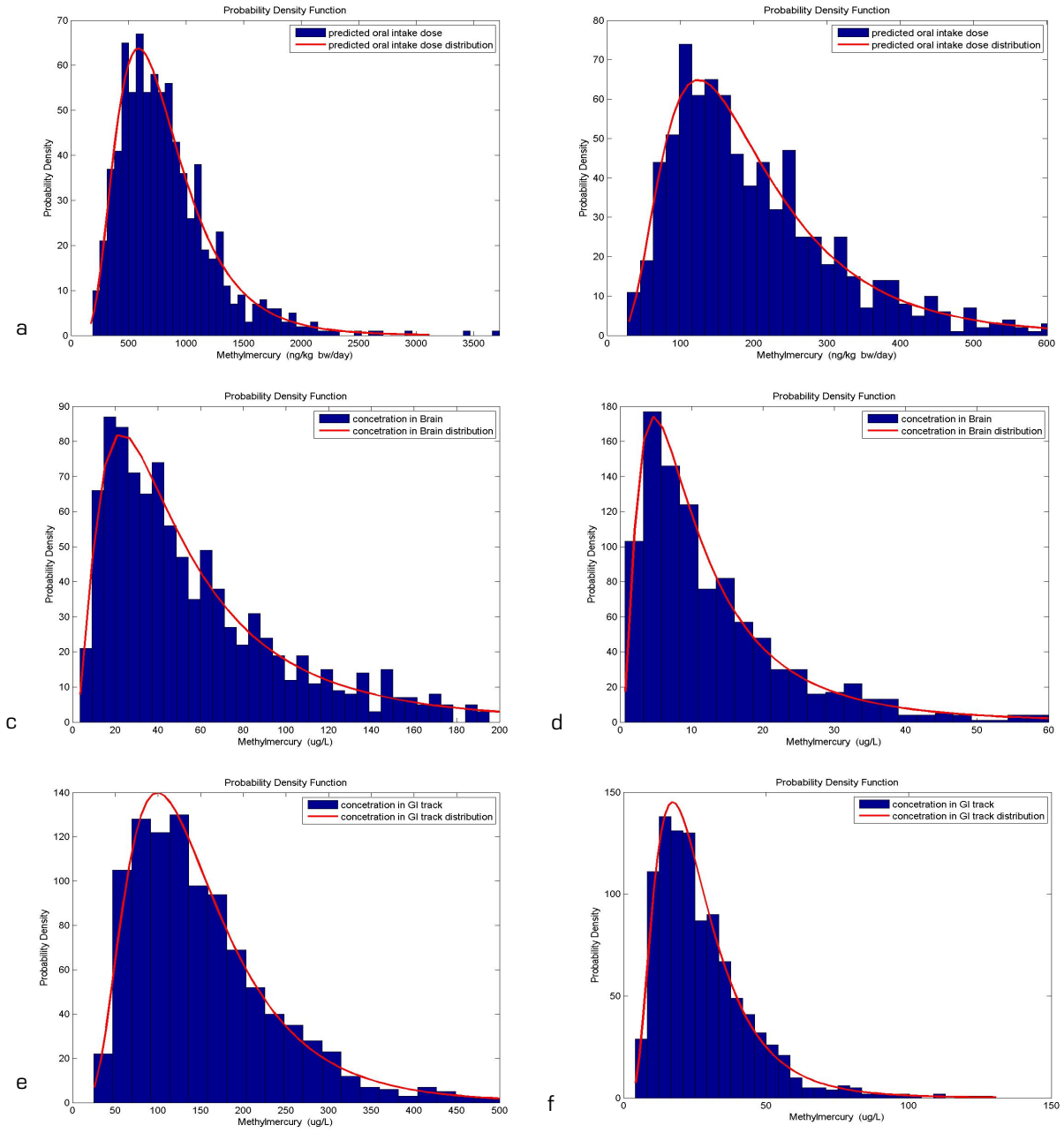

Figure 17. Methylmercury – a) predicted oral intake dose for Croatian population, b) predicted oral intake dose for Slovenian population, c) predicted concentration in brain for Croatian population, d) predicted concentration in brain for Slovenian population, e) predicted concentration in gastrointestinal for Croatian population and f) predicted concentration in gastrointestinal for Slovenian population

6.8 Arsenic

Arsenic is an element that is widely distributed in the earth's surface in small amounts. Arsenic and its compounds have had many uses in the past and present as medicines, pesticides, alloys, semiconductors, and as homicidal poisons. The exposure to inorganic arsenic can be occurred through consumption of drinking water and, to a lesser extent, meats, grain, and produce (Abernathy, Thomas et al. 2003).

An association between lung cancer and occupational exposure to inorganic arsenic has been confirmed in several epidemiologic studies (ENTERLINE, HENDERSON et al. 1987), and arsenic is considered a cause of lung (Eriksson, Jakobsson et al. 2001, Smith, Marshall et al. 2006) as well as skin cancer (Karagas, Stukel et al. 2001). In arsenic-exposed workers, there is a systematic gradient in lung cancer mortality rates, depending upon duration and intensity of exposure(Tokar, Diwan et al. 2010)

PBTK simulations for arsenic were based on scenario 2, for Italian and Slovenian population. Simulation results show that in steady state condition and at the age of 30 years, Arsenic concentration levels in skin and in lung are about 3 times higher for the population of Italy with respect to the Slovenia one (Figure 18).

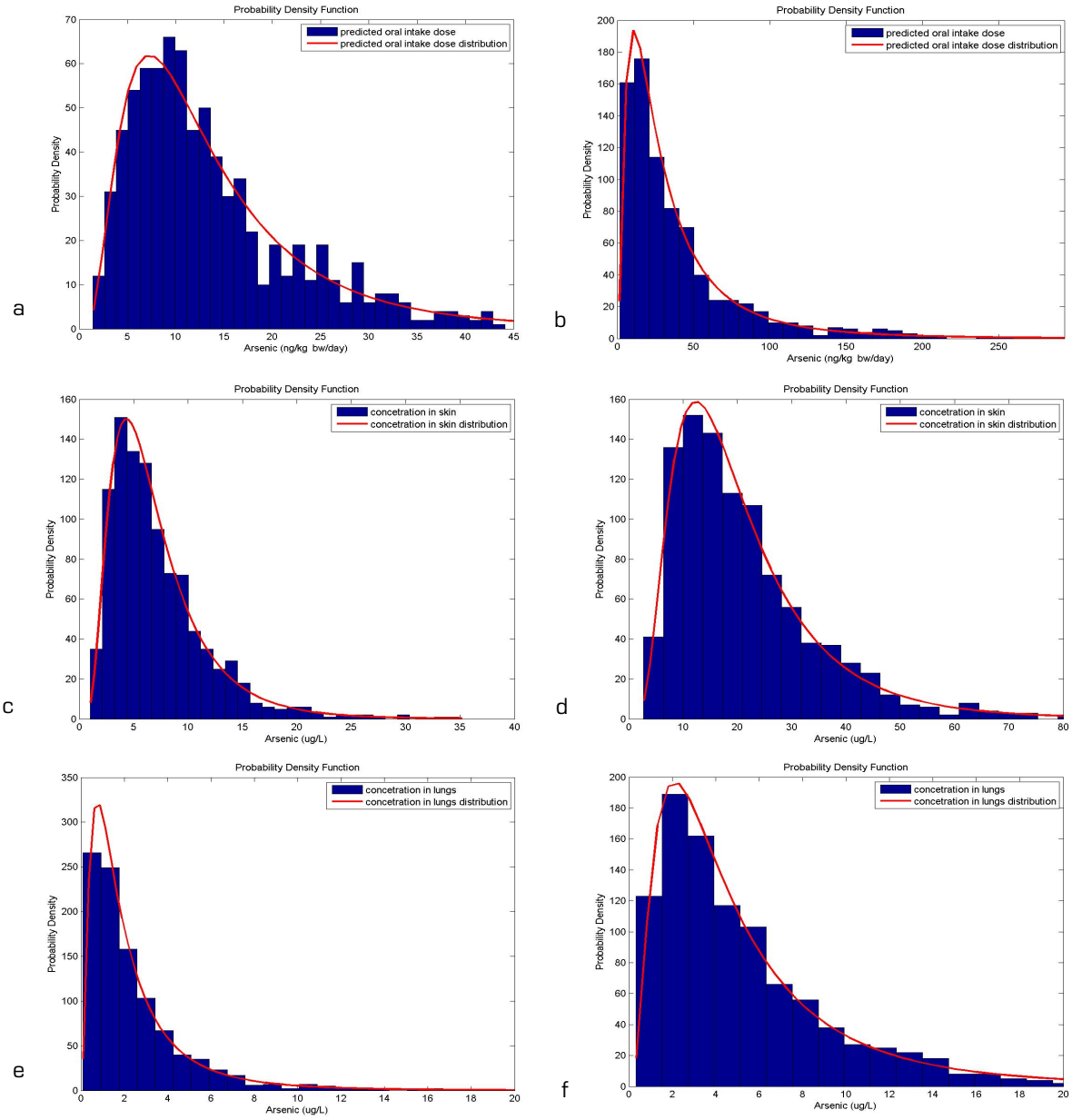

Figure 18. Arsenic – a) predicted oral intake dose for Slovenian population,

b) predicted oral intake dose for Italian population,
c) predicted concentration in skin for Slovenian population,
d) predicted concentration in skin for Italian population,
e) predicted concentration in lung for Slovenian population and
f) predicted concentration in lung for Italian population

The statistical summary of the concentrations of simulated chemical in the various target organs is reported in Table 7.

Table 7: Summary statistic of concentrations of simulated chemical in the target tissues

	Chemical	Tissue	mean	std (ug/L)	median	Q 0.05 (ug/L)	Q 0.95 (ug/L)	location
1	Arsenic	Skin	8.4	3.8	5.5	1.0	29.7	Slovenia
2	Arsenic	Lungs	3.9	1.8	1.7	0.4	7.1	Slovenia
3	Arsenic	Skin	24.8	8.7	18.0	6.8	47.3	Italy
4	Arsenic	Lungs	6.8	3.9	4.1	1.1	15.2	Italy
5	BDE47	Uterus	1.3	0.7	0.2	0.1	0.8	Spain (Valencia)
6	HCB	Brain	3.1	1.1	2.1	0.6	7.7	Spain (Valencia)
7	Mercury	Brain	16.9	12	9.2	2.8	29.0	Slovenia
8	Mercury	GI tract	37.5	15.4	23.8	9.6	58.4	Slovenia
9	Mercury	Brain	70.5	16.4	46.5	12.1	174.8	Croatia
10	Mercury	GI tract	180.5	18.5	134.9	56.0	321.1	Croatia
11	PCB153	Liver	2.1	0.72	1.3	0.4	4.2	Spain (Valencia)
12	PCB153	GI tract	0.28	0.02	0.1	0.0	0.7	Spain (Valencia)
13	PCB153	Brain	2.1	0.4	1.5	0.6	3.8	Spain (Valencia)
14	ppDDT	Uterus	0.12	0.05	0.1	0.0	0.3	Spain (Valencia)
15	ppDDT	Kidneys	0.21	0.08	0.2	0.0	0.8	Spain (Menorca)
16	ppDDT	Brain	3.2	1.1	2.0	0.8	5.0	Spain (Menorca)
17	ppDDT	Brain	2.1	0.9	1.5	0.3	6.2	Spain (Valencia)

7 Conclusions

From the methodological point of view, using an integrated exposure framework for linking mechanistically external and internal exposure, provides a comprehensive overview on how realistic exposure scenarios are translated into internal dose to humans, accounting for the age-dependent and route specific bioavailability differences. To this aim a generic PBTK modelling framework that captures lifetime internal exposure is a valuable tool with many applications in the modern risk assessment arena, by exploiting the continuously growing wealth of in vitro testing and biomonitoring data.

The developed generic PBTK model includes i) lifespan evolution in physiology, from the moment of conception till 80 years of life-time and it is differentiated by gender; ii) a detailed description of pregnancy (mother-foetus interaction) and lactation (toxicants concentration in milk) periods, iii) a detailed compartmental description of human anatomy and receptor binding; iv) a detailed description of various exposure rotes (i.e. inhalation, dermal and oral). Moreover, the generic character of the model is ensured by the capability of assessing new chemicals or chemicals with limited information. To this aim, the model is linked to quantitative structure-activity relationships (QSARs), so as to calculate chemical-specific input parameters of PBTK models (partition coefficients and metabolic parameters such as the maximal velocity (Vmax) and Michaelis affinity constant (Km) or the intrinsic clearance (Vmax/Km).

The great advantage of using an approach based on PBTK model is that it allows the estimation of internal doses of xenobiotics that exceed levels associated with biological pathway alterations and, eventually, induction of biological perturbations that may lead to health risk.. This represents a more appropriate metric to be linked with adverse health outcomes providing a more mechanistic and biology-based approach to human health risk assessment.

Assessing exposure at multiple scales across the source-to-dose continuum, needs to take into account the actual complexity of the environmental and biological/physiological processes that are critical to the proper description of the phenomena involved. This results in targeted interventions and consequently more cost efficient risk management. In addition, a comprehensive integrated exposure framework estimating tissue dosimetry for the various relevant exposure scenarios, could be of great use in exploiting the in vitro HTS results rapidly produced by ToxCast21, advancing thus both exposure science and toxicology towards serving the needs of risk assessment in the 21st century.

Starting from the application of PBTK model and /or biomonitoring data collected in Action B.2- B-3, next step will be the quantitative estimation of the effect on human health due to exposure to selected chemicals.

To this aim internal doses will be coupled to health impacts on the local population through advanced statistical methods to derive the dose – response functions which account for differences in exposure patterns, susceptibility differences and inter-individual variation (due to lifestyle, age, sex or physiological status) in health response. To estimate the health impact we will use a statistical approach based on survey-weighted logistic multivariate regression adjusted for different covariates (age, sex, socio-economic status (SES) etc.) linking internal doses with health effect considering the interdependence of the covariates (using as metric an analogy of the "linkage disequilibrium" metric used in genome-wide association studies).

Overall, our comprehensive modelling framework supports the association of a variety of environmental, exposure and biomonitoring data, as well as the incorporation of recent advances of in vitro toxicology using high-throughput systems in the risk assessment process enhancing thus significantly the artillery of environmental health science and chemical safety regulators. The necessity for associating these types of data is increased by the world-wide interest for "exposure based" risk assessment.

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9 Annex 1

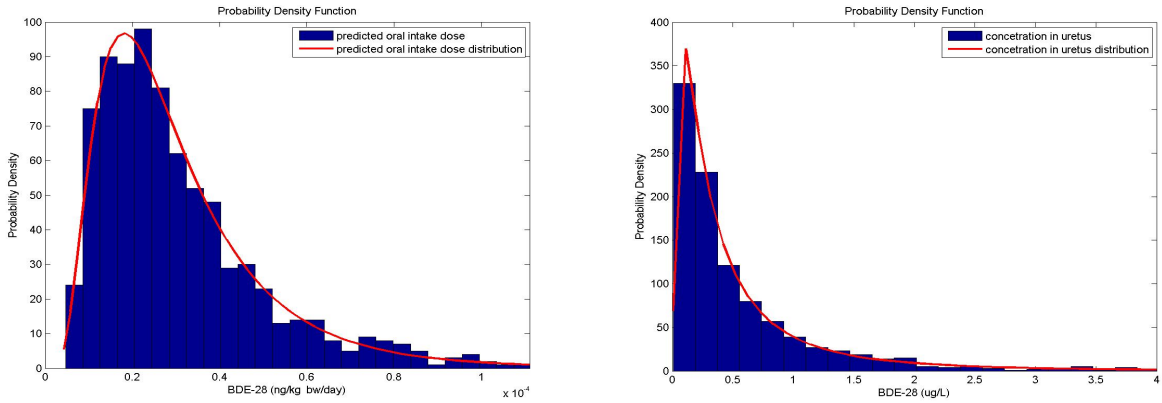

Figure 19. BDE-28 – Valencia (Spain) population – left: Predicted oral intake dose by a reconstruction simulation, Right: predicted concentration in uterus

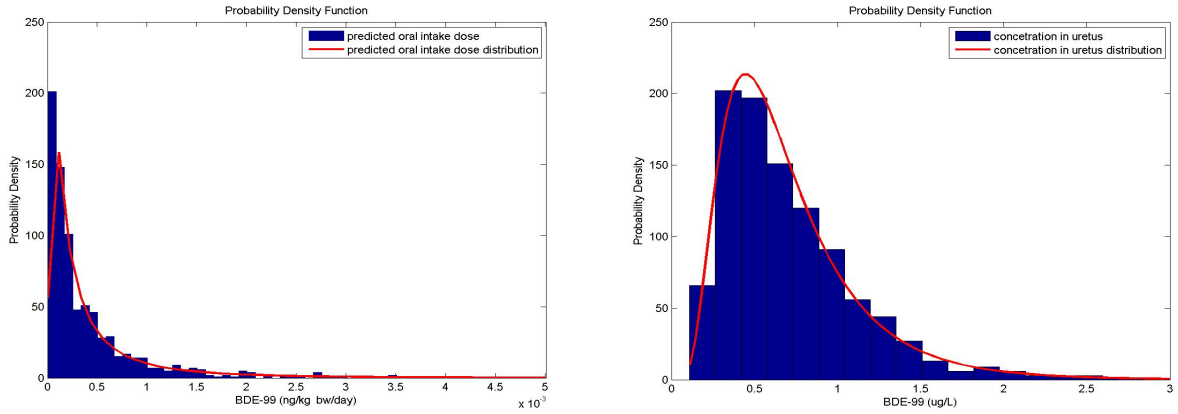

Figure 20. BDE-99 – Valencia (Spain) population – left: Predicted oral intake dose by a reconstruction simulation, Right: predicted concentration in uterus

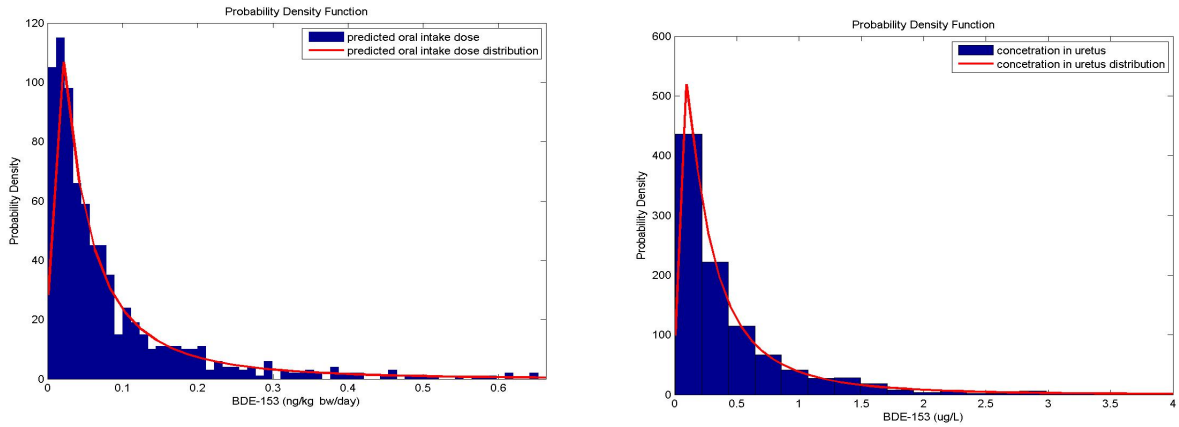

Figure 21. BDE-153 – Valencia (Spain) population – left: Predicted oral intake dose by a reconstruction simulation, Right: predicted concentration in uterus

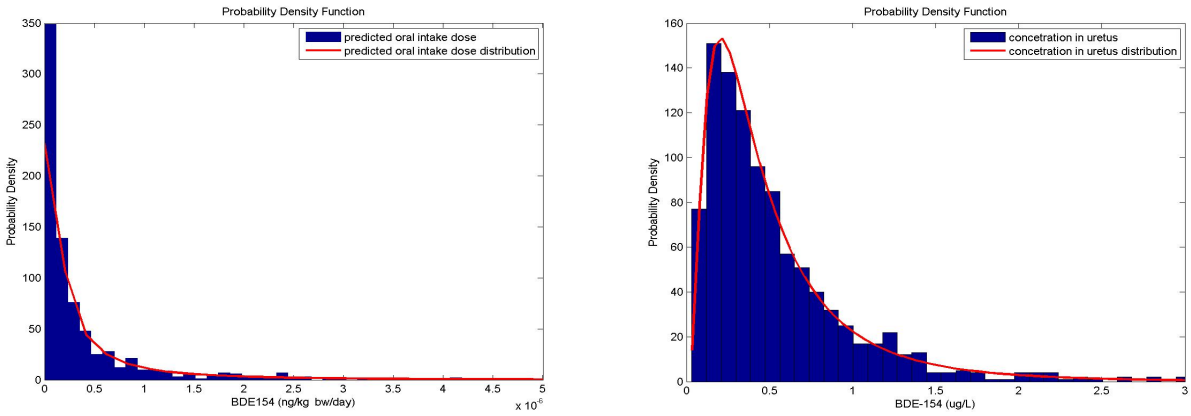

Figure 22. BDE-154 – Valencia (Spain) population – left: Predicted oral intake dose by a reconstruction simulation, Right: predicted concentration in uterus

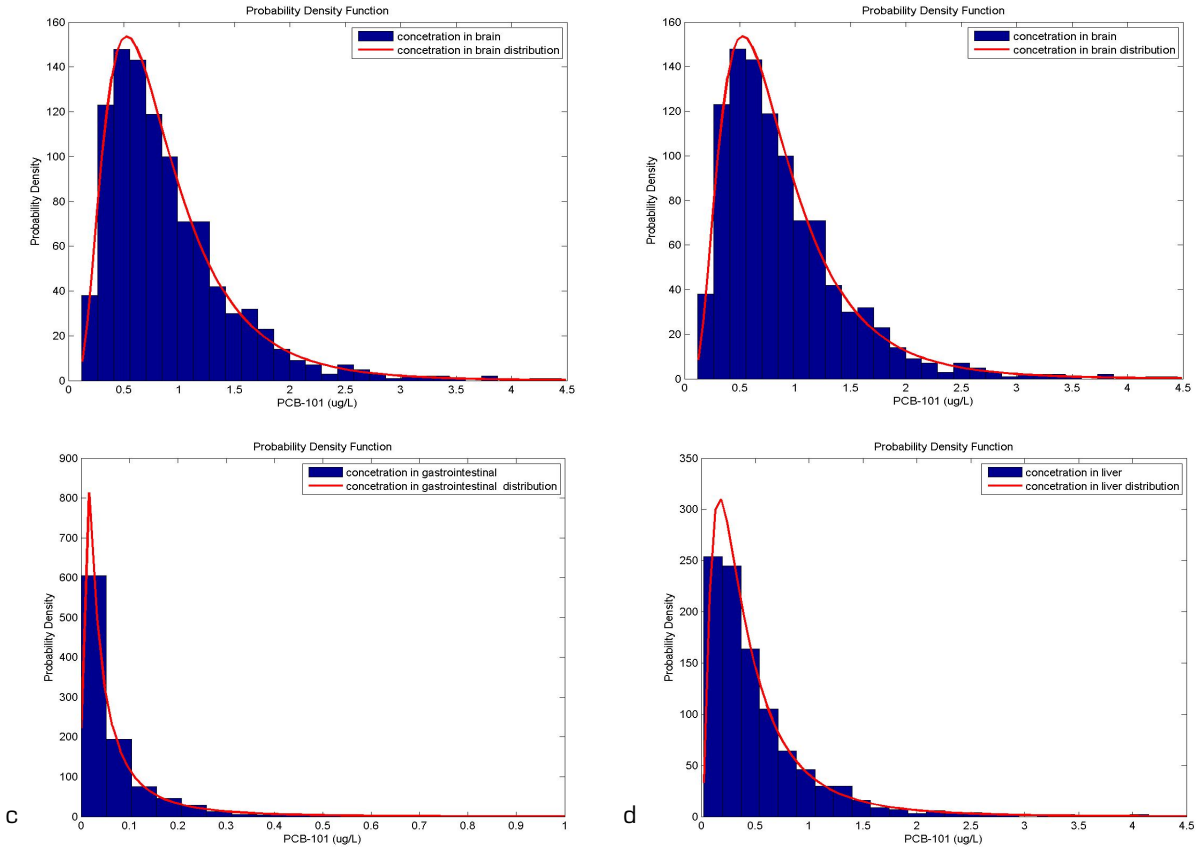

Figure 23. PCB-101 – Valencia (Spain) population – a) pedicted oral intake dose by a reconstruction simulation, b) predicted concentration in brain, c) predicted concentration in gastrointestinal and d) predicted concentration in liver

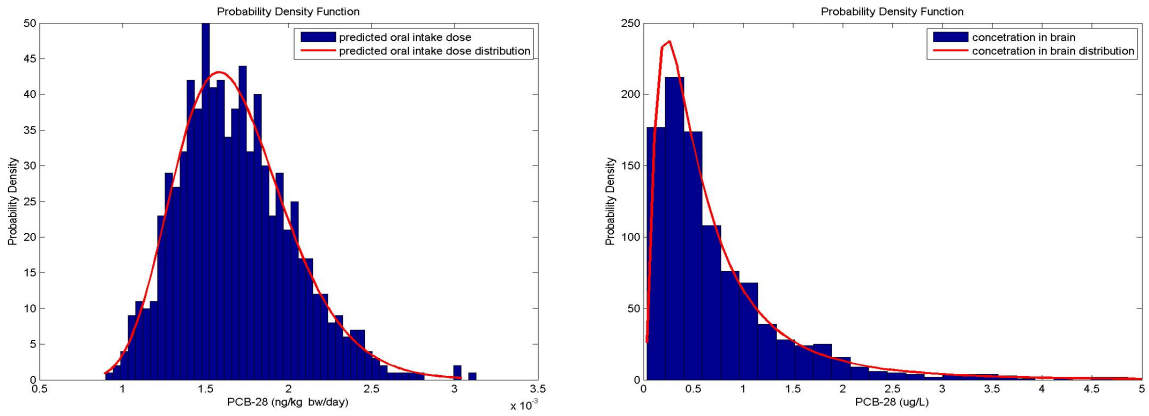

Figure 24. PCB-28 – Valencia (Spain) population – left: Predicted oral intake dose by a reconstruction simulation, Right: predicted concentration in brain

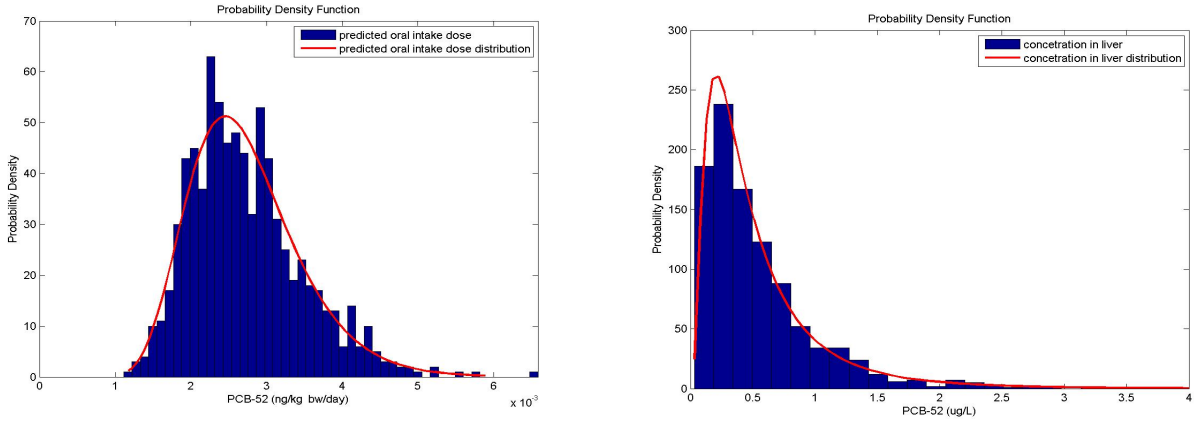

Figure 25. PCB-52 – Valencia (Spain) population – left: Predicted oral intake dose by a reconstruction simulation, Right: predicted concentration in liver

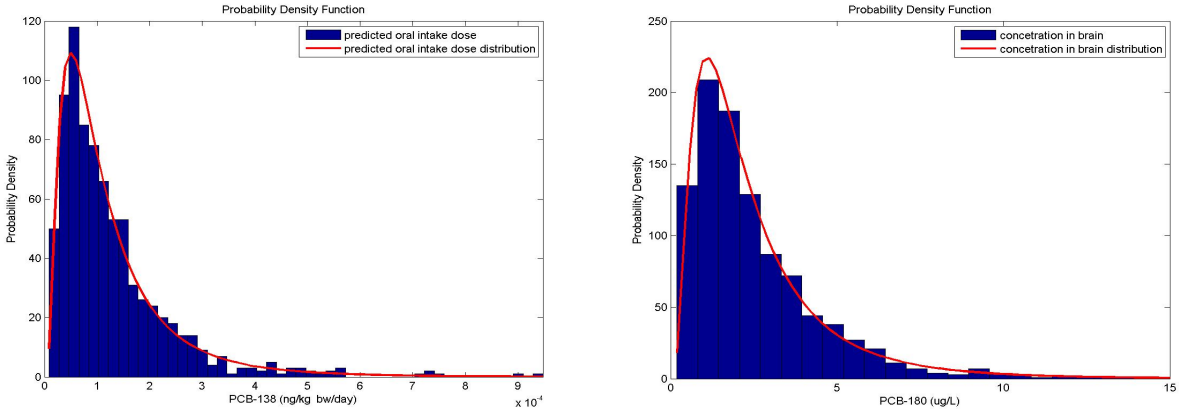

Figure 26. PCB-180 – Valencia (Spain) population – left: Predicted oral intake dose by a reconstruction simulation, Right: predicted concentration in brain