Università
Ca'Foscari
Venezia

Master's Degree programme
in Environmental Sciences

Final Thesis

Ca' Foscari
Dorsoduro 3246
30123 Venezia

Application of an environmental exposure model for Persistent Organic Pollutants in Venice lagoon

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Academic Year
2013 / 2014

Index

Abstract

The assessment of exposure to environmental pollutants requires flexible and easily applicable methods and approaches to support ecological and human health risk analysis in complex exposure scenarios. Therefore, during the last years, predictive modelling tools able to integrate and complement direct exposure assessment methodologies have gained more interest. The work presented in this thesis has been developed within the European project 4FUN (*The FUture of FUlly integrated human exposure assessment of chemicals: Ensuring the long-term viability and technology transfer of the EU-FUNded 2-FUN tools as standardized solution*). 4FUN is the follow-up project of 2FUN project, which produced a prototype software tool for ecological and human exposure assessment. The main objective of 4FUN project is to implement and validate this first prototype into a definitive and standardized tool, MERLIN-Expo, suitable to estimate time-dependent exposure in different scenarios.

MERLIN-Expo (*Modelling Exposure to chemicals for Risk assessment: a comprehensive Library of multimedia and PBPK models for Integration, Prediction, uNcertainty and Sensitivity analysis*) provides a library of exposure models for the dynamic exposure assessment (both for organic and inorganic chemicals), which can be flexibly combined to simulate complex scenarios. It allows to couple on the same platform environmental (multimedia models) and physiologically-based pharmacokinetic (PBPK) models in order to estimate chemicals environmental exposure and human intake and distribution/accumulation processes in the human body.

The main goal of thesis is to apply the “Fish model”, recently implemented in MERLIN-Expo, aimed at assessing organic pollutants bioaccumulation in fish, in order to test the model applicability and evaluate its performance and potentialities. In this thesis, the Fish model has been applied to the Venice lagoon case study to assess the exposure of two fish species (*Chelon labrosus* e *Zosterisessor ophiocephalus)* to polychlorinated biphenyls (PCBs). This work will pave the way to a future MERLIN-Expo application to assess human bioaccumulation of pollutants through the consumption of local fish and seafood.

After a review on approaches and models for environmental exposure assessment, the main chemical accumulation processes and currently available bioaccumulation models in aquatic organism have been reviewed. This work has been a help to support Fish module implementation and to understand how to approach the Venice case-study application.

After analysing and crossing available monitoring data on environmental matrices, aquatic biota and local population (human biomonitoring) to identify available data to be compared with model results, three congeners of PCBs have been selected for the application: PCB126, PCB169 and

PCB. PCB126 and PCB169 are dioxin-like congeners, while PCB180 is one of the most analysed marker in environmental studies.

For assessing adequately the bioaccumulation process, it has been necessary to reconstruct historical PCBs concentrations from the '30s (starting period of PCBs production) in the Venice lagoon. Since the final goal of the 4FUN case-study is to assess the lifetime human exposure to PCBs, and available human biomonitoring data date back to 1997-1998, it is needed to dynamically simulate concentrations in edible fish for some decades before 2000. Historical data of PCB emissions suitable to reconstruct the historical development of PCBs contamination in the lagoon of Venice are not available. As an alternative, sediment cores proved to be useful in supporting the reconstruction of temporal trends of three congeners of PCBs contamination in water and fish preys, as required by the model.

Modelling results have been compared with measured concentrations in fishes from monitoring campaigns from the end of the '90s in Venice lagoon (MAV, 2000a). Generally, it has been observed that the model can suitably predict PCBs congeners' bioaccumulation in two fish species, despite some discrepancies which could be probably related to the uncertainty in experimental input data (values below the detection limit).

The application of the Fish model to the Venice case-study demonstrated the applicability of dynamic fish bioaccumulation processes simulation, as well as the feasibility of a probabilistic application for identifying uncertainly ranges associated with model results. Furthermore, it has been possible to identify and assess fish physiological parameters and pollutants chemical-physical proprieties which influence bioaccumulation processes. Anyway, it is surely advisable to perform a sensitivity analysis of the Fish model, in order to quantify which is the contribution of each parameter to the final model outcomes.

The application of MERLIN-Expo to other classes of chemicals and its future integration with other modules able to model the entire aquatic food web will support the model validation and its application to integrate ecological and human exposure assessment.

Sommario

La valutazione dell'esposizione a contaminanti ambientali richiede approcci e metodologie flessibili e di facile applicazione per supportare l'analisi dei rischi per gli ecosistemi e per la salute umana in scenari di esposizione complessi, pertanto negli ultimi anni c'è stato un crescente interesse per strumenti modellistici di tipo predittivo, finalizzati ad integrare e completare metodi di stima diretta dell'esposizione. Il lavoro presentato in questa tesi è stato svolto all'interno del progetto europeo 4FUN (*The FUture of FUlly integrated human exposure assessment of chemicals: Ensuring the long-term viability and technology transfer of the EU-FUNded 2-FUN tools as standardized solution*). 4FUN segue a 2FUN, progetto precedente che ha portato allo sviluppo di un modello prototipale per la valutazione integrata dell'esposizione ambientale e umana. L'obiettivo principale del progetto 4FUN è di implementare e validare il software prodotto durante 2FUN per creare uno strumento completo e standardizzato, il software MERLIN-Expo, in grado di essere applicato nel tempo a vari scenari.

MERLIN-Expo (*Modelling Exposure to chemicals for Risk assessment: a comprehensive Library of multimedia and PBPK models for Integration, Prediction, uNcertainty and Sensitivity analysis*) presenta una libreria di modelli per la valutazione dell'esposizione a inquinanti (sia organici, che inorganici), facilmente combinabili tra loro per simulare gli scenari più complessi. Sulla stessa piattaforma, infatti, si possono selezionare una serie di modelli ambientali (Multimedia Models) e il modulo farmaco-cinetico basato sulla fisiologia umana (PBPK), in modo da valutare la distribuzione di inquinanti nell'ambiente, l'assunzione da parte degli esseri umani e i processi di distribuzione nell'organismo.

E' in questo contesto che la tesi si pone come obiettivo principale l'applicazione di un modello recentemente implementato in MERLIN-Expo per la stima del bioaccumulo di contaminanti organici persistenti nei pesci ("Fish model"), con l'obiettivo di testarne l'applicabilità e valutarne le performance e le potenzialità. In questa tesi, il modello Fish in MERLIN-Expo è stato quindi applicato al caso di studio della laguna di Venezia valutando l'esposizione ambientale di due specie ittiche (*Chelon labrosus* e *Osterisessor ophiocephalus*) a policlorobifenili (PCB). Ciò pone le basi per una futura applicazione di MERLIN-Expo con lo scopo di valutare l'accumulo di inquinanti da parte dell'uomo attraverso il consumo di prodotti ittici locali.

Dopo un approfondimento sui metodi e i modelli di valutazione dell'esposizione ambientale, sono stati studiati i principali processi implicati nell'accumulo di inquinanti da parte di organismi aquatici e analizzati i modelli di bioaccumulo attualmente disponibili. Ciò è stato indispensabile per supportare l'implementazione del modulo Fish, per capirne il funzionamento e procedere con la sua applicazione nel contesto del caso di studio della laguna di Venezia.

Analizzando e incrociando i dati di monitoraggio disponibili riguardanti le varie matrici ambientali, la catena trofica acquatica e la popolazione locale (dati di biomonitoraggio umano) al fine di identificare i dati disponibili per la validazione dei risultati modellistici, sono stati selezionati tre congeneri di PCB per l'applicazione del modello Fish al caso di studio di Venezia: PCB126, PCB169 e PCB180. I PCB126 e PCB169 sono congeneri diossina-simili, mentre il PCB180 è uno dei marcatori più analizzati negli studi ambientali. Per un corretto approccio nella valutazione del processo di bioaccumulo, è stato necessario ricostruire le concentrazioni di PCB nella laguna di Venezia dagli anni '30 (periodo in cui è iniziata la loro produzione). Considerando che l'obiettivo finale di 4FUN è la valutazione dell'esposizione umana a PCB e i dati di biomonitoraggio umano disponibili derivano da una campagna del 1997-1998, è essenziale simulare dinamicamente le concentrazioni in organismi aquatici eduli per qualche decennio prima del 2000. In assenza di dati storici delle emissioni di PCB, le concentrazioni degli inquinanti in carote sedimentarie radiodatate prelevate dal fondale della laguna veneziana si sono dimostrate essere utili per la ricostruzione delle concentrazioni storiche dei tre congeneri di PCB in acqua e nelle prede, richieste come input nel modello.

Dopo aver parametrizzato il modello per una sua applicazione sia deterministica che probabilistica attraverso una review di letteratura e l'applicazione di modelli QSAR, è' stato stimato il bioaccumulo dei tre congeneri di PCB nei due organismi selezionati per un periodo di circa 50 anni.

I risultati modellistici sono stati confrontati con quelli misurati in campioni di pescato durante le campagne di monitoraggio di fine anni '90 nella laguna di Venezia (MAV, 2000). In generale si è potuto osservare che il modello riesce a predire in maniera soddisfacente la concentrazione di PCB nei tessuti delle specie ittiche considerate, nonostante alcune discrepanze probabilmente attribuibili all'incertezza associati ai dati sperimentali (valori al di sotto del limite di rilevabilità).

L'applicazione al caso di studio di Venezia ha permesso di dimostrare l'applicabilità dello strumento per la ricostruzione dinamica dei processi di bioaccumulo nei pesci, nonchè la fattibilità di una sua applicazione probabilistica per identificare il range di incertezza associato ai risultati modellistici. Inoltre è stato possibile approfondire il ruolo che i parametri fisiologici per le specie ittiche e i parametri chimici possono avere nell'influenzare il bioaccumulo, anche se si suggerisce di effettuare un'analisi di sensitività del modello per poter quantificare più in dettaglio tali contributi.

L'applicazione del modello Fish ad altre classi di contaminanti e la sua futura integrazione con ulteriori modelli per modellizzare l'intera catena trofica aquatica potranno essere di aiuto per una validazione del modello e per la sua applicazione integrata con i modelli per la stima dell'esposizione umana.

Motivations and aims of the thesis

An appropriate exposure assessment is necessary and useful to manage and assess chemical risk, implementing environmental monitoring and control. In fact, exposure assessment consists in quantifying the levels of chemicals to which ecological and human targets are exposed, in terms of magnitude, duration and frequency. Many different approaches can be used for quantifying environmental exposures: direct methods (measuring, monitoring or biomonitoring) or indirect methods, involving exposure estimations from measurements and existing data, like environmental monitoring, questionnaires and exposure models. In many case-study approaches, direct methods cannot be applied, so, it is necessary for risk managers and decision makers to apply flexible tools which can assess environmental and human exposures in realistic way considering complex scenarios.

Considering this context, European 4FUN project aims to validate a innovative software tool, called MERLIN-Expo, for the integrated human exposure assessment, including on the same platform environmental multimedia models and PBPK model.

The activities in 4FUN project aims to improve MERLIN-Expo considering bioaccumulation and biomagnification processes in a thophic chain, to finally assess human health exposure. So, Fish module has been developed considering bioaccumulation processes in an aquatic food web.

The main goal of this thesis is to apply Fish model, in order to verify its applicability and performance on case-study of the Venice lagoon, POPs (in particular PCBs) pollution affected area. Assessing fish exposure to Polychlorinated Biphenyls in the lagoon of Venice through MERLIN-Expo application is the step before application to bioaccumulation process assessment in local human population through fish diet.

In particular, the model application of this thesis aims to assess the concentrations of three PCBs congeners in two fish species of Venice lagoon. Finally, MERLIN-Expo performance will be evaluated through the comparison between model results and monitoring data of Venice lagoon.

The main steps in order to run and verify the Fish model applicability to Venice case study are: parametrization of the model, according to the specific case-study conditions and the three target PCBs congeners; reconstruction of the input data require by the model; definition of simulations settings; analysis and evaluation of model through comparison with measured data in biota.

PART A

METHODS AND THEORY

1. ENVIROMENTAL EXPOSURE ASSESSMENT

1.1 RISK ASSESSMENT

Chemical risk assessment could be defined as “the evaluation of the potential for adverse effect on human health or ecosystem from exposures to chemicals” (RATSC, 1999). The environmental risk assessment is finalized to describe the relationship between exposure rate to chemicals and toxic human health effects (Human Health Risk Assessment, HHRA) or ecological effects (Ecological Risk Assessment, ERA) from chemical contacts (Nieuwenhuijsen, 2006; Van Leeuwen and Vermeire, 2007).

The process of chemical risk assessment can be divided into four steps: (1) hazard identification; (2) exposure assessment; (3) dose-response characterization; (4) risk characterization (National Research Council, 1989; RATSC, 1999). The exposure assessment step is very important and consists of quantifying the levels of chemicals to which environmental and human targets are exposed, in terms of magnitude, duration and frequency (RATSC, 1999).

Many different approaches can be used for quantifying human exposures (Fig 1.1). Direct methods include measurements of exposure taken at the point of contact or at the moment it occurs (monitoring or biomonitoring). Indirect methods involve exposure estimations from measurements and existing data, like environmental monitoring, questionnaires and exposure models (Nieuwenhuijsen, 2003).

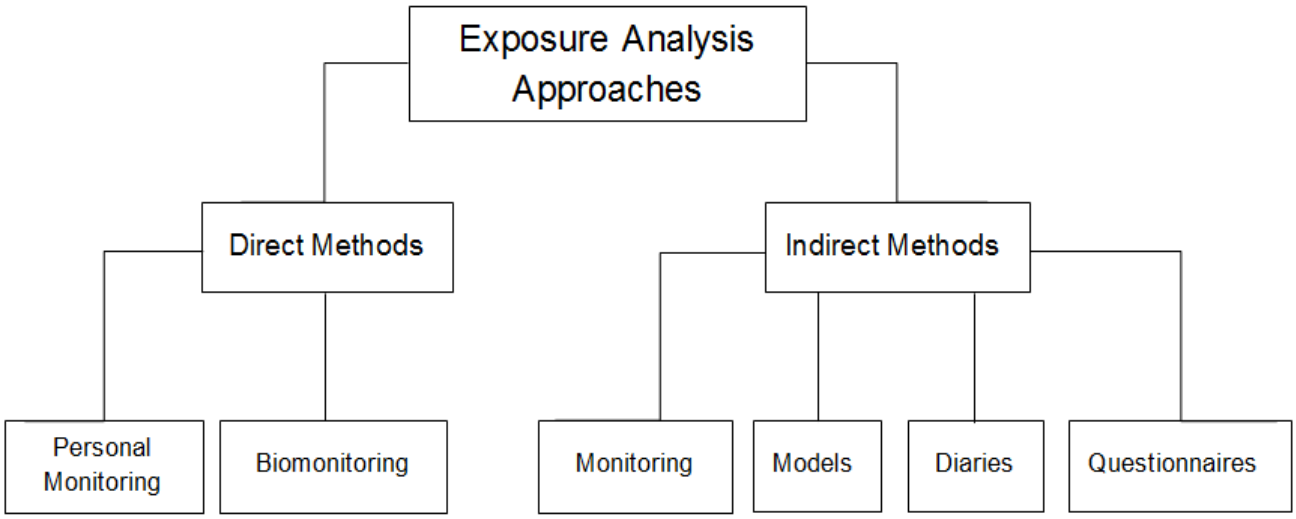

Fig. 1.1. Summary of approaches to determining personal exposure to pollutants (Adapted from Risk Assessment and Toxicology Steering Committee, 1999).

1.2 EXPOSURE MODELS

Exposure models are important tools for indirect exposure assessments. An exposure model is “a logical or empirical construct which allows estimation of individual or population exposure parameters from available input data” (WHO, 2000).

Models are used as instruments in risk assessment and risk management to describe the relationship between emissions and concentrations and to predict the results of risk management measures. They are usually used where direct measurements of exposure or biological monitoring data are not available or where these techniques are not applicable for the exposure assessment conditions. Moreover, there are a lot of benefits associated with the use of exposure models for quantifying human exposures: they can predict potential exposures for future or imaginary releases or contact events, and they allow the utility of existing data to be maximized by combining different sources into one structure (RATSC, 1999).

The degree of complexity of an exposure model can vary according to what is required by the assessment. The different exposure routes and pathways considered and included in a model depend on the complexity and the specific aim of the case study. So, a wide variety of exposure models are currently employed all over the world, characterized by different evaluating phases. Specific models have been developed to meet the requirements set for chemical exposure assessment by responsible authorities and agencies (e.g. ECETOC TRA, Stoffenmanager, Risk of Derm, ConsExpo are models developed in the context of REACH project). The existing exposure models, taking into account human exposure models and ecological risk assessment, can be categorized according to the following types of exposure source: environmental, dietary, consumer product, occupational, or aggregate and cumulative. Aggregate exposure models consider multiple exposure pathways, while cumulative models consider multiple chemicals (Fryer, 2006).

A crucial stage in creating and using models is the conceptualization stage, i.e. deciding what kind of representation of the reality has to be created. Obviously, model builders need to do this process of fundamental decision-making very carefully, while model users should realize that, in selecting an existing model for their specific purpose, decisions of the same kind are implicitly made. During conceptualization, modellers (builders and users) need to reflect on the purpose of their model and on what is modelled. Conceptualization involves making fundamental choices about what aspects of reality are relevant to the purpose of the specific modelling process and which aspects of reality are to be left out. At this stage, the modeller chooses the level of complexity required to meet the objectives of the modelling task. In general, simple models are preferred over sophisticated models, since the more sophisticated the model is, the more data and labour-intensive (and therefore expensive) the modelling activity, and the more difficult the interpretation of the results becomes (Commission of the European Communities, 1994).

The great benefit of using models is that they allow conceptualizing our knowledge of the exposure processes in order to describe the relationship in terms of the characteristics of the environment and properties of the chemical. The utility of models is that they allow us to evaluate the results of many processes occurring at the same time which would not otherwise be apparent. The processes influencing the concentration of a chemical are relatively well understood and may look simple if they are studied one by one. It is the multitude of processes acting together that makes the results difficult to understand (ECETOC, 1992).

A relevant issue concerning the development of exposure models consists in their validation. If generic fixed environmental values are used, the results might be inconsistent because the described environment does not exist in reality and so it is hard to validate the evaluation. However, the regional generic characteristics can be modified during the application phases of the model and region-specific parameters (from direct methods: measurements, monitoring, biomonitoring, etc.) can be introduced in order to validate a specific model setting (Mackay et al., 1985).

Exposure to chemicals can be evaluated through the use of direct measurements in the environment (monitoring methods). It may seem that direct measurements give more reliable results than model estimations. However, even measured exposure concentrations can have a considerable uncertainty, due to temporal and spatial variations. Therefore, when carrying out an exposure assessment it may be very useful to compare and integrate the estimated and measured concentrations in order to select the “right” data for use in the risk characterization phase (Commission of the European Communities, 1994).

This comparison can be done in three steps:

1. Selection of reliable monitoring data by evaluation of the analytical techniques used and the time scale of the measurements.
2. Correlating these data to the appropriate emission and modelling scenarios.
3. Comparing representative data with corresponding model estimates and undertaking a critical analysis of the differences between the two.

In principle, for exposure assessment, data from direct measurements in the environment should be given more weight than model calculations, remembering that they should be representative of the emission scenario and have adequately measured. A comparison with model estimations, however, is probably always advisable since it is the only way to validate the assumptions made in models. Each time model predictions are validated by monitoring or laboratory data, confidence in the model’s predictive power will increase (Van Leeuwen and Vermeire, 2007).

1.2.1 ENVIROMENTAL EXPOSURE MODELS

After contaminant concentrations have been measured in environmental media or estimated using transport and fate models, it is necessary to estimate the exposure to contaminants of selected targets (i.e., species in the ecosystems or human populations/groups). For most ecological risk assessments, the exposure corresponds to the concentration in water, sediment, or soil expressed as total concentration or as concentration in a period of time. Exposure models are necessary because the measured concentrations can vary in time and models help to give more realistic values (Suter, 2007).

Two wide categories of environmental exposure models can be distinguished: environmental concentration models and human intake models.

Environmental concentration models simulate environmental processes in order to generate chemical concentrations in particular media to which humans may come into direct contact (Pilling et al., 2004).Environmental exposure models have been developed in an effort to quantify human exposures to chemicals via the surrounding natural environment. A wide range of existing exposure models fall into this category, with individual models tending to focus on human exposures from a restricted range of environmental media (Fryer, 2006).

Exposure assessment has recently been restricted to evaluating, at various levels, the relationship between the source and concentrations of a hazard and a receptor (e.g. humans) in the environment by studying the exposure pathways and routes (Environment Agency UK, 2001).

Human intake models go one-step further by quantifying human chemical intake from contact with the relevant environmental media. Human intake models differ from environmental concentration models because they require data about human activities and physiology in addition to parameters related to chemical and environmental characteristics (Fryer, 2006).

1.2.2 MULTIMEDIA MODELS

If a chemical is released into one medium and resides there until it is removed by degradation or advection, single-media models may be perfectly suitable for estimating the environmental concentration. If, however, a chemical is released into several compartments at the same time, or after being released into one compartment is transported to other compartments, it becomes necessary to analyse the intermedia transport processes in order to understand its last fate in different environments. Multimedia models are specifically designed to accomplish this task (Van Leeuwen and Vermeire, 2007).

Multimedia fate models are typical examples of compartment mass balance models. The environment is represented as a group of homogeneous compartments, one compartment for each environmental medium in which the chemical is assumed to be uniformly distributed. The most typical compartments considered in models are: air, water, suspended solids, sediment, soil and biota (Den Hollander et al., 2004). Multimedia mass balance modelling was started in the early

1980's by Mackay and co-workers, and then more recently the use of multimedia fate models has become quite common for exposure assessment at different spatial and time scales. Generally, multimedia models can account for emissions into one or more compartments, exchanges by import and export between compartments and degradation in all.

In all the models, the user has to set parameter values (Fig. 1.2) for mass flows to provide input for the model (Mackay and Diamond, 1989). The main utility of multimedia models, as a first step in exposure assessment, is to determine to what extent intermedia partitioning may occur. As intermedia transfer is usually slow, its effect on the fate of chemicals is significant only over long periods of time. Therefore, the exposure assessment of chemicals on regional and larger spatial scales is one of the main applications of these models. Multimedia models are very useful especially for calculating the predicted environmental concentration of chemicals with a very diffuse release pattern (Berding and Matthies, 2002).

Essential model input data	Supporting substance properties
Henry's law constant	Molecular weight
Sediment-water partition coefficient	Water solubility
Soil-water partition coefficient	Octanol-water partition coefficient
	Vapour pressure
Half-life air	(Estimated) constant OH-radical attack
Half-life water	Readily biodegradable (yes/no)
Half-life sediment	
Half-life soil	

Fig. 1.2. Typical data requirements for multimedia models (Mackay and Paterson, 1981).

2. BIOACCUMULATION

2.1 BIOACCUMULATION PROCESS

Bioaccumulation is a fundamental process to be considered in environmental toxicology and risk assessment, because it controls the internal dose of potential toxicants (Mackay and Fraser, 2000). Information regarding chemical accumulation in organisms is important in determining environmental-quality guidelines, establishing total maximum daily loadings, categorizing substances that are potential hazards, and quantifying the risk of chemicals on ecosystems and human health (Government of Canada, 1999). Bioaccumulation is a complex environmental process influenced by many factors. When pollutants are released into the environment, most aquatic and terrestrial organisms are exposed to these chemicals. Some xenobiotics are taken up from organisms which can bioaccumulate in higher concentrations than the matrix where they live (Van Leeuwen and Vermeire, 2007).

Bioaccumulation is the result of biomagnification (the process which occurs when food is the major source of bioaccumulation in an organism) and bioconcentration (related to the direct uptake of chemicals by organisms from the surrounding matrix via respiratory surface and/or skin).

Particularly in aquatic organisms, bioconcentration describes the process of accumulation of chemical in the organism through the direct exposure to the polluted water. Biomagnification describes the process of accumulation of chemical along the food chain. It refers to those cases where concentrations in an organism exceed concentrations in the consumed prey (EXTONET, 1993).

The bioaccumulation factor (BAF) (Eq. 2.1) is the ratio of the concentration of the chemical in the organism (CB) to the total chemical concentration in the water (CWT), or to the freely dissolved chemical concentration in water (CWD) (Gobas and Morrison, 2000) and can be expressed as follows:

BAF = CB/CWT or CB/CWD *(Eq. 2.1)*

Uptake and elimination processes

There are several processes leading to the uptake and elimination of chemicals by organisms (Fig. 2.1). Each uptake process involves the passage of compounds across a biological membrane, mediated by a carrier or as a single solute (Block, 1991).

Organisms usually have a much higher capacity to store xenobiotics per unit of volume than the matrix where they live in. In fact chemicals are stored in lipids or proteins and they may reach higher concentration than in the matrix where they came from.

Metals, particularly, can be taken up by complex permeation, by carrier mediated processes or by ion channel. While there is no regulation in the uptake of chemicals by passive diffusion, organisms are able to regulate the uptake of chemicals by other, active uptake processes (Phillip, 1993).
Different routes lead to a significant reduction in the concentration of chemicals in an organism. Similarly to uptake processes, many mechanisms are responsible for the elimination of chemicals. Most hydrophobic chemicals are eliminated by passive diffusion, via water or via faeces. Growth is another way of diluting chemicals: the same number of moles of a compound in an organism results in a higher concentration than in a bigger organism. Reproductive transfer of chemicals via mammals 'milk or via the eggs can reduce the concentration in the organism. Biotransformation processes can also convert some chemicals into others, usually more hydrophilic and toxic than the original or sometimes into no effect substances (Sijm et al., 1992).

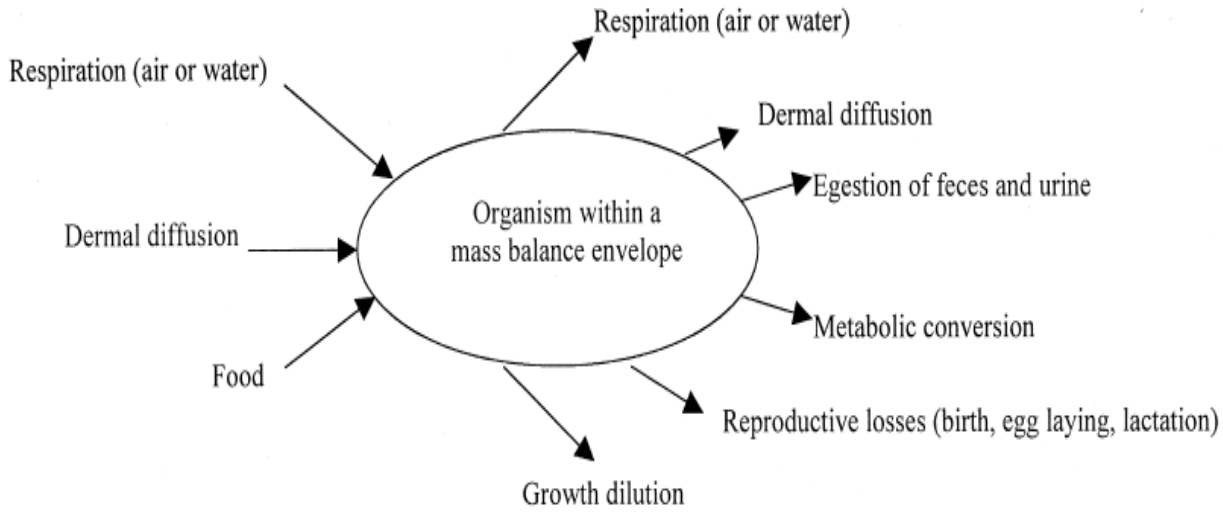

Fig. 2.1. Different processes of uptake and elimination applicable to a general organism with a mass balance envelope (Mackay and Fraser, 2000).

2.2 BIOCONCENTRATION

Bioconcentration is the result of the uptake, distribution and elimination processes of a chemical after the direct exposure to a polluted matrix. Many physicochemical factors regulate the bioconcentration process and the consequent chemical concentration in organisms.
For organic chemicals, which bioconcentrate in lipid tissues mainly by exchange processes from and to water, the degree of bioconcentration depends mostly on the hydrophobicity valuated by the

octanol-water partition coefficient (K_{ow}) and the lipid content of the organism (Van Leeuwen and Vermeire, 2007).

For metals, bioconcentration depends more on physiological processes. Some heavy metals influence bioconcentration processes because of their similarity to essential ions, like that of cadmium to calcium (Adams et al., 2000). Many aquatic organisms can show some physiological adaptation systems and can control internal metal concentrations through active regulation or storage, for example by synthetizing a storage protein, i.e. metallothionein (McGeer et al., 2003).

Factors that influence metal uptake and bioaccumulation act at every level of abiotic and biotic complexity, including: water geochemistry; membrane function; vascular and intercellular transfer mechanisms; and intracellular matrices. In addition, physiological processes (first of all renal, biliary or branchial) generally control elimination and detoxification processes. Storage improves additional controls on steady-state concentrations inside the organism.

The bioconcentration factor (Eq. 2.2) (BCF) is defined as the ratio of the chemical concentration in an organism (CB), to the total chemical concentration in the water (CWT), or the freely dissolved chemical concentration in water (CWD), and is expressed as:

BCF = CB/CWT or CB/CWD *(Eq. 2.2)*

The use of CWD is preferred because it only takes into account the fraction of the chemical in the water that is biologically available for uptake (Gobas and Morrison, 2000).

2.2.1 FACTORS AFFECTING BIOCONCENTRATION

Bioconcentration is a process depending on a lot of aspects linked to chemicals and organisms' characteristics. When assessing bioconcentration, it is strictly necessary to take into account the following chemical proprieties: molecular weight, molecular size, molecular charge, speciation, surface/volume ratios and morphology.

The main biological aspects influencing bioconcentration are: surface/volume ratios and morphology of chemicals molecules and the rate to which the chemicals are biotransformed (Van Leeuwen and Vermeire, 2007).

The bioavailability of chemicals is one of the most important characteristic than influences bioconcentration. Bioavailability is strictly related to the octanol-water partition coefficient, so K_{ow} can be useful for bioaccumulation assessment. For chemicals characterised by $\log K_{ow}$ values between 2 and 6, bioaccumulation could be estimated with precision (Connell et al., 1990). For chemicals characterised by $\log K_{ow}$ values between 5 and 6.5 biomagnification can be relevant, under 5 is improbable and over 8 is very significant (Thomann, 1989). One crucial factor in bioconcentration is the exposition time to the chemical. Assessing bioaccumulation depends on the time organisms are exposed to pollutants. During the exposition, the concentration of chemicals in the environment may change, consequently influencing organism's bioaccumulation and sometimes it becomes hard to assess a precise trend of bioaccumulation.

2.2.2 BIOTRANSFORMATION

Biotransformation is one of the processes which decrease the concentration of a chemical in an organism. In general, it transforms the chemical to more polar products (Sijm et al., 1989). In bioaccumulation models, biotransformation is considered as an elimination process, together with elimination through physicochemical processes, growth dilution, excretion by lactation and reproduction. Biotransformation is a process that takes place after the chemical has been transported to a site where it can be transformed through enzymatic catalytic action. In this process, the compound must reach the enzyme and then bind with it. Consequently, both transport rate or internal distribution and the capacity of the enzyme to fix and biotransform the chemical will determine the biotransformation rate. In addition, the enzyme requires other factors to enable the transformation. Species differ widely in their capacity for biotransformation, which largely depends on the presence or absence and specific activity of enzymes.

2.3 BIOMAGNIFICATION

Bioaccumulative substances have the potential to biomagnify via the food chain and affect organisms at higher trophic levels. Biomagnification takes place when the concentration of a chemical becomes higher in the organism than in its food (and the major uptake route is food) (Van Leeuwen and Vermeire, 2007). Therefore the concentration of pollutant increases along the food chain. Biomagnification is usually important only for chemicals reaching relatively high concentrations in food in comparison to very low concentrations in other surrounding environments, such as water for aquatic organisms, air for terrestrial organisms and soil and sediment for soil and benthic organisms. A biomagnification factor (BMF) can be defined (Eq. 2.3) as the ratio of the concentration of chemical in the organism (CB) to that in the organism's diet (CA) (Gobas and Morrison, 2000) and can be expressed as:

$$BMF = CB/CA \qquad (Eq.\ 2.3)$$

Uptake from food occurs in the gastrointestinal tract (GIT). After release of the contaminants in the GIT lumen, the chemicals may cross the lipid membranes. Food digestion is the process that leads to a thermodynamic gradient between the gut content and the organism (Kelly et al., 2004), which is responsible for biomagnification. Some aquatic organisms, such as many aquatic invertebrates, are sediment-dwelling organisms or deposit feeders. They are able to digest sediment or detritus, which serves as a food source. Uptake from sediment may be significant for these organisms. Biomagnification can only be measured when sufficient information is available on the type of food, the amount of food ingested, the uptake efficiency from food as well as excretion processes (Van Leeuwen and Vermeire, 2007).

2.4 BIOACCUMULATION MODELS

Assessing bioaccumulation is a relevant component of research activities aimed at identifying and controlling chemicals of environmental concern. It is generally accepted that substances which are persistent (P), bioaccumulative (B), and toxic (T) and are subject to long-range transport (LRT) are of particular concern (UNEP, 1998). International agreements (e.g. UN-ECE: agreement on a POPs protocol under the Geneva Convention on Long-Range Transboundary Air Pollution or the United Nations Environmental Program from Stockholm Convention) have been formulated to identify, ban or regulate these PBT/LRT substances from among the tens of thousands of chemicals of commerce (Teran et al., 2012), and those produced inadvertently as by-products of processes (AMAP, 1998) such as combustion, incineration, or various industrial operations (Vallack et al. 1998).

Several procedures for estimating bioaccumulation potential have been developed, mainly based on molecular structure or measurable properties of the substance. Typical measures used for assessing bioaccumulation include the octanol–water partition coefficient (K_{ow}), bioconcentration factor (BCF), bioaccumulation factor (BAF), and biota–sediment accumulation factor (BSAF). These can be obtained from empirical measurements and from mathematical models. Empirical information is often preferable, but models are also quite useful, particularly when empirical measurements do not exist or cannot be obtained for technical or economic reasons (Gobas el al., 2004). Chemicals are taken up by biota via different routes, from air, water, soil and sediment, and each process depends on environmental and physiological factors. Different models are used to describe and predict bioaccumulation, bioconcentration and biomagnification. Each type of bioaccumulation is measured differently and depends on the type of organism and chemical involved (McLachlan, 1996). Food chain or food web models can be used to predict bioaccumulation in aquatic and terrestrial organisms (Hendriks et al., 2001) and humans (Kelly et al., 2004). Concentrations in organisms in a food chain can be modelled by linking a set of equations to describe uptake from water and consecutive food sources. Rates for ventilation, food uptake, growth and reproductive effort are usually estimated with the help of allometric equations based on the relationships of these parameters to body size (Nielsen-Schmidt, 1984). Such models are mathematically very similar to environmental multimedia models (described in Chapter 1). The great benefit of these models is that food webs of different dimensions can be described with many food sources and the concentrations in all species can be calculated at the same time (Sharpe et al., 2000). In general, food web models successfully predict steady-state concentrations of persistent halogenated organic pollutants which are gradually metabolized (Arnot and Gobas, 2004; Traas et al. 2004). However, these models are relatively difficult to use for a large number of chemicals. A simpler approach consists in estimating the BAF of species at different trophic levels that account for water and food uptake with empirical regressions. These are calibrated on

measured BAF data and calculate a maximum BAF for persistent organic chemicals in selected trophic levels (e.g. algae, invertebrates and fish). The only required input is the K_{ow} value for the chemical. The main differences between model predictions and measured BAF values are due to biotransformation of a chemical by the organism and to an overestimation of bioavailable concentrations in the water column and in sediment (Voutsas et al. 2002).

2.4.1 MODELLING BIOACCUMULATION IN AQUATIC ORGANISMS

There are two general model approaches for quantifying bioaccumulation phenomena in aquatic organisms, as illustrated in Fig. 2.2.

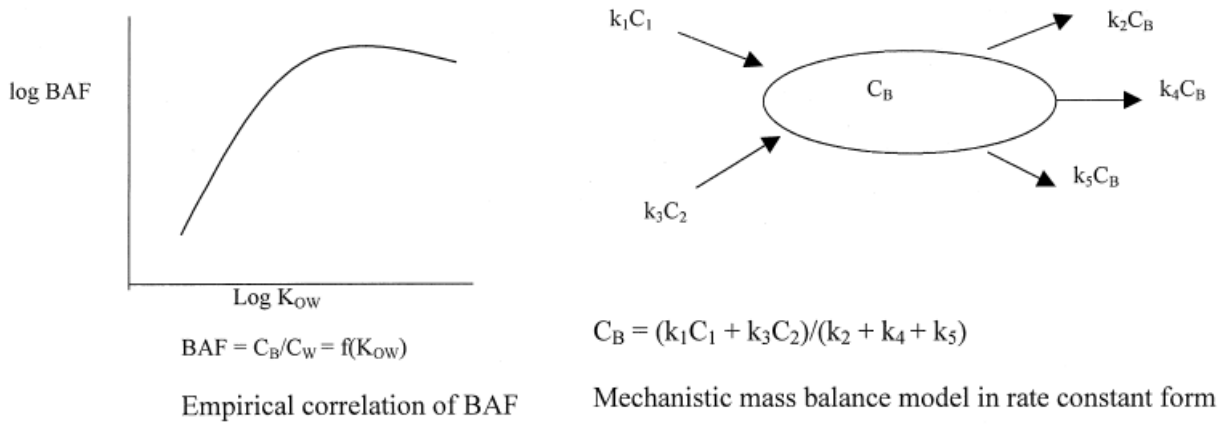

Fig. 2.2. The empirical correlation and mechanistic model approach for quantifying the concentration in the organism Cb as a function of environmental concentrations C1, C2, etc. (Mackay and Fraser, 2000).

In the “empirical approach”, BCF or BAF are estimated as the ratio between concentration in aquatic organism and a measured concentration. The usual empirical approach is to plot logBCF or logBAF versus logK_{ow}, and obtain a regression which can be used as a predictive correlation. These values are inevitably subject to error and variability but however they represent the real conditions. The main goal of these models is to use the model parameter values as a basis for prediction (Mackay and Fraser, 2000).

In the second approach, a mass balance model quantifying uptake and loss processes is developed. Such models require lots of data about the chemicals and the organism to be modelled, such as information to describe respiration processes, feeding rates and absorption. Nowadays, it can be stated that a validated mechanistic model provides quite good data to describe bioaccumulation processes and has the potential to give the most reliable predictions. Along with the improvement of bioaccumulation studies, there has been a shift from the empirical to the mechanistic approach. Simple correlations of BAFs against measurable quantities such as K_{ow} are

being substituted by a series of more complete equations and models able to describe and quantify the most important processes (Mackay and Fraser, 2000).

The first step to quantify uptake and clearance mechanisms is to identify the relevant processes for the specific organism and then try to quantify the uptake and elimination rates. So it is possible to compile a mass balance equation that describes the processes from external concentrations to internal concentrations or body loads. It is advised to consider the organism as one compartment and draw a mass balance envelope. After this, it is calculated the total mass of chemical in the envelope which gives the total mass and the average concentration (Mackay and Fraser, 2000). In some mass balance models, the gut and lungs or gills are treated as separate compartments from the internal tissues. It is important that measurements of organism body burden and model assumptions are referred to the same envelope. In the most sophisticated models, the organism is treated as a set of connected organs or tissue group. Most refined models are the physiologically based pharmacokinetic models (PBPK) which are widely used in human and rodent pharmacology (Reddy et al.,2005).

To assess bioaccumulation considering also biomagnification, it is suggested that a simple food chain be modelled, similar to those described by Gobas (1993), Carbonell (2000) or Campfens and Mackay (1997). The result will be a food web bioaccumulation model considering the aquatic food chain or treating also higher organisms (e.g. mammals and birds).

An attractive possibility is the development of a general model which could treat a large number of organisms and physiological and chemical data. The individual organisms could then be linked using predator/prey relationships to describe food webs. To be complete, such a model must include vegetation as a food and contaminant source. It could be possible to develop a model of contaminant migration considering entire ecosystems (Mackay and Fraser, 2000).

Higher organisms, such as mammals and birds, are called top predators and form the end point of biological pathways along which contaminants may accumulate in increasing concentrations. So they may be subject to adverse effects. Food is the major route of uptake for mammals and birds. Crucial to bioaccumulation process is the choice of food. Plants and lower organisms are the prey of mammals and birds. An essential difference between birds/mammals and fish/plankton is represented by elimination rates. The elimination rate drives biomagnification: if it is high, it does not matter how much an organism eats, and no biomagnification will occur. Thus, biomagnification of hydrophobic chemicals which are very slowly excreted will be most pronounced in birds and mammals that prey on organisms which are already relatively highly contaminated (Travis and Arms, 1988). Since the concentration of contaminants vary significantly between the preys, the choice of food largely determines the concentration of contaminants in higher organisms. When the exact composition of the diet, the concentration of the contaminant in the diet items and the uptake efficiencies are available, uptake from food can be modelled. Some models take a different approach, focusing on bioenergetics. Bioenergy based models try to assess bioaccumulation

relating the amount of energy a higher organism requires for growth, reproduction, warmth, migration, etc., to the amount, caloric content and digestive efficiency of the food they ingest (Norstrom et al., 1979; DeBruyn and Gobas, 2006).

2.4.2 REVIEW OF BIOACCUMULATION MODELS

This paragraph provides a review of existing bioaccumulation models, focusing on the most known and widely used for modelling aquatic and human food chain. This thesis is focused on aquatic and human food chain models in relation to the implementation of MERLIN-Expo and its application to the Venice lagoon case study. The main objective of the case-study application is to estimate human exposure to POPs, through the local seafood diet, in order to support the characterization of health risks associated with local fish and seafood consumption and bioaccumulation process. Accordingly, the following models have been reviewed:

a) Aquatic food chain:
   - EU Technical Guidance Document/EUSES
   - Foodweb model
   - Food Chain Bioaccumulation/ECOFATE models
   - AQUATOX
   - Prediction of Bioaccumulation in Aquatic Food Webs model
   - OMEGA
   - BASS
   - System dynamic model
   - TRIM.FaTE

b) Human food chain (including bioaccumulation in human population):
   - EU Technical Guidance Document/EUSES
   - ACC-Human model
   - TRIM.FaTE

Hereunder a short description of each of the most used models for aquatic and humans food chain is provided.

The **Technical Guidance Document** (frequently abbreviated to TGD) is used for the risk assessment of both new and existing substances in the EU (European Commission, 2003). The TGD contains a detailed description of methods to carry out a risk assessment considering exposure of aquatic organisms, wildlife and humans to a chemical through environmental pathways. The methods are presented as a series of equations to predict contaminants

concentration in human aquatic and terrestrial organisms. To calculate aquatic food chain bioaccumulation is used the fish bioconcentration factor (BCF). Equation by Veith et al. (1979) and by Connel and Hawker (1988) are included in the TGD for estimating BCF. The methods proposed in the TGD are also implemented in a computer program called **EUSES** (European Uniform System for Evaluation of Substances), which is also recommended for chemical risk assessment in compliance with REACH regulation (European Parliament, 2006). The TGD method uses the concentrations in air and soil and the dissolved concentration in surface water and marine water as inputs. These can either be estimated using the TGD method/EUSES or can be input as known concentrations. The method allows the calculation of concentrations at two spatial scales: the local scale (which represents concentrations in the vicinity of a point source of release) and a larger, regional scale. The method takes into account degradation in soil, water, sediment and air.

The **Foodweb model** has been developed by the Canadian Environmental Modelling Centre at Trent University. The model is available free of charge from their modelling website. Details of the theory behind the model are given in Campfens and Mackay (1997). The model is a fugacity-based mass balance model of the contaminant flux through an aquatic food web. Uptake by organisms in the food web is assumed to occur by diffusion from water (water column or sediment pore water) and via diet (both benthic and pelagic food organisms are considered). Clearance of the chemical from the organisms is modelled as a result of respiration, egestion and metabolism. The model also takes into account growth dilution. The food web can consist of any number of organisms, and each organism can be specified to feed on any others within the food web (including their own species).

The **Food Chain Bioaccumulation model** and the **ECOFATE** model have been developed by the Simon Fraser University in Canada. The following three models (all working on very similar principles) are available for download free of charge:

- Food Chain Bioaccumulation model version 1.0
- Food Chain Bioaccumulation model version 1.1
- ECOFATE model version 1.0

The models were originally developed for the Lake Ontario ecosystem but can be adapted to other ecosystems. The ECOFATE model is a beta-test version. The model is used within the USEPA methodology to assess ambient water quality criteria for the protection of human health (USEPA, 2000). The model is a non-steady state mass balance model for hydrophobic organic chemicals based on a Lake Ontario food chain. The Food Chain Bioaccumulation model (version 1.0) is based on the work published by Gobas (1993) but it has recently updated by a new study by Arnot and Gobas (2004) that introduces new kinetics models and allometric relationships obtained from laboratory experiments.

The main assumptions used in the model are as follows:

• the bioconcentration factor for a chemical in aquatic macrophytes, phytoplankton and zooplankton can be estimated directly from the K_{ow} value and the lipid content of the macrophytes/phytoplankton/zooplankton;

• the bioaccumulation in benthic invertebrates results from an equilibrium partitioning of the chemical between the lipids of the organism, the organic carbon fraction of the sediment and the interstitial pore water;

• uptake into fish can occur directly from water via the gills and from the consumption of food via the gastrointestinal tract;

• loss of chemical from the fish can occur via the gills to water, via egestion to faecal matter and as a result of metabolic transformation.

The model core is that the change in concentration of a chemical with time in an organism is a result of the rate of uptake and loss of the chemical. The effect of growth of the organism is also taken into account.

The **AQUATOX** model was produced by the United States Environmental Protection Agency to assist in the performance of ecological risk assessments for aquatic ecosystems (USEPA, 2004). The model is an ecological risk assessment model for aquatic ecosystems that takes into account the combined environmental fate and effects of toxic chemicals, and also pollutants such as nutrients and sediment. It considers several trophic levels including attached and planktonic algae, submerged aquatic vegetation, invertebrates, forage-feeding fish, bottom-feeding fish and game fish. The model simulates the transfer of biomass, energy and chemicals from one compartment of an ecosystem to another using a process-based or mechanistic model. A database of species-related data is contained within the model. This database covers numerous species including fish, aquatic invertebrates, benthic organisms and aquatic plants and algae. Many outputs from the model are available including BAFs and predicted concentrations in the aquatic organisms considered. The model also has built-in routines to carry out uncertainty analysis (Brooke and Crookes, 2007).

Prediction of Bioaccumulation in Aquatic Food Webs method was published as a research paper (Voutsas et al., 2002). The model consists of a series of regression equations relating field bioaccumulation factors to $\log K_{ow}$ for four trophic levels. The data used covered a wide variety of persistent, non-metabolized organic chemicals including polychlorinated biphenyls, other chlorinated hydrocarbons and polyaromatic hydrocarbons. Many of the data referred to field studies in the Great Lakes area but also included some data from the Canadian Arctic, an estuarine system in Louisiana and various water systems in the Netherlands. The data used consisted of measurements of concentrations in water and concentrations in various aquatic

organisms from the same location. In order to facilitate the analysis, organisms were assigned to one of four generalized trophic levels. These were:

- plankton, including both phytoplankton and zooplankton;
- benthic invertebrates;
- planktivorous fish;
- piscivorous fish.

The **OMEGA** bioaccumulation model is a kinetic model for food chain transfer, based on chemical fugacity and biological allometry. It estimates the internal chemical concentration in an organism based on the uptake and elimination rate constants of the chemical. These rate constants are a function of the chemical property K_{ow} and the species wet weight, lipid content and trophic level (Hendriks et al., 2001). The OMEGA model helps to assess bioaccumulation rates of many chemicals, in contrast to most other bioaccumulation models which depend on experimental data. This method based on fugacity, provided by Hendriks (2001) does not need a lot of parameters (e.g. gut uptake efficiencies or ventilation rates) which are necessary in other models. The OMEGA model has been successfully applied to estimate the internal concentrations of metals and several organic pollutants for many invertebrate and vertebrate species from aquatic or terrestrial food chain (De Laender et al., 2010; Hauck et al., 2007).

The **BASS** (Bioaccumulation and Aquatic System Simulator) model is developed by the United States Environmental Protection Agency (USEPA). The BASS model incorporates an earlier bioaccumulation model developed by the USEPA called FGETS (Food and Gill Exchange of Toxic Substances; Barber et al., 1988 and 1991). A user guide is available (Barber, 2005) outlining full details of the model. The BASS model can be used to predict both the population and bioaccumulation dynamics of age-structured fish populations. It consists of a bioaccumulation model coupled to a growth model and a model for population dynamics. The community's food web is specified by defining one or more foraging classes for each fish species based on its body weight, body length, or age. The dietary composition of each of these foraging classes is specified as a combination of benthos, incidental terrestrial insects, periphyton/attached algae, phytoplankton, zooplankton, and one or more fish species. The model can be used for hydrophobic organic chemicals and some types of metals. The overall model is based on mass balance differential equations.

The **System Dynamic model** is based on the paper by Carbonell et al. (2000). The model was developed with the goal of modelling the food chain taking into account the bioaccumulation and biomagnification processes. The paper by Carbonell et al. (2000) is focused on aquatic food chain model, but the described principles in the paper can be applied to other food webs. The model is

flexible and there are no limits to the number of organisms to be modelled. One of the two versions available is intended to represent a worst-case calculation and assumes an instantaneous equilibrium between water, sediment and all organisms considered in the food chain. This version requires information on bioconcentration factors (BCFs), and biota-food (BFAF) and biota-sediment (BSAF) accumulation factors (Brooke and Crookes, 2007). The other version takes into account uptake and elimination rates of chemicals in the organisms.

The **ACC-Human model** is a food chain model for predicting the levels of lipophilic organic chemicals in humans. The model was published in a paper by Czub and McLachlan (2004) and a computerized version is available. The model is a fugacity-based, non-steady state, mechanistic model that considers the bioaccumulation of lipophilic organic chemicals by humans exposed through air, water, soil and food. The model incorporates recent advances in the scientific understanding of bioaccumulation processes in agricultural and aquatic food chains, as well as in humans. The model predicts human tissue levels, like levels in various parts of the food chain, from concentrations of a chemical in air, water and soil. Within the model a representative food chain is constructed for an agricultural soil system and a marine water system. The top predator in each system is considered to be humans. Each link in the food chain is treated as being composed of one or several homogenous compartments that are assumed to be in equilibrium with each other. Each link in the food chain is interconnected with the appropriate abiotic environmental compartments (air, water, soil) and the next link below it in the food chain. A mass balance is defined for each compartment. For the marine water system, the model assumes a simple pelagic food chain consisting of zooplankton, planktivorous fish and piscivorous fish.

The **Total Risk Integrated Methodology** (TRIM.FaTE) was produced by the United States Environmental Protection Agency to assist in the performance of ecological risk assessments. TRIM.FaTE is a spatially explicit, compartmental mass balance model that can be used to predict pollutant concentrations in multiple environmental media (including biota) and pollutant intakes for biota. The actual food chains considered can be user-defined. The model contains information for various defined compartment types that can be used to construct an ecosystem; some of these compartments contain the necessary input data for the model, others have to be input by the user (Brooke and Crookes, 2007).

3. MERLIN-Expo

3.1 4FUN PROJECT

The work presented in this thesis has been developed within the European project "4FUN" (*The FUture of FUlly integrated human exposure assessment of chemicals: Ensuring the long-term viability and technology transfer of the EU-FUNded 2-FUN tools as standardized solution*). 4FUN project is funded by the European Union's Seventh Framework Programme for Research, it started in October 2012 and will continue until the end of 2015 (www.4funproject.eu), and includes 14 partner institutions from different EU countries (Fig. 3.1). 4FUN is the follow-up project of "2-FUN" project (2007-2011), which produced a prototype software tool containing a library of exposure models, combining environmental multimedia and pharmacokinetic models. The 2-FUN software resulted to be an innovative and useful instrument for the assessment of human health risks from exposure to chemicals, but it was only a prototype and not a standardised software. The main objective of 4FUN project is to transform this first prototype into a standardized tool and to ensure the long term applicability and visibility of the tool (4FUN Project, 2014c).

Fig. 3.1. 4FUN project partners (www.4funproject.eu).

The tool developed during 4FUN project is called “MERLIN-Expo”, an acronym standing for: *Modelling Exposure to chemicals for Risk assessment: a comprehensive Library of multimedia and PBPK models for Integration, Prediction, uNcertainty and Sensitivity analysis*.

4FUN project activities include the improvement of the software tool, its benchmarking through comparison with similar existing models, its demonstration in real case-studies and the development of a detailed documentation for end-users. The demonstration of MERLIN-Expo will include the parameterisation of the environmental multimedia and PBPK models in order to tailor the assessment to site-specific conditions, the comparison between the model outcomes and actual monitoring data for the full chain of models, and uncertainty and variability analysis.

To demonstrate the reliability of the model and its applicability to complex exposure scenarios, three case studies were selected:

1. Assessment of body burden of metals (lead and arsenic) for adults and children in Belgium regions, using environmental contamination data in several media (air, dust, soil samples) and considering population behaviour (diet, activity pattern, health status). Metals concentrations in blood and urine samples from local population will be used to compare with model results.
2. Assessment of population exposure to POPs (particurarly PCBs) through the diet in the Venice region, considering the bioaccumulation of chemicals along the aquatic food web (and in particular in edible organisms). Human biomonitoring data on PCBs concentration in serum and breast milk in mothers are available for comparison with the internal exposure estimated by the model.
3. Assessment of human exposure to halogenated emerging pollutants focalising on endocrine disruptors, namely brominated flame retardants (BFRs) and perfluorinated compounds (PFCs) in Ebro River (Spain). Human biomonitoring datasets (chemical concentrations in breast milk, blood, cord blood) will be used to assess the agreement between simulations and measured data.

3.2 MERLIN-Expo TOOL

MERLIN-Expo is a tool integrating on the same platform a multimedia model, an intake model and a physiologically-based pharmacokinetic (PBPK) model, allowing to cover all the exposure assessment chain (from concentration in water, air and/or soil to internal dose to target organs) over a wide range of time periods. A set of environmental models (Fig. 3.2) can be combined to calculate the distribution of target pollutants in multiple environmental media (air, water, soil, vegetation) by considering inter-media pollutant transfers. Combined with the information about human behaviours (diet, time outdoor, etc.) the multimedia model can provide an estimation of the daily chemical intake by inhalation, ingestion, and dermal intake by the population of interest.

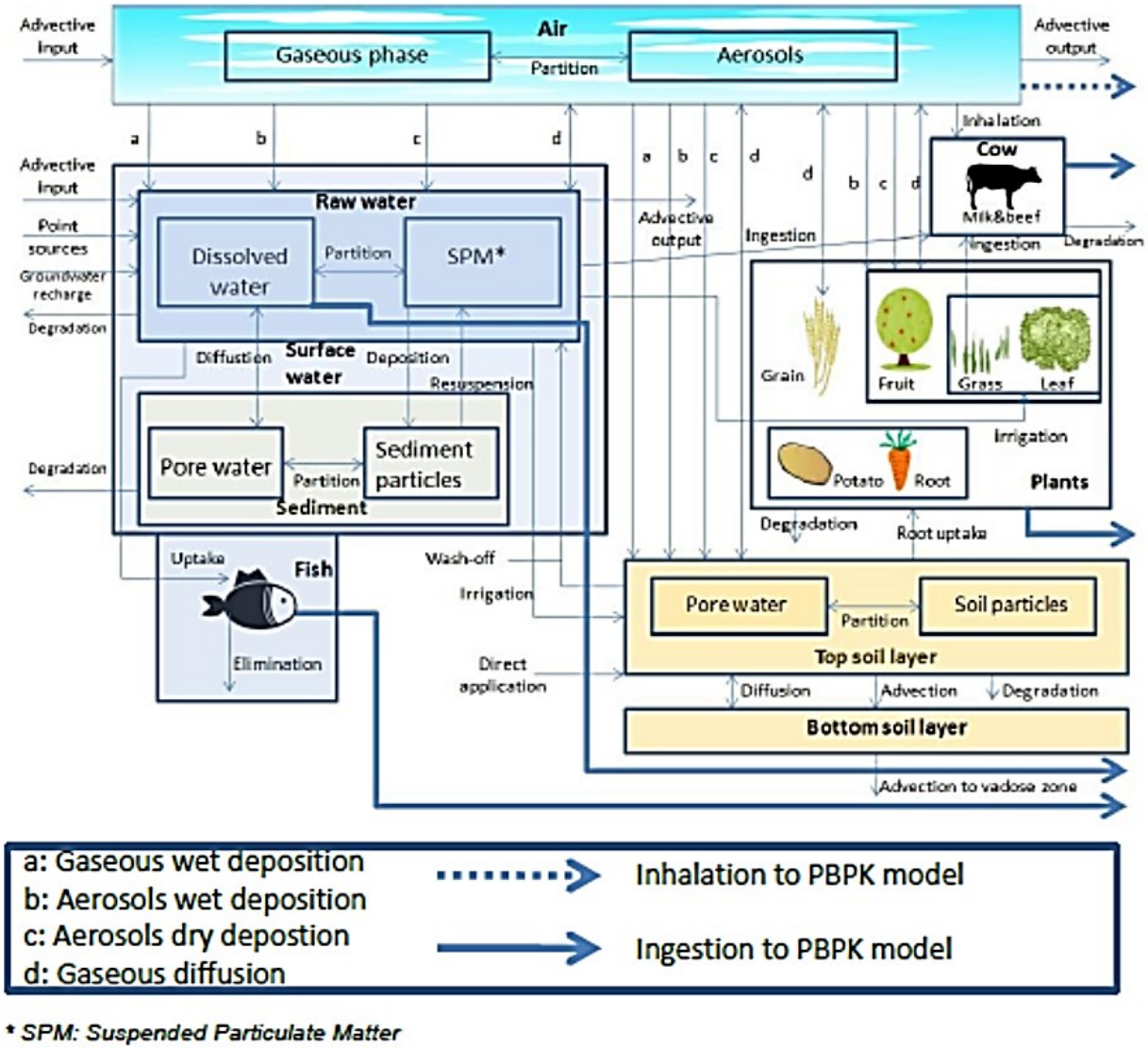

Fig. 3.2. Multimedia Model structure (4FUN Project, 2014b).

The Intake model estimates human daily chemical intake for the target chemicals, using as inputs the environmental concentrations provided by the environmental models. It considers information on the diet composition (daily intake rates of different food items), on lifestyles at different age classes, and inhalation rates.

The PBPK model (Fig. 3.3), using as input the chemical daily intake provided by the intake model, predicts the internal effective concentrations in the target internal tissues where toxic effects arise. It considers quantitative descriptions of the absorption, distribution, metabolism and excretion (ADME) of chemicals in biota based on interrelationships among key physiological, biochemical and physicochemical determinants of these processes (WHO, 2010). It can consider the influence of age-or gender-dependent changes on the internal dosimetry (Clewell et al. 2004; Beaudouin et al. 2010).

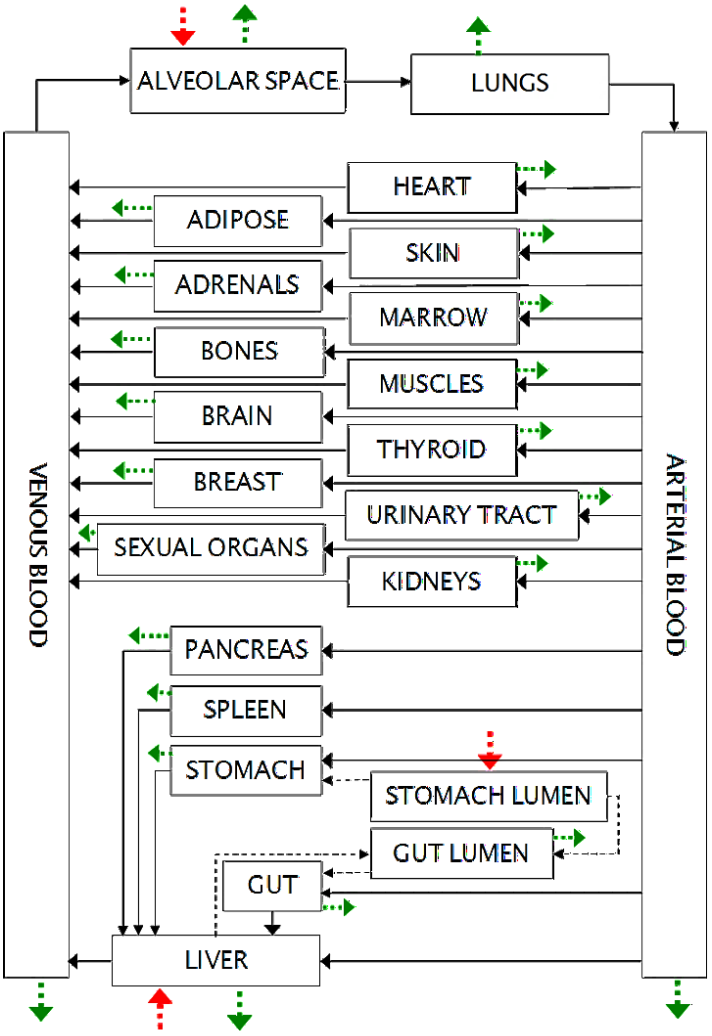

Fig. 3.3. PBPK model structure (4-FUN Project, 2014b).

MERLIN-Expo tool includes functionalities for creating different types of charts and tables for representing input data and results, and allows creating exportable reports with selected input and output data.

Finally, MERLIN-Expo tool contains a set of functionalities for uncertainty and sensitivity analysis that are in line with the tiered approach recommended by WHO (2005).

MERLIN-Expo can run model simulations for:

- deterministic application: inputs and outputs are single values non including elements of uncertainly and randomness; in deterministic application mode, when the model runs with the same initial input values, the results will be the same.
- probabilistic application: in order to take into account the parametric uncertainty in the model inputs, some parameters values are given by probability density functions (PDFs) (instead than by single value). In MERLIN-expo uncertainly can be propagated through random sampling procedure, i.e. Monte Carlo method (Ciffroy et al., 2011).

3.3 LIBRARY OF MODELS IN MERLIN-Expo

MERLIN-Expo tool allows carrying out lifetime exposure assessments for different human populations (general population, children at different ages, pregnant women) considering exposure through multiple pathways.

Multimedia, Intake and PBPK models are implemented on the same platform, i.e. Ecolego (http://en.wikipedia.org/wiki/Ecolego), in order to facilitate integrated full-chain assessments for combined exposures. One of the main characteristics of Ecolego is the use of Interaction Matrices to create and visualize complex dynamic models with many interactions between compartments. The interaction matrix allows combining different independent compartments into hierarchical sub-systems to simulate complex scenarios. Ecolego provides powerful solvers for complex dynamic system and allows performing both deterministic and probabilistic simulations. The graphical user interface supports the user in defining and managing building blocks, parameters, species and simulation settings.

Available “modules” in MERLIN-Expo library are:

- Environmental modules: River, Atmosphere and Soil;
- Food modules: Root, Fruit, Grain, Leaf and Grass, Tuber, Cow milk, Beef meat, Fish, Invertebrates and Phytoplankton;
- Human Intake module;
- PBPK (Physiology Based Pharmaco-Kinetic) model.

The following section presents the modules (sub-models) that are currently available in the MERLIN-Expo library, with a short description of the goals, outputs (and their regulatory relevance), and main processes incorporated in the model (4-FUN Project, 2014a). Since this thesis is focused on the application and testing of the Fish model, this model is described more in detail in the next paragraph (Par. 3.4).

The River Model

The goal of the River Model is to dynamically simulate the distribution of organic contaminants and metals in abiotic media (i.e. water, suspended particulate matter and sediments) of river systems. It provides an estimation of the time-dependent concentration of the targeted contaminant(s) in raw water, filtered water and bottom sediments, which can be used for evaluating the risk to exceed a given regulatory threshold for environmental risk (e.g. Predicted Non Effect Concentration (PNECs), Environmental Quality Standards (EQS) for individual pollutants defined by the European Water Framework Directive). It can also be used to estimate contaminant inputs onto soils and

plant leaves originating from irrigation practices and to calculate contaminant concentrations in potential drinking water.

The main processes considered in the River model are: sorption/desorption between water and Spm; deposition of particulate contaminants to bed sediments; resuspension of particulate contaminants from bed sediments; diffusion between water and sediment pore water; diffusion between water and atmosphere; degradation.

The Atmosphere Model

The goal of the Atmosphere model is to dynamically simulate the distribution of organic contaminants and metals in the atmospheric systems. This model can provide an estimation of the time-dependent concentration of the targeted contaminant(s) in total atmosphere and the air column, for example for evaluating the residence time of contaminant(s) in air and the risk over time to exceed a given regulatory threshold for environmental risk. Moreover it can be used to estimate atmospheric inputs of contaminants to soil or river system and time-dependent concentration of the targeted contaminant(s) in inhaled air (e.g. to provide an input for PBPK models).

The main processes modelled by the Atmosphere model are: partition between aerosol and gas; diffusion within air; dry and wet deposition of aerosols; gaseous exchanges at the atmosphere-soil interface; interception by leaves; degradation.

The Soil Model

The goal of the Soil Model is to dynamically simulate the distribution of organic contaminants and metals in abiotic media (i.e. soil particles, pore water) of soil systems, with a description of their depth profile in the root zone. Its outputs can be used for evaluating the risk to exceed a given regulatory threshold for environmental risk and to evaluate the residence time of contaminant(s) in soil. Moreover, it provides an estimation of contaminant inputs into plant crops originating from root uptake; an estimation of contaminants emitted from soils to the atmosphere, an estimation of the time-dependent concentration of the targeted contaminant(s) in soil available for human ingestion (pica behaviour in children).

The main processes considered in the Soil Model are as follows: sorption/desorption between pore water and soil particles; evapotranspiration; water mass balance in soil and loss by infiltration; retardation factor and advection within soil; diffusion between water and atmosphere; bioturbation; diffusion within soil; wash-off from soils to river; degradation.

The Fruit, Leaf, Grass and Grain Models

The goal of Fruit, Leaf, Grass and Grain Model is to estimate the time-dependent accumulation (as mass and concentration) of organic/metals in the edible part in fruit, grain and leafy crops at harvest.

Coupled with the information about the ingestion rate of fruit, leafy, root, grass or grain crops (expressed as $kg_{freshweight}$ d^{-1}), these food models can estimate the human exposure to organic substances/metals through the ingestion. This output can be used for evaluating the risk to exceed regulatory thresholds for human health or used as an input for the PBPK model.

The main processes considered in these models are: sorption/desorption between pore water and soil particles; partition between concentrations in roots and in water (xylem water), and concentrations in leaves or fruit and in water; xylem influx from soil to root driven by plant transpiration; xylem outflux from root to leaves, fruit or grain or driven by plant transpiration; phloem outflux from leaves to grain or fruit driven by fruit growth; diffusion between leaves, grain, fruit and air; dry and wet deposition from air to leaves, fruit and grain; degradation in roots, grain, fruit and leaves; root uptake (for metals).

The Root and Tuber Models

The goal of Root and Tuber Models is to estimate the time-dependent accumulation (as mass and concentration) of organic/metals in the edible part in root (e.g., carrots) and in tubers (e.g., potatoes) at harvest. Coupled with the information about the ingestion rate of root and tubers crops, the root and tubers model can estimate the human exposure to organic substances/metals through the diet. This output can be used for evaluating the risk to exceed regulatory thresholds for human health or used as an input for PBPK models.

The main processes considered in the model are: sorption/desorption between pore water and soil particles; partition between concentrations in tubers or roots and in water; diffusion from soil to tubers driven by concentration gradient; xylem influx from soil to root driven by plant transpiration; degradation in root and tubers; root uptake (for metals).

The Cow Milk and Meat Model

The Cow Model is aimed at estimating the time-dependent accumulation (as in mass and concentration) of organic/metals in cow milk and meat. The estimated time-dependent concentration of the targeted contaminant(s) in cow milk and meat available for food ingestion can be used for evaluating the risk to exceed a given regulatory threshold for human health (e.g. Daily Reference Dose) or to provide an input for PBPK models.

The main processes considered are: dietary uptake/elimination of chemicals; water uptake/elimination of chemicals; soil uptake/elimination of chemicals; metabolic biotransformation.

The Human Intake Model

The Human Intake Model can dynamically simulate the intake of organic contaminants and metals by humans from intentional ingestion of drinking water, crops and animal products, non-intentional ingestion of dust and soil particles (pica behaviour for children) and inhalation of air contaminants. It is aimed at linking environmental/food models with the PBPK model, because this latter requires the daily ingested or inhaled dose(s) of the targeted contaminant as input.

The PBPK Model

The Physiologically-Based Pharmaco-Kinetic (PBPK) model is aimed at simulating the toxicokinetics of contaminants in humans, i.e. the time-dependent amounts (or concentrations) in different human organs/tissues following ingestion or inhalation.

The PBPK Model provides the computation of toxicokinetic properties in each compartment (e.g. the maximal concentration C_{max}, the time at maximal concentration T_{max}) and the prediction of the amount of contaminant that will be excreted or/and metabolised. The outputs of the PBPK model can be used for evaluating the risk to exceed a given regulatory threshold for human health (e.g. Equivalent Biomonitoring Reference Doses).

The main processes simulated by the PBPK model are: growth of human individuals (influencing anatomy, physiology and metabolism, and thus PBPK parameters); absorption by inhalation; absorption by ingestion; distribution of chemicals among organs (i.e. partitioning of a compound into the various tissues of the body from the systemic circulation); metabolism (i.e. irreversible transformation of a parent compound into metabolites by enzymatic reactions); excretion (i.e. removal of the compound and its metabolites from the body, occurring predominantly via the kidneys in urine).

3.4 THE FISH MODEL

In order to expand the available library of models in MERLIN-Expo and to allow the estimation of bioaccumulation of contaminants in aquatic organisms, a Fish Model has been recently developed by the University Ca' Foscari of Venice in collaboration with Electricitè De France (EDF) and it has been subsequently implemented in MERLIN-Expo tool. Since the objective of this thesis is to verify and test the applicability and the performance of the Fish Model, an analysis of the model has been performed and in this paragraph a detailed description of this model is provided, including its applicability domain, its conceptual structures and parameters, input data required to apply the model.

3.4.1 MODEL PURPOSE AND APPLICATION

The goal of the Fish model is to dynamically simulate the bioaccumulation of organic contaminants and metals in fish and to calculate the concentration of chemicals accumulated in fish caught for human food.

The Fish Model can provide an estimation of the time-dependent concentration of the targeted contaminant(s) in fish. This/these output(s) can be used for evaluating the risk to exceed a given regulatory threshold for environmental risk (e.g. Environmental Quality Standards (EQS) in fish for individual pollutants).

The Fish model can be coupled with some of the modules described in Paragraph 3.3 in order to simulate different exposure scenarios. It will be possible to couple the Fish model with Phytoplankton model and Aquatic Invertebrate model (currently under development and not yet implemented in MERLIN-Expo) to simulate the transfer of contaminants along an aquatic food chain including organisms at different trophic levels (e.g. planktonic species, benthic organisms such as molluscs and crustaceans, fish). Coupling them together with the River model it will be possible to simulate a realistic aquatic scenario to assess bioaccumulation and biomagnification processes useful for ecological risk assessment.

Coupled with the model dedicated to Human intake, the Fish model can provide an estimation of the time-dependent concentration of the targeted contaminant(s) in fish available for food ingestion. This output can be used for evaluating the risk to exceed a given regulatory threshold for human health (e.g. daily Reference Dose) or to provide an input for the PBPK model.

Spatial scale and resolution: spatial scale and resolution are governed by the homogeneity of the water body under investigation, in which Fish is assumed to live. It is then advised to use the Fish model for river zones that show low variations in their geometry. For water bodies showing significant relative variations in their dimensions (e.g. under the effect of tributaries affecting dilution of contaminants in water), it is possible to subdivide these latter in several successive homogeneous zones and to couple them.

The River model assumes that contaminants are homogeneously distributed along the transect of the river (i.e. laterally and vertically). The distribution of Fish among several zones presenting different contamination levels must be defined. In the MERLIN model, no migration of fish among different zones is assumed. So, Fish are assumed to stay in the same water box during their entire life.

Temporal scale and resolution: there is no limitation for temporal scale (i.e. duration of the simulation). As far as temporal resolution is concerned, several processes included in the model are relevant at daily (or less) resolution for fish submitted to exposure from sediments (e.g. benthic fish, fish ingesting significant quantities of SPM). Physical exchanges at the water column-

sediment interface are highly dependent on water velocity and concomitantly on flow rate. This is especially the case for resuspension processes during flood events. As the flow rate can be subject to rapid variations, especially during flood periods, the estimation of particles resuspension is poorly relevant for large temporal resolution (e.g. weekly or monthly). In conclusion, it is highly recommended to run the model for daily (or less) temporal resolution.

Chemical considered: the Fish model can be used for all organic contaminants (e.g. PAHs, PCBs, pesticides, etc.). Some parameters are estimated from QSAR models. For some compounds, partition coefficients are theoretically related to pH and the applicability domain of existing QSAR must be checked with cautious. The Fish model can be used for metals for which Bioconcentration Factors are provided (i.e. As, Cd, Cu, Pb, Zn, Ni, Ag).

Steady-state vs dynamic processes: some existing models imply constancy of parameters in time (steady-state models) (Mackay, 2001). The Fish model in MERLIN-Expo represents all the exchanges processes dynamically. Processes representing exchanges of contaminants between fish and its surrounding environment (i.e. overlying water, sediment and other biota system representing its food) are dynamically simulated, with uptake and elimination rates.

3.4.2 FISH MODEL COMPONENTS

Media considered: A Medium is defined as an environmental or human compartment assumed to contain a given quantity of the chemical. The quantity of the chemical in the media is governed by loadings/losses from/to other media and by transformation processes (e.g. degradation). The Fish model includes two media that correspond to two input/output pathways for chemical accumulation in Fish, i.e. the Fish respiratory system and the Fish gastro intestinal tract (GIT) system (Fig. 3.4).

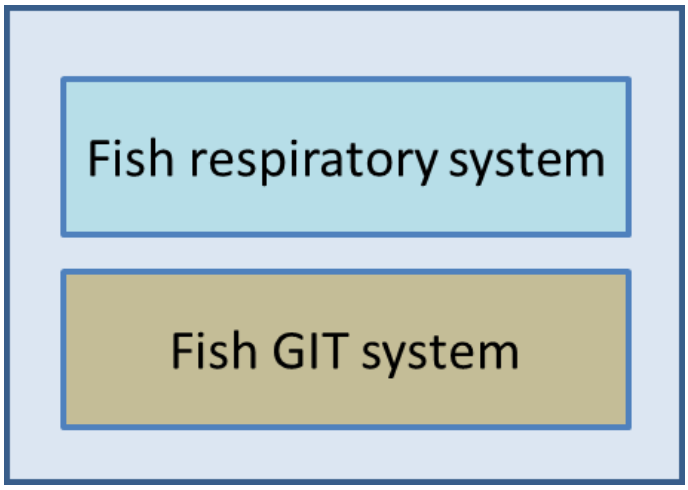

Fig. 3.4. Media considered in the Fish model (4-FUN Project, 2014a).

Loadings: a Loading is defined as the rate of release/input of the chemical of interest to the receiving system, here the Fish system.

The inputs of contaminant(s) into the Fish system can have the following origins:

- contaminant originating from “Direct uptake from water” through membrane diffusion via the respiratory area (i.e. gills and skin);
- contaminant originating from “Uptake from ingestion of food” (prey ingestion). By default, in the MERLIN-Expo Fish model, the number of food sources is 10, i.e. Fish can eat 10 different preys according to food availability and preferences.

If the Fish model is used alone (i.e. not coupled to other models available in the MERLIN-Expo library), these loadings are defined by the end-user as time series (see Chapter 5). If coupled to other models (e.g. the River model able to calculate contamination in water and sediments; and/or Phytoplankton and Invertebrate models representing prey for fish), some of these loadings can be calculated by these models (i.e. the outputs of the coupled models are used as loading inputs for the Fish model). The loading inputs are represented in Fig. 3.5.

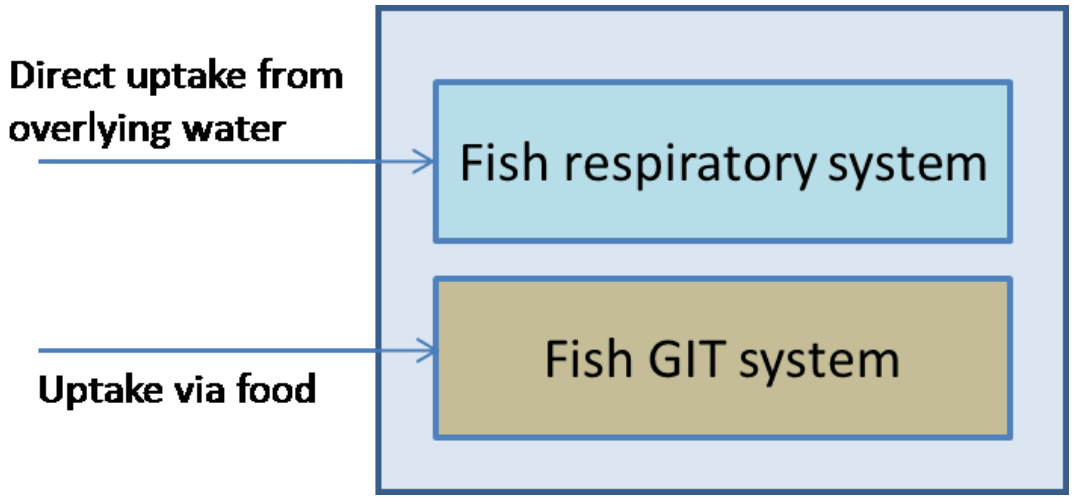

Fig. 3.5. Loading Inputs and Media considered in the Fish model (4-FUN Project, 2014a).

Losses: a Loss is defined as the rate of output of the chemical of interest from the receiving system, here the Fish system. The potential losses of contaminant(s) from the Fish system can be:

The potential losses of contaminant(s) from the Fish system can be:

- contaminant leaving the Fish system by elimination via the respiratory area (i.e. gills and skin);
- contaminant leaving the Fish system via elimination into egested feces;
- contaminant leaving the Fish system via metabolism (i.e. metabolic transformation of the chemical);
- contaminant concentration decreasing via growth of fish mass (should be regarded as dilution of chemicals and not loss of chemicals).

For metals, some losses are not relevant because these chemicals are assumed not to be subject to metabolism. The losses of contaminants (organics) from the Fish model are represented in Figure 3.6.

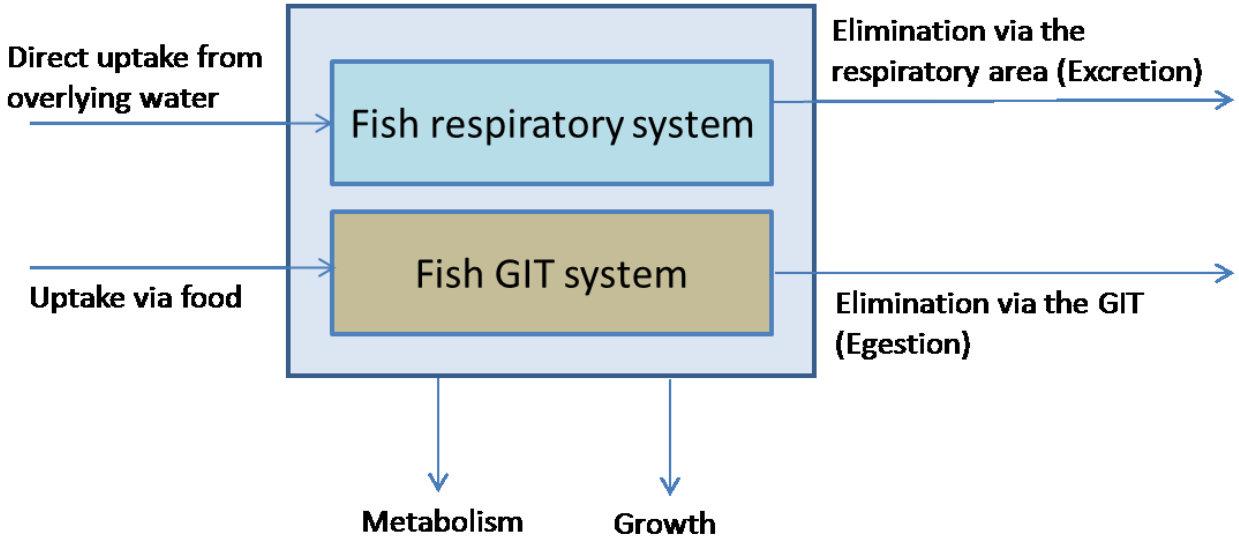

Fig. 3.6. Losses, Loading Inputs and Media considered in the Fish model (4-FUN Project, 2014a).

Exchanges between model media: an Exchange is defined as the transfer of the chemical of interest between two media of the system, here the Fish system. As the Fish system is based on one media only, there is no exchange between model media in Fish model.

Potential coupled models: Coupled models are defined as models that can generate loadings to the investigated system (here the Fish system) or receive losses from the latter. The Fish model can be coupled to other models of the MERLIN-Expo library (Figure 3.7).

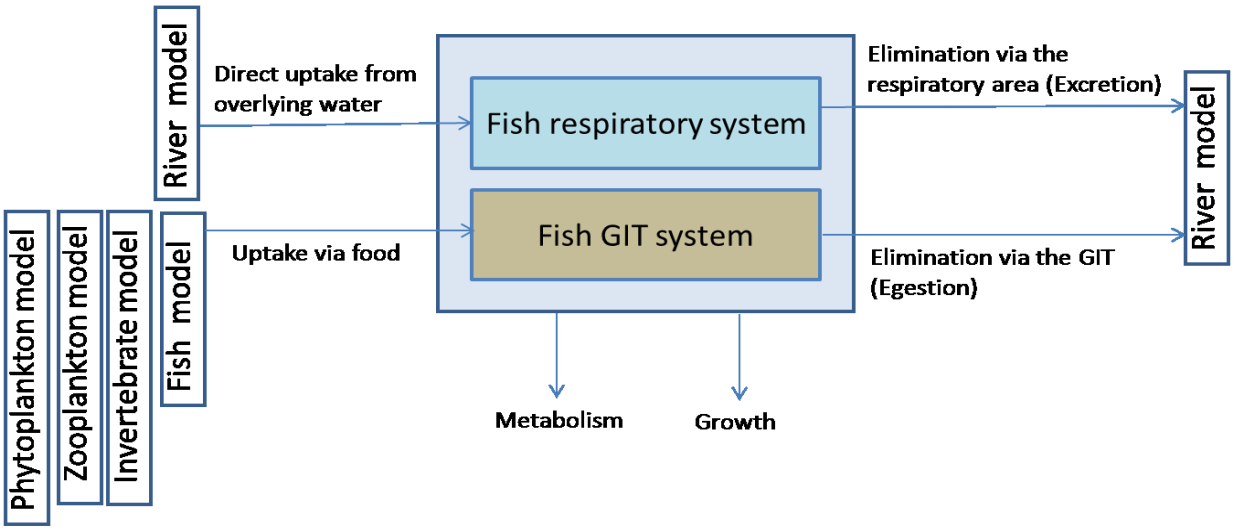

Fig. 3.7. Models which can be coupled with Fish model in MERLIN-Expo (4-FUN Project, 2014a).

Forcing variables: a Forcing variable is defined as an external or exogenous (from outside the model framework) factor that influences the state variables calculated within the model. Such variables include, for example, climatic or environmental conditions (e.g. temperature of water) or water chemicals concentrations changing in time. The forcing variables needed to run the Fish model are shown in Table 3.1.

Name	Abbreviation and unit	Purpose
Water temperature	T_river (°C)	Temperature affects a fish's feeding, assimilation, respiration, and excretion
Concentration of the chemical in dissolved river water	C_dis_water ($mg.m^{-3}$)	Is used to calculate the input through respiratory uptake. Can be calculated (instead of being defined by the end-user) if the River model is coupled to the Fish model.
Concentration of the chemical in prey i, i=1,10	C_prey_i ($mg.kg^{-1}_{fw}$)	Is used to calculate the input through dietary uptake through other species. Can be calculated (instead of being defined by the end-user) if another Biota model is coupled to the Fish model.

Table 3.1. Forcing Variables included in the Fish model.

Parameters: a Parameter is defined as a term in the model that is fixed during a model run or simulation but can be changed in different runs as a method for conducting sensitivity analysis or to achieve calibration goals. The parameters required by Fish model are Site-specific parameters (Tab. 3.2), parameters related to degradation of chemicals (Tab. 3.3), parameters related to partition between phases (Tab. 3.4) and physiological parameters of fish (Tab. 3.5).

Name	Abbreviation and unit	Purpose	Used for calculating the following state variable(s)
Fish diet preference for food item i	Pref_diet_i (unitless) (i=1 to 10)	Fish diet preferences for certain foods used to calculate the concentration of the chemical substance that is absorbed with ingested diet. Ten potential diets are arbitrary defined in the MERLIN-Expo model.	C_fish C_diet_total p_lipid_food

Table 3.2. Site-specific parameters.

Name	Abbreviation and unit	Purpose	Used for calculating the following state variable(s)
Metabolic half-life of chemicals	hl_metabolic_ norm (d^{-1})	Defines time after which amount of chemical in the organism decreases to half of its starting amount, due to metabolic activity.	Biotransformation rate constant

Table 3.3. Parameters related to degradation of chemicals.

Name	Abbreviation and unit	Purpose	Used for calculating the following state variable(s)
Bioconcentration Factor	Log10_BCF_organic ($L \cdot kg\ fw^{-1}$)	Represents the partitioning at equilibrium of organic chemicals between fish organism and water in absence of diet contribution.	Respiratory elimination rate constant
Octanol-water partition coefficient	Log10_K_ow (-)	K_{ow} partition coefficient is used as a measure of hydrophobicity of the organic substance at equilibrium concentrations between octanol and water phase.	Respiratory uptake rate constant Dietary uptake rate constant Dietary elimination rate constant
Water-layer diffusion resistance for uptake of chemicals from food	ρ _water_layer_food ($kg.d.kg^{-1}$)	Represents time of diffusion of organic contaminant from ingested food through aqueous layer.	Dietary uptake rate constant Dietary elimination rate constant
Water-layer diffusion resistance for uptake of chemicals from water	ρ _water_layer ($kg.d.kg^{-1}$)	Represents time of diffusion of organic contaminant contained in water through aqueous layer.	Respiratory uptake rate constant
Lipid-layer permeation resistance	ρ_lipid_layer ($kg.d.kg^{-1}$)	Represents time of passive diffusion of organic contaminant through lipid membranes.	Respiratory uptake rate constant Dietary uptake rate constant Dietary elimination rate constant

Table 3.4. Parameters related to partition between phases.

Name	Abbreviation and unit	Purpose	Used for calculating the following state variable(s)
Fish age at maturity	time_fishlife (d)	It is used for correcting concentration of contaminant in fish body for a given age of fish and for calculating growth rate.	C_fish k_growth
Fish length at maturity	L_fish (cm)	Used in calculating weight of fish at maturity according to an allometric relationship.	W_fish
Intercept of weight-length relationship	a_W_fish (unitless)	The relationship between weight and length is expressed by an allometric formula including intercept a_W_fish. Used in calculating weight of fish at a given age, based on a given fish length.	W_fish
Slope of weight-length relationship	b_W_fish (unitless)	The relationship between weight and length is expressed by an allometric formula including intercept a_W_fish.	W_fish
Allometric rate exponent	κ (-)	Allometric relationships provide body-size specific parameters instead of values that are arbitrary or taken from a well-known species. Allometric regression exponent κ expresses body size correlation with animals physiological characteristics i.e. rates, transport coefficients.	All rate constants
Lipid fraction of food item i	p_lipid_i (-)	Lipid fraction of food item i. Used to calculate the fraction of lipid ingested by fish with its diet. It is related to trophic level of the prey.	Dietary uptake rate constant Dietary egestion rate constant Mean lipid fraction in food
Lipid fraction of fish	p_lipid_fish (-)	Lipid fraction of fish. Used to calculate the egestion rate from GIT	Dietary egestion rate constant
Fraction of assimilated food	Assimilated_food (-)	This parameter represents fraction of ingested food that is absorbed or digested by the organisms in the gastro intestinal tract. Its estimation depends on prey (food) position in the food web. Used in calculating fish's inflow and outflow rates of chemicals through water/food and feces, respectively.	Dietary uptake rate constant Dietary elimination rate constant
Food transport coefficient	γ_food ($kg \cdot kg^{-1} \cdot d^{-1}$)	Food transport coefficient represents delay in advective transport of chemical substances through organism due to limited supply of new food.	Dietary uptake rate constant Dietary elimination rate constant

Table 3.5. Physiological parameters.

Intermediate State variables: an Intermediate State variable is defined as a dependent variable calculated within the model (Tab. 3.6; Tab. 3.7; Tab 3.8). Some State variables are fixed during a model run or simulation because they are calculated only from parameters. Some others are time-dependent because they are calculated from parameters, but also from time-dependent forcing variables. We distinguish 'Intermediate State variables' and 'Regulatory State variables'. The first ones are generally not used by decision-makers for regulatory purposes but can be used as performance indicators of the model that change over the simulation. The second ones can be used by decision-makers for regulatory purposes.

Name	Abbreviation and unit	Purpose
Fish weight at maturity	W_fish (kg fw)	Represents weight of fish at a maturity time of its life. Weight of the fish is estimated from its length. It should be used when user does not have data on weight, but on length instead.

Table 3.6. State variables related to fish physiology.

Name	Abbreviation and unit	Purpose
Mean lipid fraction of food	p_lipid_food	Defines lipid fraction of food ingested with fish diet
Mean concentration of the chemical in ingested diet items	C_diet_mean	Defines the mean chemical concentration in food ingested with fish diet

Table 3.7. State variables related to site-specific parameters.

Name	Abbreviation and unit	Purpose
Respiratory uptake rate constant for organics	k_uptake_resp_organic ($L \cdot kg_{fw}^{-1} \cdot d^{-1}$)	Defines rate at which chemicals are absorbed via the respiratory surface e.g. gills, skin.
Dietary uptake rate constant for organics	k_ingestion_organic ($kg_{fw} \cdot kg_{fw}^{-1} \cdot d^{-1}$)	Defines rate at which organic chemicals are absorbed via ingestion of food.
Respiratory elimination rate constant	k_excretion (d^{-1})	Defines rate at which chemicals are eliminated via the respiratory surface
Dietary elimination rate constant	k_egestion_organics (d^{-1})	Defines rate at which chemicals are eliminated via egestion (undigested food).
Metabolism rate constant	λ_metabolism (d^{-1})	Defines rate at which chemicals are eliminated via metabolism.
Growth rate constant	k_growth (d^{-1})	Defines rate at which chemicals are diluted via growth of the organism.

Table 3.8. State variables related to kinetic rate constants.

Regulatory State variables: a Regulatory State variable is defined as a dependent variable calculated within the model. It is generally time-dependent because it is calculated from parameters, but also from time-dependent forcing variables and loadings. Intermediate State variables are generally not used by decision-makers for regulatory purposes but can be used as performance indicators of the model that change over the simulation. Regulatory State variables can be used by decision-makers for regulatory purposes.

In the scheme reported in Figure 3.8 the flow chart for calculating the regulatory state variables and the processes included in the model is presented. The following symbols are adopted for representing parameters, state variables and forcing variable respectively:

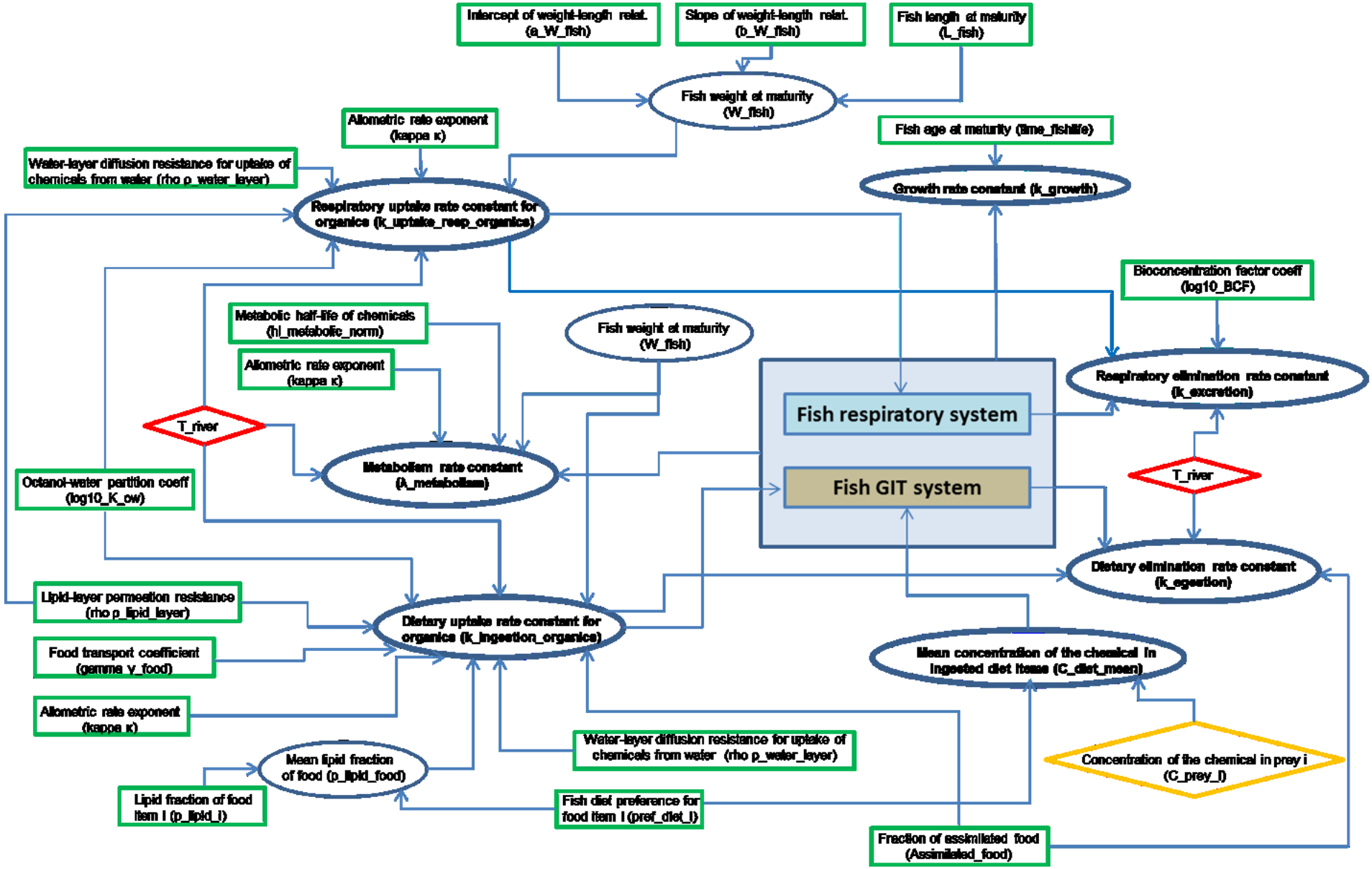

Fig. 3.8 Flow chart for calculating the regulatory state variables and the processes included in the Fish model (4-FUN Project, 2014a).

3.4.3 PROCCESSES AND ASSUMPTIONS

The ratio of concentration to storage is the fugacity of a chemical (Mackay and Fraser, 2000). Fugacity, which is essentially partial pressure, is a principle of equilibrium analogous to temperature in the case of heat transfer. When a chemical equilibrates between two phases, such as air and water, its fugacity in each phase are equal. Using fugacity as a substitute measure for concentration could be an approach to quantify uptake and clearance mechanisms. Details of this approach are described by Mackay (1991). So, it is assumed that chemicals are transported from high to low fugacity by passive diffusion (Van Leeuwen and Vermeire, 2007). Following classical fugacity theory rate constants for contaminant fluxes were considered inversely proportional to a series of resistances in water and lipid layers ρ ($d \cdot kg^{-K}$) and flow delays 1/γ ($d \cdot kg^{-K}$).
Influx rate constants can generally be denoted as influx rate constant (Eq. 3.1):

$$\text{influx} = \frac{\text{organism weight}^{-\kappa}}{\text{water layer resistance} + \text{lipid layer resistance} + \text{flow delay}} \qquad \textit{Eq. 3.1}$$

Efflux rate constants are basically described as efflux rate constant (Eq. 3.2)

$$\text{efflux} = \frac{1}{\text{accumulation}} \times \frac{\text{organism weight}^{-\kappa}}{\text{water layer resistance} + \text{lipid layer resistance} + \text{flow delay}} \qquad \textit{Eq. 3.2}$$

indicating the resistances and delays are similar in both directions (Hendriks et al., 2001). The accumulation ratio reflects the affinity of substances for different body compartments. The derivation of the appropriate equations has been described in detail in Hendriks, 1995 and Gobas et al., 1986. The following section presents the six processes taken into account in the Fish Model.

Respiratory uptake of chemicals (**k_uptake_resp**) defines rate at which chemicals are absorbed via the respiratory surface e.g. gills, skin (Eq. 3.3). Used for calculating gill elimination rate constant (k_excretion).

$$\mathbf{k\_uptake\_resp} = \frac{\text{W\_fish}\text{\^{}}(-\kappa)}{\rho\text{\_water\_layer} + \frac{\rho\text{\_lipid\_layer}}{10\text{\^{}log10\_K\_ow}}} \qquad \textit{Eq. 3.3}$$

Fish weight at maturity: W_fish
Water-layer diffusion resistance for uptake of chemicals from water: ρ_water_layer
Allometric rate exponent: K
Lipid-layer permeation resistance: ρ_lipid_layer
Octanol-water partition coefficient: 10^log10_K_ow

Dietary uptake of chemicals (k_ingestion): defines rate at which chemicals are absorbed via ingestion (Eq. 3.4).

$$\mathbf{K\_ingestion} = \frac{\text{Assimilated\_food}}{1-\text{Assimilated\_food}} \cdot \frac{1}{\left(\text{p\_lipid\_food}\cdot(10\text{^log10\_K\_ow}-1)+1\right)} \cdot \qquad \textit{Eq. 3.4}$$

$$\cdot \frac{\text{w\_fish}\text{^} - \kappa}{\rho\text{\_water\_layer\_food} + \frac{\rho\text{\_lipid\_layer}}{10\text{^log10\_K\_ow}} + \frac{1}{(\text{p\_lipid\_food} \cdot 10\text{^log10\_K\_ow} \cdot (1 - \text{Assimilated\_food}) \cdot \gamma\text{\_food})}}$$

Water-layer diffusion resistance for uptake of chemicals from food: ρ_water_layer_food

Lipid-layer permeation resistance: ρ_lipid_layer

Octanol-water partition coefficient: 10^log10_K_ow

Food transport coefficient: γ_food

Mean lipid fraction of food: p_lipid_food

Fraction of assimilated food: Assimilated_food

Allometric rate exponent: K

Fish weight at maturity: W_fish

Respiratory excretion rate constant (k_excretion): defines rate at which chemicals are eliminated via the respiratory surface (Eq. 3.5). Used for calculating chemical concentration in fish (C_fish).

$$\mathbf{k\_excretion} = \frac{\text{k\_uptake\_resp}}{10\text{^log10\_BCF}} \qquad \textit{Eq. 3.5}$$

Respiratory uptake rate constant: k_uptake_resp

Bioconcentration factor: 10^log10_BCF

Growth rate constant (k_growth): defines rate at which chemicals are eliminated via dilution due to growth (Eq. 3.6)

$$\mathbf{k\_growth} = \frac{1}{\text{time\_fishlife}} \qquad \textit{Eq. 3.6}$$

Fish age at maturity: time_fishlife

Dietary egestion rate constant (k_egestion): defines rate at which chemicals are eliminated via egestion (Eq. 3.7).

$$\mathbf{k\_egestion} = \frac{1}{(\mathrm{p\_lipid\_fish}\cdot(10\hat{}\log10\_\mathrm{K\_ow}-1)+1)}. \cdot \frac{\mathrm{w\_fish}\hat{} - \kappa}{\rho\_\mathrm{water\_layer\_food} + \frac{\rho\_\mathrm{lipid\_layer}}{10\hat{}\log10\_\mathrm{K\_ow}} + \frac{1}{(\mathrm{p\_lipid\_food}\cdot 10\hat{}\log10\_\mathrm{K\_ow}\cdot(1-\mathrm{Assimilated\_food})\cdot\gamma\_\mathrm{food})}} \quad \textit{Eq. 3.7}$$

Water-layer diffusion resistance for uptake of chemicals from food: ρ_water_layer_food

Lipid-layer permeation resistance: ρ_lipid_layer

Octanol-water partition coefficient: 10^log10_K_ow

Food transport coefficient: γ_food

Mean lipid fraction of food: p_lipid_food

Fraction of assimilated food: Assimilated_food

Allometric rate exponent: K

Fish weight at maturity: W_fish

Fraction of lipid in fish: ρ_lipid_fish

Metabolic biotransformation (λ_metabolism): defines rate at which chemicals are eliminated via metabolism (Eq. 3.8).

$$\boldsymbol{\lambda\_metabolism} = \frac{\ln 2}{\mathrm{hl\_metabolic\_norm}} . \frac{\mathrm{W\_fish}}{0.01} . \exp(0.01(\mathrm{T}-15)) \quad \textit{Eq. 3.8}$$

Chemical half-life of chemicals: hl_metabolic_norm

Allometric rate exponent: K

Fish weight at maturity: W_fish

Water Temperature: T_water

3.5 HOW TO APPLY MERLIN-Expo

In this paragraph the procedure for the application of MERLIN-Expo is presented, through the different windows which allow accomplishing all the steps needed to achieve the final simulation results.

1. Start MERLIN-Expo program: in the first window ("Information") the user can create a new assessment file and add specific information to be attached to this file (Fig. 3.9). From this window it is also possible to open an Ecolego file with a previously developed assessment and simulation.

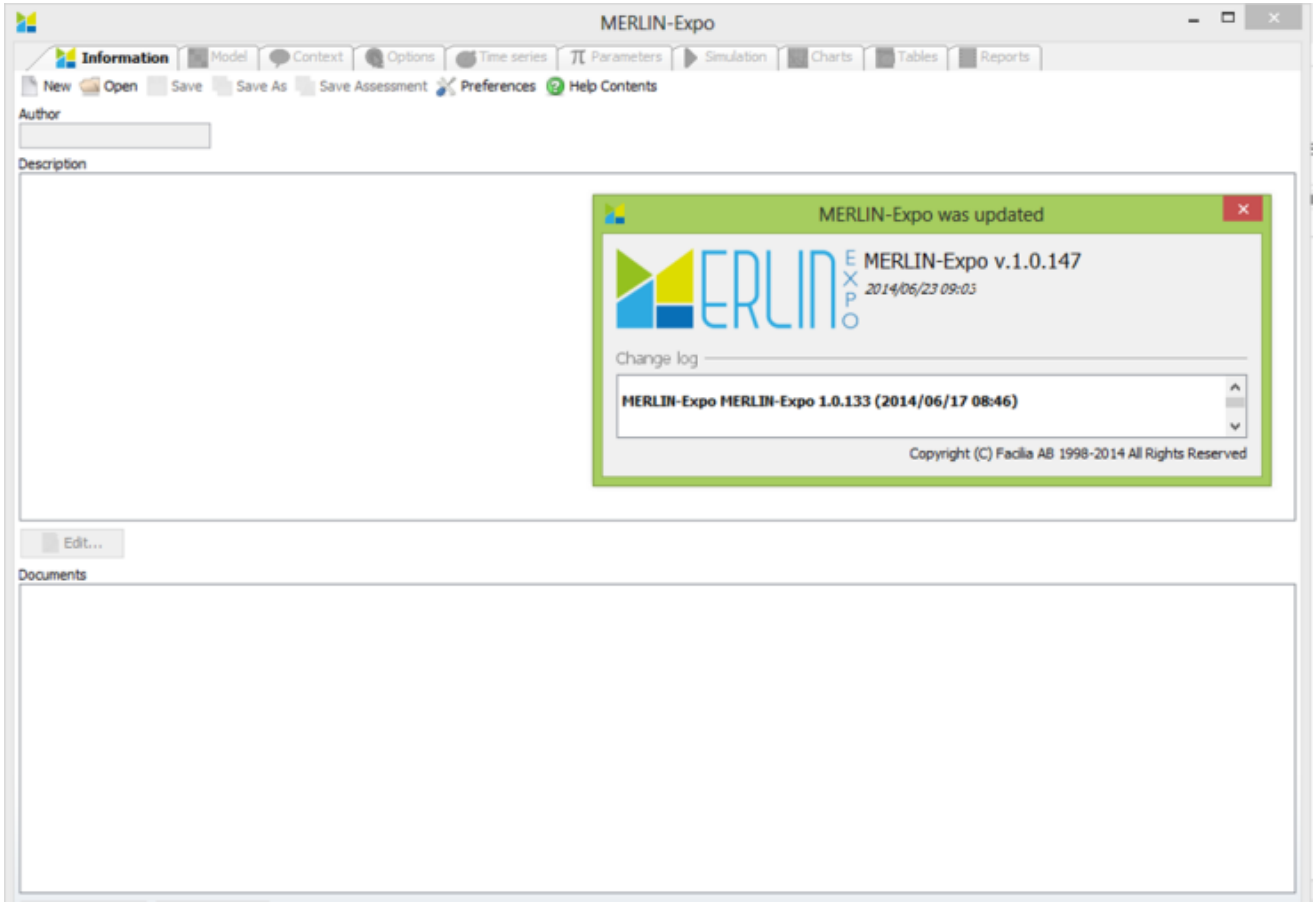

Fig. 3.9. First MERLIN-Expo starting window.

2. Building the chain of models: the MERLIN-Expo tool stores all the sub-models in a library. The effective graphical interfaces of the platform system can facilitate a comprehensive identification and visualization of the exposure pathways and of the roles of different sub-models in terms of their transfer relationships. From the library, model users can flexibly select and connect the sub-models (Fig. 3.10) depending on the exposure scenarios selected by the users.

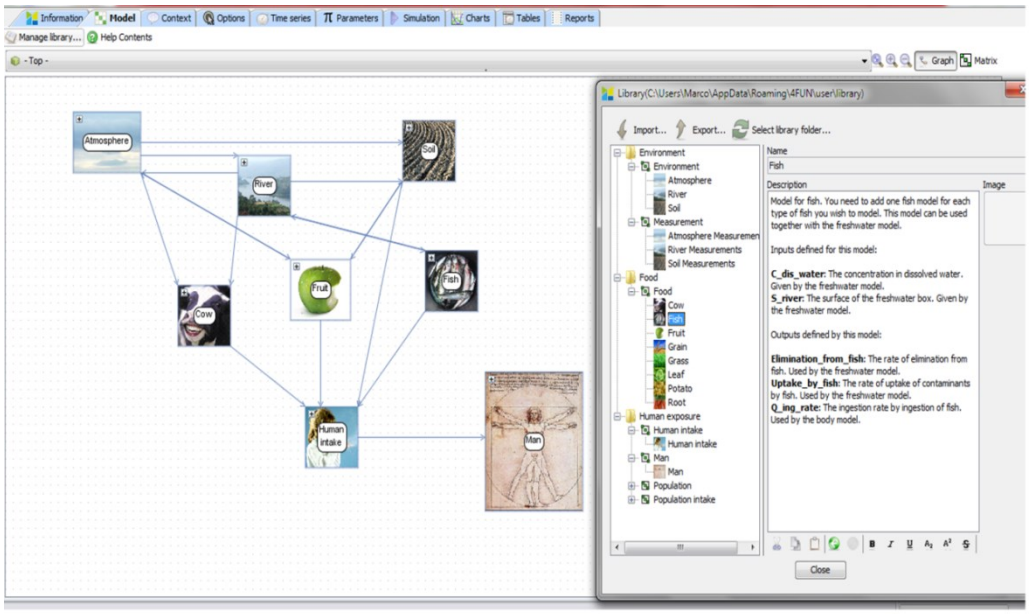

Fig. 3.10. Example of selected sub-models from MERLIN-Expo library.

3. Setting the context: in the "Context" window, the user can select the chemical substance of interest and can define the exposure scenario (e.g. by selecting food items to be considered in the human diet) (Fig. 3.11).

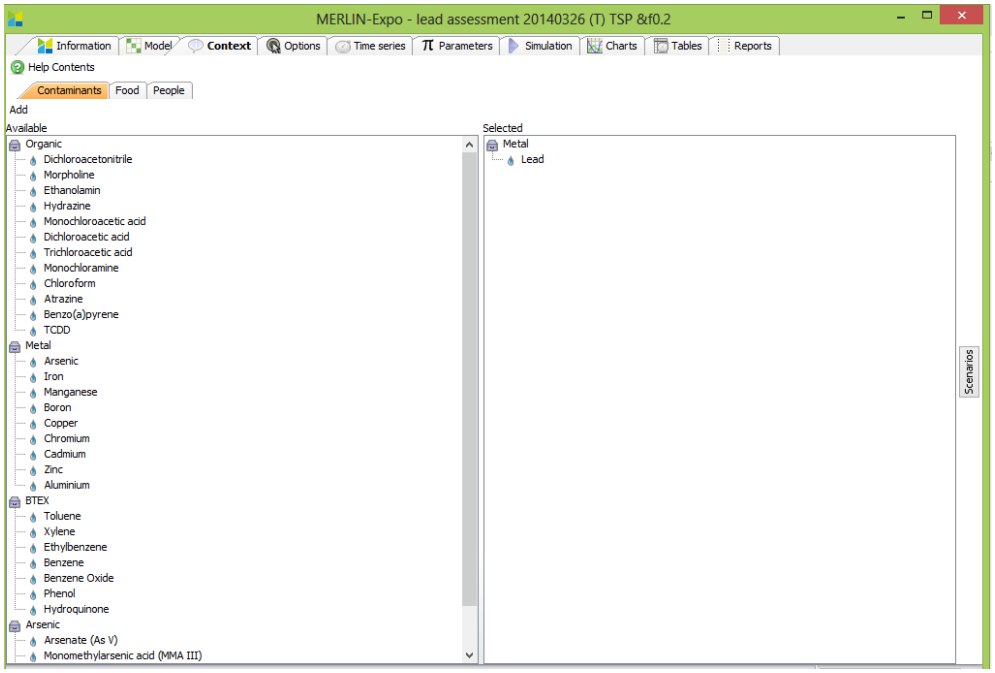

Fig. 3.11. Screen showing the "Context" window.

4. Enter time series: in the “Time series” window, the user can provide the time dependent input values for model inputs which can vary in time (Fig 3.12).

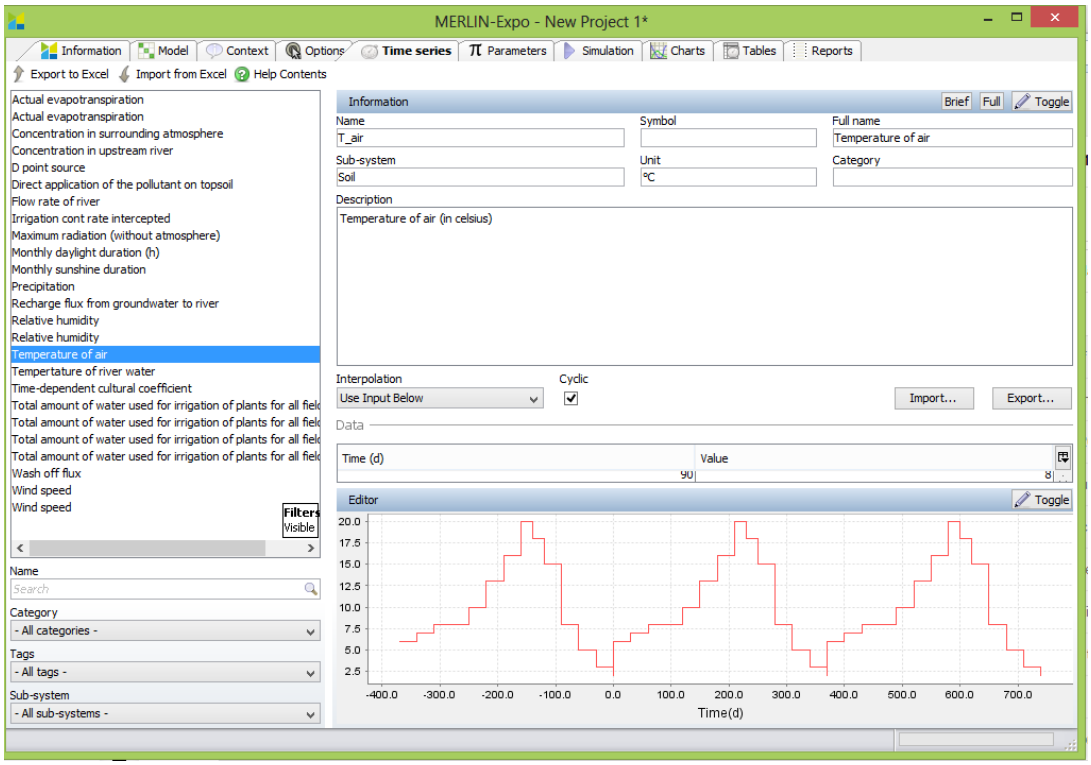

Fig 3.12. “Time series” window in MERLIN-Expo

5. Enter parameters: in the “Parameters” window, the user can add input values for those parameters which do not vary in time during model simulations (Fig. 3.13).

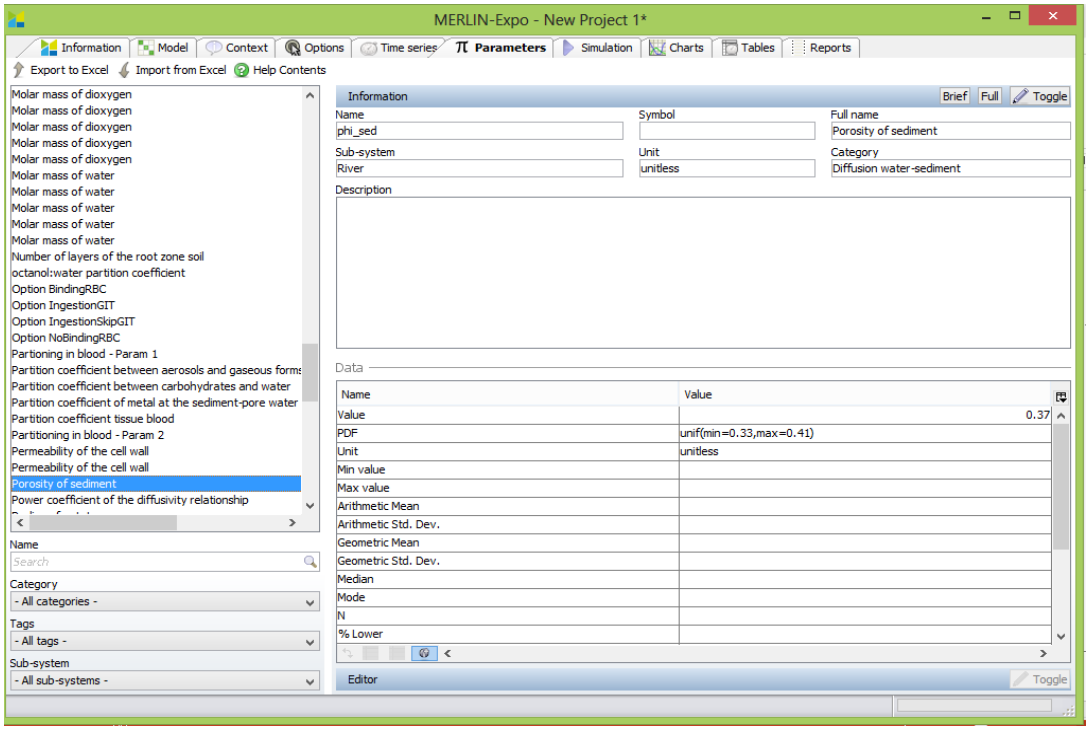

Fig. 3.13. “Parameters” window.

6. Run simulation: in the "Simulation" window, the user can define the simulation settings (deterministic or probabilistic simulation, selection of outputs of interest) and after that he can start the simulation (Fig. 3.14).

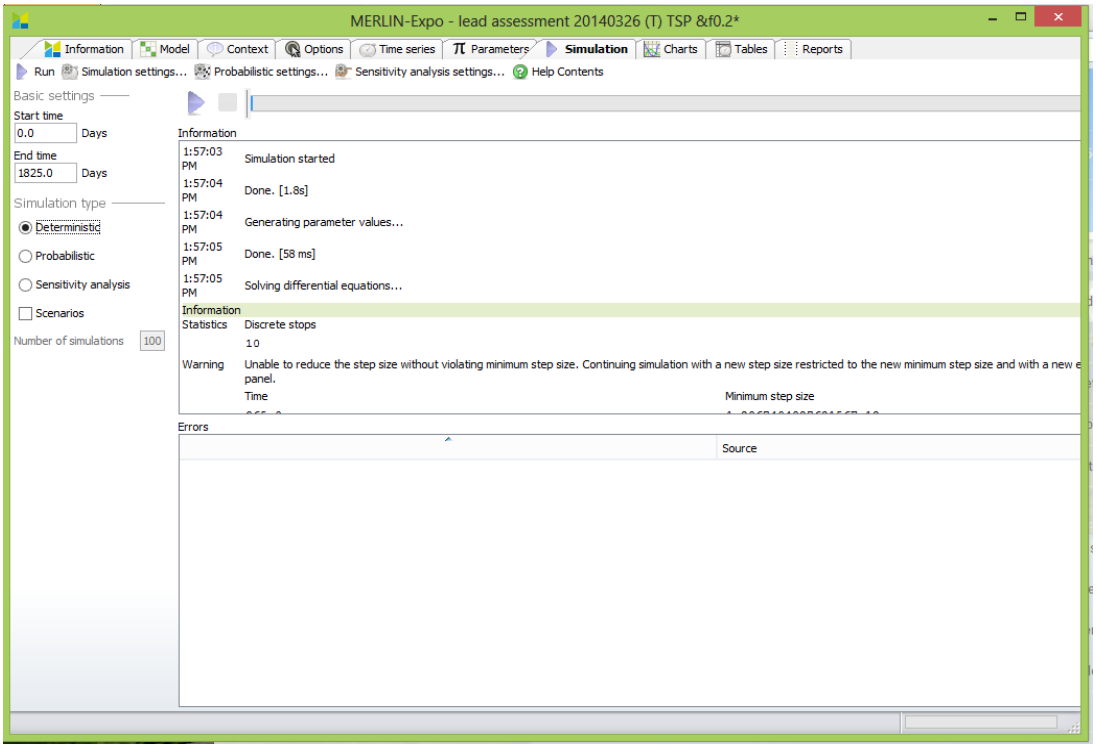

Fig. 3.14. Screen of a "Simulation" running in MERLIN-Expo.

7. Create charts and tables: when simulation is finished, data charts and tables results are automatically created by the tool and can be displayed and exported by the user (Fig. 3.15).

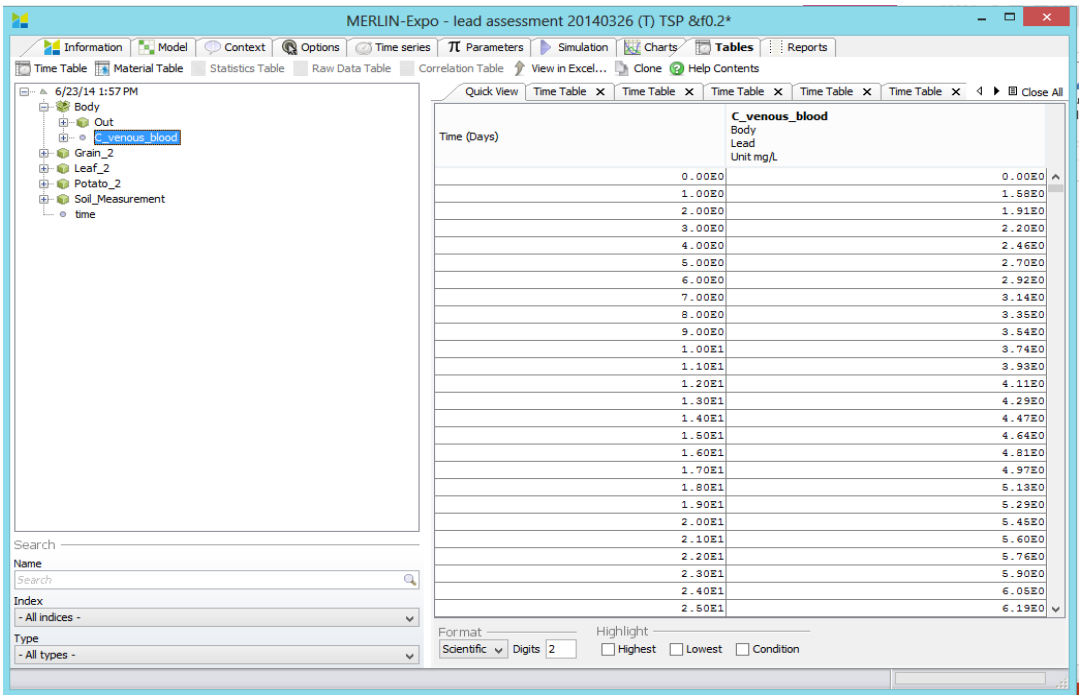

Fig. 3.15. Table results screen.

8. Generate report: MERLIN-Expo can automatically generate a final report including a summary of input and output values and the setting of the simulation (Fig. 3.16).

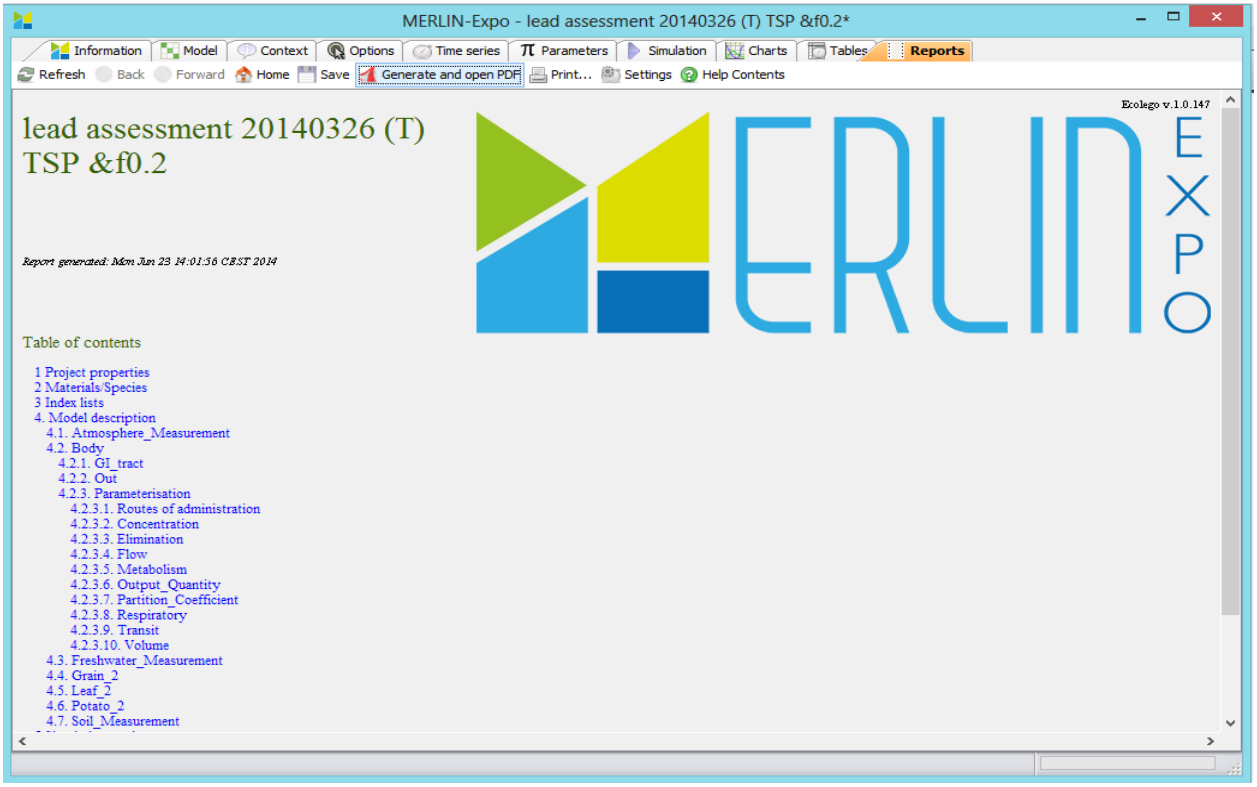

Fig. 3.16. Automatic report generator in MERLIN-Expo.

PART B

CASE-STUDY APPLICATION

4. CASE STUDY APPLICATION: FISH EXPOSURE TO PCBs IN THE LAGOON OF VENICE

In order to verify the Fish model recently implemented within the MERLIN-Expo tool (described in Chapter 3) and to test its applicability and performance on a real case-study, the Fish model has been applied for the assessment of fish exposure to Persistent Organic Pollutants (and specifically to Polychlorinated Biphenyls) in the lagoon of Venice. Therefore in this chapter a description of the main characteristics and environmental behaviour of the target chemicals is firstly presented, then information on the studies and selection criteria regarding the input data are described.

4.1 POLYCHLORINATED BIPHENYLS (PCBs)

In this paragraph, a description of the main characteristics of Persistent Organic Pollutants (POPs) and in particular of Polychlorinated Biphenyls (PCBs), selected as target contaminants for the Venice case study, will be provided.

4.1.1 PERSISTENT ORGANIC POLLUTANTS (POPs)

Persistent organic pollutants (POPs) are organic compounds that, to a varying degree, resist photolytic, biological and chemical degradation. POPs are often halogenated and characterised by low water solubility and high lipid solubility, leading to their bioaccumulation in fatty tissues. They are also semi-volatile, therefore they can cover long distances in the atmosphere before deposition (Stober, 1998). Although many different natural sources of POPs may exist, such as volcanic activity and vegetation fires, most POPs are created by humans in industrial processes, either intentionally or as byproducts (El-Shahawi et al., 2010).

POPs, which are known for their persistence and bioaccumulative potential, include many of the first generation organochlorine insecticides such as dieldrin, DDT, toxaphene and chlordane and several industrial chemical products or byproducts, including polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (dioxins) and dibenzo-p-furans (furans). These compounds are toxic because of their environmental persistency and the ability to bioaccumulate and biomagnify (Ritter et al., 2007; Wania et Mackay, 1996; Vallack at al., 1998). POPs are also semi-volatile compounds, therefore these compounds can occur either in the vapour phase or adsorbed on atmospheric particles, thereby facilitating their long range transport through the atmosphere (Kelly et al., 2007). POPs are represented by two important subgroups including both the polycyclic aromatic hydrocarbons and

some halogenated hydrocarbons. Halogenated compounds include organochlorines which are very resistant to degradation. In general, it is known that the more highly chlorinated biphenyls tend to accumulate to a greater level than the less chlorinated PCBs; metabolism and excretion processes are also more rapid for the less chlorinated PCBs than for the highly chlorinated.

Humans can be exposed to POPs through the diet, occupational accidents and the environment (including indoor). Exposure to POPs, either acute or chronic, can be associated with a wide range of adverse health effects, including illness and death (Ritter et al., 2007).

Since this thesis is focused on the application of the MERLIN-Expo Fish model to PCBs in the Venice lagoon, the following paragraphs are focused on the description of physico-chemical and toxicological characteristics of this group of POPs.

4.1.2 PHYSICO-CHEMICAL PROPERTIES OF *PCBs*

PCBs belong to a family of organic chemicals called chlorinated hydrocarbons. PCBs were produced from the '30s. Due to their non-flammability, extreme chemical stability and electrical insulating properties, PCBs were used in lots of industrial applications as heat transfers, plasticizers, plastics and more. (www.epa.org).

The generic chemical formula for a PCB is $C_{12}H_{10-x}Cl_x$.

PCBs are a class of chemical compounds in which from 2 to 10 chlorine atoms are attached to the biphenyl molecule. Monochlorinated biphenyls (i.e., one chlorine on the biphenyl molecule) are often included when describing PCBs. The general chemical structure of chlorinated biphenyls is shown in Fig. 4.3. It shows that a large number of chlorinated compounds are possible (Lundgren, 2003). Each of the 209 possible compounds is called "congener", and congeners are named from PCB1 to PCB209 according to an identification system developed by Ballschmiter and Zell (1980) and based on the order of PCB structural names. PCBs can also be categorized by degree of chlorination. The term "homologs" is referred to PCBs having the same number of chlorines. Homologs with different substitution structures are called isomers. The numbering system for the PCBs is shown in Fig. 4.3. *Ortho* positions are 2, 2', 6, and 6'; *meta* positions are 3, 3', 5, and 5'; *para* positions are 4 and 4'. The benzene rings can rotate developing the two configurations: *planar* (the two benzene rings in the same plane) and *nonplanar* (the benzene rings are at a 90° angle to each other).

The degree of planarity is mainly determined by the number of substitutions in the ortho positions. When hydrogen atoms in the ortho positions are replaced by larger chlorine atoms, benzenic rings are pushed to rotate out of the planar configuration. The benzene rings of non-ortho substituted PCBs, mono-ortho substituted PCBs, assuming a planar configuration are referred to planar or coplanar congeners; non-planar congeners are characterized by non-planar benzene rings configurations. (Leifer at al., 1983).

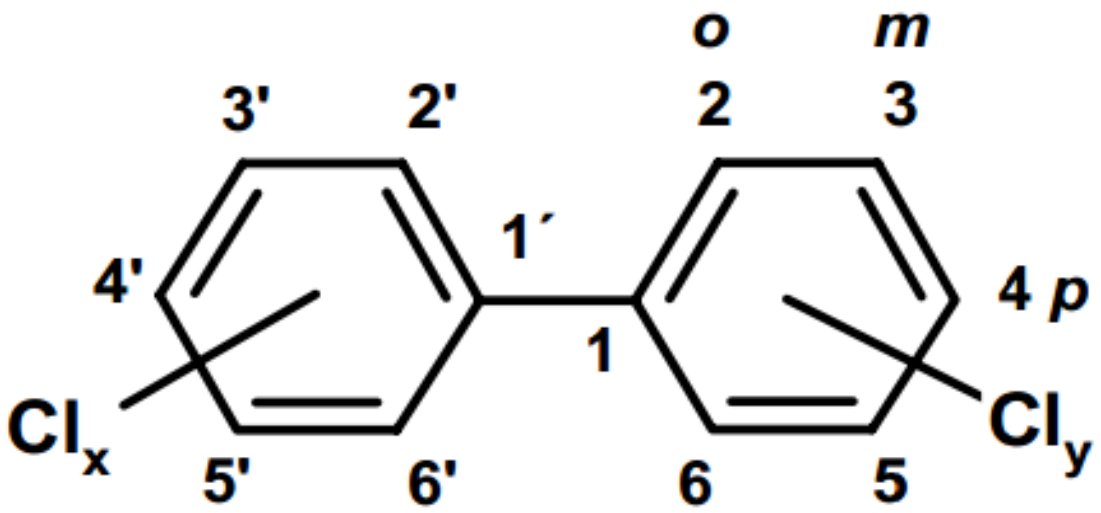

Fig. 4.3. General chemical molecular structure, numbering, and nomenclature of the PCBs (o, ortho; m, meta; and p, para) (Lundgren, 2003).

Generally, the water solubility and vapour pressure decrease as the degree of substitution increases (PCB 1, $\log K_{OW}$ = 4.46; PCB 209, $\log K_{OW}$ = 8.18) (Hawker and Connell, 1988).

Dioxins and PCBs have similar chemical and toxic proprieties, although their sources may be different. In contrast with dioxins, which are unwanted by-products of chemical and combustion processes, PCBs were produced consciously as components of many industrial products until the '80s. Then in that period their sale and use was prohibited, due to their proven toxic effects on the reproductive system (Feeley and Brouwer, 2000). Production of PCBs in Italy was banned starting from 1983.

Only twelve out of the 209 congeners, and specifically those with chlorine atoms in a non-ortho position (coplanar PCBs) or with a single chlorine atom in one of the four ortho positions (mono-ortho chlorinated PCBs), show a level of toxicity similar to dioxins. For this reason, these congeners are known as dioxin-like PCBs (dl-PCBs) (Harrard et al., 2003).

4.1.3 RELEASE AND EXPOSURE OF PCBs

PCBs entered the environment during their production and use. PCBs have been used in all of sort of products. Chemical mixtures composed by many congeners of PCBs, mostly known by their industrial trade names (e.g. Aroclor or Apirolio), have been commercialized for a long period of time in the 1900s. The total production of PCBs has been estimated at 1.5 million tonnes (De Vooght and Brinkman, 1989).

PCBs in the environment can be transported for long distances and they are expected to be founded in soils, sediments, water and biological tissues. Due to their stability, they do not degrade very easily and they can be carried in all environmental matrix. PCBs volatilize from water surfaces

because of their low vapour pressure and also their hydrophobicity (Hansen, 1999); atmospheric transport may therefore be a significant pathway for the distribution of PCBs in the environment (Manahan, 2000; Gambaro et al., 2009). In general, lighter congeners of PCB can be transported farther from the source of contamination (Dickhut and Gustafson, 1995). PCBs have been founded in regions where they have never been used or produced, as in oceans, deserts, Arctic and Antarctic. PCBs have been reported in air, in all areas of the world, at concentrations up to 15ng/m^3; in industrialized areas, concentrations may be several orders of magnitude greater (Kelly et al., 2007).

It is well know that PCBs can accumulate in foliage of plants and food crops (Bohme et al., 1999). They have also founded into the bodies of small organisms and fish. People who eat edible contaminated part of fishes, can be exposed dangerously to PCBs. The degradation of PCBs in the environment depends mostly on the degree of chlorination of the biphenyl. Persistence increases as the degree of chlorination increases, influencing therefore bioaccumulation processes in organisms (UNEP, 1999).

4.1.4 HEALTH EFFECTS OF PCBs

The number of substituted chlorine atoms on the ring structures influences the toxicity of PCBs. (Kogevinas, 2000). PCBs have been demonstrated to cause a variety of adverse health effects on humans and animals. Elevated exposures to PCB mixtures are associated to modifications in liver enzymes, hepatomegaly, and dermatological effects in humans (Lundgren, 2004). PCBs have been shown to have strict relations to cancer and to cause a number of serious non-cancer health effects in animals and humans (ATSDR, 2000).

Reproductive Effects

Studies analysing reproductive effects of PCBs in many animal species show a reduction of birth weight in monkeys and reduced sperm amounts in rats (ATSDR, 2000)..

Some studies on humans demonstrate that children born to women who worked with PCBs in factories or belonging to a fishing population showed reduced birth weight (Henry and de Vito, 2003).

Immune Effects

The immune system behaviour changes in exposed human populations have been observed. Studies in monkeys and other animals have revealed a number of serious effects on the immune system following exposures to PCBs. The studies suggest that low PCBs concentrations could be dangerous to the immune systems of exposed individuals. The immunological effects were noticed in humans who usually eat fish, oil and rice contaminated by PCBs. (WHO, 2008).

Neurological Effects

Neurological effects related to PCBs exposure are widely analysed in humans and animals. Studies in humans have suggested similar effects to those observed in animals exposed to PCBs, including changes in activity and learning diseases. Neurological problems have been reported by studies on human babies exposed to low PCBs concentrations (Ritter et al., 2007).

Endocrine Effects

Endocrine system ("endocrine disruption") is subject of many discussions and researches on the effects of environmental contaminants. PCBs have been demonstrated to exercise bad effects on thyroid in animals and humans. Thyroid hormone levels are fundamental to growth and development.

It has been shown that PCBs decrease thyroid hormone levels in many species. Some studies on humans show also changes in thyroid hormone levels in infants exposed to PCB. (Henry and DeVito, 2003).

Cancer

PCBs are one of the most widely studied classes of environmental contaminants, and many studies in animals and human populations have been performed to assess the potential carcinogenicity of PCBs. Studies on animals report that exposures to PCBs can cause cancer. As regard the humans, PCBs are supposed to be carcinogenic compounds. The International Agency for Research on Cancer (IARC) has classified PCBs compounds as a probably carcinogenic to humans (IARC, 2011; NIOSH, 2012).

PCBs have general tendency to bioaccumulate in fishes and other animals. Mortality studies demonstrate how the ingestion of contaminated food or the direct contact with contaminated matrix are more dangerous than PCBs mixture contacted by workers and released into the environment. (UNEP, 1999).

A few of other non-cancer effects of PCBs have been reported in animals and humans, including dermal, ocular effects and increase of blood pressure.

TEF

Toxicity Equivalence Factor (TEF) was developed by USEPA to evaluate potential health risks associated with exposure of organochlorine compounds such as dioxins and mixtures of PCB congeners (DeVito et al., 2000). This factor indicates the degree of toxicity of a compound compared to 2,3,7,8-TCDD, which is given a reference value of 1 (Van den Berg et al., 2006) (Table 4.1).

Considering TEFs, the toxicity of dioxins and dioxin-like compounds can be expressed as a number, the Toxic Equivalency (TEQ). It is a single value resulting from the product of the

concentration and individual TEF values of each congener. The TEQ concept has been developed to facilitate human health risk assessment and regulatory control (Diletti, 2007).

Compound	1998 TEF	2005 TEF
Polychlorinated dibenzo-*p*-dioxins (*PCDDs*)		
2,3,7,8-Tetrachloro-dibenzo-*p*-dioxin (TCDD)	1	1
1,2,3,7,8-Pentachloro dibenzo-*p*-dioxin (PeCDD)	1	1
1,2,3,4,7,8-Hexachloro- dibenzo-*p*-dioxin (HxCDD)	0.1	0.1
1,2,3,6,7,8-Hexachloro- dibenzo-*p*-dioxin (HxCDD)	0.1	0.1
1,2,3,7,8,9-Hexachloro- dibenzo-*p*-dioxin (HxCDD)	0.1	0.1
1,2,3,7,8,9-Heptachloro- dibenzo-*p*-dioxin (HpCDD)	0.01	0.01
Octachloro-dibenzo-*p*-dioxin (OCDD)	**0.0001**	**0.0003**
Polychlorinated dibenzofurans (*PCDFs*)		
2,3,7,8-Tetrachlor-dibenzofuran (TCDF)	0.1	0.1
1,2,3,7,8-Pentachloro-dibenzofuran (PeCDF)	**0.05**	**0.03**
2,3,4,7,8-Pentachloro-dibenzofuran (PeCDF)	**0.5**	**0.3**
1,2,3,4,7,8-Hexachloro-dibenzofuran (HxCDF)	0.1	0.1
1,2,3,6,7,8-Hexachloro-dibenzofuran (HxCDF)	0.1	0.1
1,2,3,7,8,9-Hexachloro-dibenzofuran (HxCDF)	0.1	0.1
2,3,4,6,7,8-Hexachloro-dibenzofuran (HxCDF)	0.1	0.1
1,2,3,4,6,7,8-Heptachloro-dibenzofuran (HpCDF)	0.01	0.01
1,2,3,4,7,8,9-Heptachloro-dibenzofuran (HpCDF)	0.01	0.01
Octachloro-dibenzofuran (OCDF)	**0.0001**	**0.0003**
Polychlorinated biphenyls (*PCB* congener number)		
3,3',4,4'-Tetrachloro-biphenyl (77)	0.0001	0.0001
3,4,4',5-Tetrachloro-biphenyl (81)	**0.0001**	**0.0003**
3,3',4,4',5-Pentachloro-biphenyl (126)	0.1	0.1
3,3',4,4',5,5'-Hexachloro-biphenyl (169)	**0.01**	**0.03**
2,3,3',4,4'-Pentachloro-biphenyl (105)	**0.0001**	**0.00003**
2,3,4,4',5-Pentachloro-biphenyl (114)	**0.0005**	**0.00003**
2,3',4,4',5-Pentachloro-biphenyl (118)	**0.0001**	**0.00003**
2',3,4,4',5-Pentachloro-biphenyl (123)	**0.0001**	**0.00003**
2,3,3',4,4', 5-Hexachloro-biphenyl (156)	**0.0005**	**0.00003**
2,3,3',4,4',5'-Hexachloro-biphenyl (157)	**0.0005**	**0.00003**
2,3',4,4',5,5'-Hexachloro-biphenyl (167)	**0.00001**	**0.00003**
2,3,3',4,4',5,5'-Heptachloro-biphenyl (189)	**0.0001**	**0.00003**

Table 4.1.Toxicity Equivalence Factors (TEFs) of PCDDs, PCDFs and Dioxin-Like PCBs calculated by WHO in 1998 and in 2005 (USEPA, 2013).

4.2 PCBs CONTAMINATION IN THE VENICE LAGOON

The Venice Lagoon is a superficial basin, located along the north-western coast of the Adriatic Sea, with an area of 549 km^2 (Guerzoni and Tagliapietra, 2006). The lagoon of Venice can be defined as a transitional environment, characterized by shallow waters (i.e., mean water depth is 1.2 meters) (Guerzoni and Tagliapietra, 2006), and affected by several anthropogenic activities such as industry, tourism, and fishery (Marcomini et al., 1997). The lagoon of Venice and the surrounding urban area have been and are still affected by activities, which caused the release in environmental media of a wide range of chemical substances, such as POPs, including PCBs and PAHs (Pavoni et al., 2003; Manodori et al., 2006). The most significant sources of POPs can be identified in the industrial settlement of Porto Marghera (where chemical industries, oil refining plants, and waste incineration plants were present, today partially dismissed), the treated and untreated municipal effluents from the city of Venice and surrounding centres, the freshwater loads from the catchment area and atmospheric depositions. Despite the implementation of environmental protection regulations and the use of technologies for emissions control in recent years, the presence of polychlorinated dibenzo-p-dioxins and dibenzo–furans (PCDD/Fs) and polychlorinated biphenyls (PCBs) in lagoon sediments might still represent a hazard to ecosystems and population health.

Research projects and monitoring surveys concerning the Venice lagoon and the surrounding areas have been reviewed and relevant environmental data for the case-study have been extracted.

Studies conducted starting from the end of the ‘80s in the lagoon of Venice showed the presence of high concentrations of Persistent Organic Pollutants in the sediments. Particularly the central part of the Lagoon, the zone between Porto Marghera and Venice, was chosen as a case study area, on account of its long and strong influence from industrial activities and human settlements.

In Pavoni et al. (1987) the evolution of concentrations of PCBs is analysed from the sedimentation rate taking into account the industrial district evolution, focussing on the central area of lagoon.

Di Domenico and co-workers during the ‘90s focused their work on the presence of organic contaminants in the sediments of the entire lagoon of Venice.

As described in Di Domenico et al. (1996), Moret at al. (2001), Frignani et al. (2001), PCBs concentrations in sediments are showed to be more marked in the areas next to anthropogenic activities (Porto Marghera and Venice town) than the others. This trend is reflected in superficial water as reported by Moret el al. (2005) and Manodori et al. (2006).

The concentrations of lightest PCBs congeners are influenced in some areas of lagoon not only by direct emission of factories in Porto Marghera, but mainly by the atmospheric deposition, such as in the northern area (Gambaro et al., 2005, 2009; Manodori et al., 2007; Dalla Valle et al., 2005b).

Historical trends show how POPs concentrations in time have been changing. During the '70s and the '80s Porto Marghera reached its maximum activity and this played a fundamental role in the increase of POPs emissions in the lagoon environment (Piazza et al., 2003). As shown in historical reconstructed trends of concentrations for PCBs by Marcomini et al. (1999) and Frignani et al. (2005) higher concentrations in central lagoon during '70s can be observed.

Secco et al. (2005) described how the concentrations of PCBs in upper sediments changed in time. Starting from the end of '80s there has been a decrease of PCBs concentrations, probably due to the improvement of depuration systems in Porto Marghera and natural degradation processes and also as a consequence of PCB production control and ban.

Dalla Valle et al. (2005a) applied a dynamic multimedia model to estimate PCDD/Fs and PCBs concentrations from 1900. According to the estimated values there is a peak between the end of '60s and the '70s, in good accordance with monitoring values.

Concentrations of dioxins and PCBs in Venice lagoon organisms can be correlated with those found in sediments (Guerzoni et, 2007). A lot of studies assessed concentrations in Venice food chain organisms highlighting a severe ecological risk from bioaccumulation of POPs (Micheletti et al., 2007, 2008; Sfriso et al. 2004).

Data reconstruction of PCBs concentration in Lagoon waters back to 1940s is required to perform ecological exposure assessment. Starting point for reconstructing past PCBs concentration trends are monitoring data derived from analysis of PCBs contamination in lagoon sediments. In particular, two projects funded by the Venice Water Authority provide useful information for the characterization of lagoon contamination levels (MAV, 2000a; MAV, 2000b).

PCBs concentration in environmental media from the mentioned projects can be used as input data to the MERLIN-Expo model, while PCBs concentrations in organisms can be used for the final validation of the results of the bioaccumulation modelling. Moreover, in order to estimate population long-life exposure to the chemicals of interest, it is necessary to reconstruct the historical trends of contamination in the study area. No information is available on the historical development of POPs contamination in the lagoon of Venice (Marcomini et al., 1999). For this purpose, sediment accumulation rate and chronologies can be obtained from activity depth profiles of specific isotopes (e.g. ^{210}Pb, ^{137}Cs). Therefore, studies reporting chemical concentrations measured at different depth in dated sediment cores from the Venice lagoon were reviewed (e.g., Marcomini et al., 1999; Piazza et al., 2003; Frignani et al., 2005) in order to collect suitable input data (as described in Chapter 5).

4.3 SELECTION OF TARGET PCBs CONGENERS FOR THE CASE-STUDY APPLICATION

4.3.1 SELECTION CRITERIA

The ultimate aim of the Venice case-study within 4FUN project is the assessment of dietary exposure of local population to Polychlorinated Biphenyls (PCBs) through the application of the full chain of models provided by MERLIN-Expo (from environmental multimedia models to PBPK model to estimate human internal exposure). The simulation of PCBs bioaccumulation in aquatic organisms and biomagnification along the aquatic food chain may therefore provide useful inputs to reconstruct the overall human exposure and to understand better the possible contribution to exposure from the consumption of local food.

The specific objective of the case-study application presented in this thesis consists in a preliminary demonstration of the Fish model to test its applicability and to evaluate its performance through the comparison of model results with real biomonitoring data (i.e., chemical concentrations in fish species) collected in the Venice lagoon.

The selection of target PCB congeners was mainly driven by the availability of monitoring data for specific PCBs congeners to be used as input data to the model and as reference for comparing intermediate and final model results. In particular, since the ultimate aim of the Venice case-study within 4FUN project is the estimation of human internal exposure to PCBs, not only environmental data (concentrations in environmental media and in aquatic biota), but also human biomonitoring data have been considered. Therefore, available environmental contamination data (PCBs concentrations in sediment cores) have been crossed with existing chemical concentrations in aquatic biota (required for the evaluation of Fish model results) and with existing human biomonitoring data (needed in a further step for the comparison with human internal concentrations estimated by the PBPK model).

According to the available datasets, three PCBs congeners have been selected for the testing application of the MERLIN-Expo Fish model to the Venice case study, namely PCB126, PCB169 and PCB180. Their chemical-physical and toxic proprieties are described in Paragraph 4.3.2.

From the project “Mapping of pollutants in the lagoon bottom sediment” (MAV, 2000a), **environmental monitoring data** on chemical concentrations of POPs for the years 1997-1998, 95 superficial sediment samples (0-15 cm depth) and 20 sediments cores (2 m depth) are available. Moreover, during the same campaign, chemicals concentrations were measured in 52 samples of edible organisms, specifically: Mussel (*Mityilus galloprovincialis*); Clam (*Tapes philippinarum*); Crab (*Carcinus mediterraneus*); Mullet (*Chelon labrosus*); Goby (*Zosterisessor ophiocephalus*). These organisms are significantly part of the Venice lagoon food web (Micheletti et al., 2007) and a part of these available data are compared with the application results in Chapter 6.

Human biomonitoring data are available for the Venice area from two studies on persistent organic contaminants. In the first study, funded by the Municipality of Venice, serum samples were collected in 1998 from 41 volunteers (adult men, 24-76 years old) living in Venice area (Frangipane, 1999; Raccanelli et al., 2007). Chemical concentrations in serum samples were individually measured in each person for some PCDDs, PCDFs and PCBs congeners. The second study, funded by the Italian National Health Institute, was focused on the monitoring of POPs in breast milk of mothers from different Italian cities. 29 primiparous mothers (21-40 years old) were enrolled in Venice between 1998 and 2000 (Abballe et al. 2008; Ingelido et al., 2007). The specimens of breast milk were pooled to obtain 3 samples according to the fish consumption levels of mothers, as follows: low fish consumption (10 donors; mean age = 31 years); medium fish consumption (13 donors; mean age = 30 years); high fish consumption (6 donors; mean age = 27 years). In a future phase of the project (not included in this thesis), the described human biomonitoring data will be compared with the results provided by the last step of the MERLIN-Expo modelling chain, i.e. the estimate of human internal exposure provided by the PBPK model.

4.3.2 CHARACTERISTICS OF SELECTED PCBS

Two of the three selected congeners, namely PCB126 and PCB169, are dioxin-like PCBs with a non-*ortho* coplanar structure. They are among the most toxic PCBs congeners, therefore it is very relevant to take these compounds into account in the exposure assessment. The third congener, PCB180, is a no dioxin-like substance with a di-*ortho* coplanar structure. It is generally used as a marker in a lot of studies about POPs exposure. All of them have been reported in various commercial PCB mixtures e.g. Aroclor1254 or Aroclor1260 (Kannan et al., 1988). In Table 4.2 some the main physico-chemical properties of the three selected PCBs congeners are reported, while Figure 4.4 presents the chemical structures of the three congeners (www.chemspider.com).

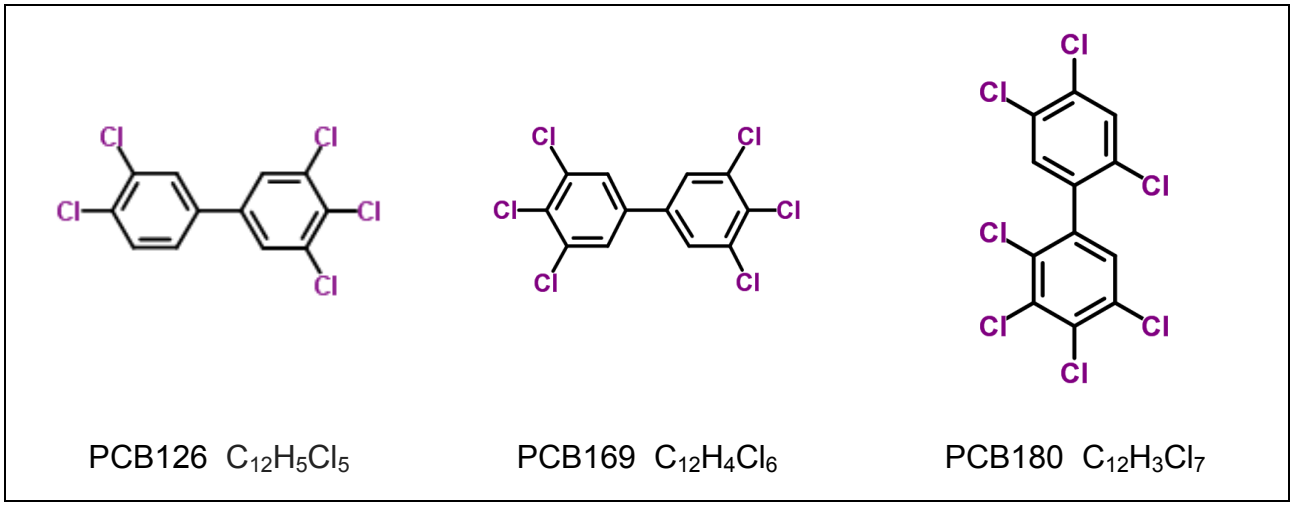

Fig. 4.4. Chemical structures of PCB126, PCB169 and PCB180 (www.chemspider.com).

PARAMETER	UNIT	PCB 126		PCB 169		PCB 180	
		VALUE	REFERENCE	VALUE	REFERENCE	VALUE	REFERENCE
Molecular mass	g mol^{-1}	326,433	Mackay et al., 2006	360,878	Mackay et al., 2006	395,323	Mackay et al., 2006
Melting Point	°C	106	Brodsky et al., 1988	201	Brodsky et al., 1988	109	Hutzinger et al. 1974
Henry's Law constant	Pa m^{-3} mol^{-1}	5,47	Sabljic & Güsten, 1989	5,98	Sabljic & Güsten, 1989	8,96	Bamford et al., 2000
Log Octanol-water partition coefficient	-	6,98	EpiSuite*	7,41	EpiSuite*	8,27	EpiSuite*
Log Water - Organic carbon partition coefficient	-	6,18	Burkhard, 1984	6,6	Burkhard, 1984	6,92	Burkhard, 1984
Global degradation rate in sediments	d	3650	Sinkonnen and Passivirta, 2000 (T = 7°C)	6875	Sinkonnen and Passivirta, 2000 (T = 7°C)	9125	Geyer et al., 2000
Global degradation rate in water	d	2500	Sinkonnen and Passivirta, 2000 (T = 7°C)	5000	Sinkonnen and Passivirta, 2000 (T = 7°C)	10000	Sinkonnen and Passivirta, 2000 (T = 7°C)
Bioconcentration Factor	-	4,86	EpiSuite*	4,51	EpiSuite*	4,09	EpiSuite*

Table 4.2. Physico-chemical proprieties of the three selected PCBs congeners.

**Episuite is a software developed by USEPA to estimate chemical-physical proprieties of chemicals and environmental fate based on a set of QSAR models (www.epa.gov).*

4.4 THE AQUATIC FOOD WEB AND SELECTION OF FISH SPECIES

To understand better the biomagnification processes influencing the chemicals concentration in fishes, a site-specific food web for the Venice lagoon is needed. The fish diet matrix is considered in the Fish model and it might play a relevant role in influencing final results (4FUN Project, 2000a). Some existing studies and bioaccumulation models on Venice food chain have been reviewed (Micheletti et al., 2007; 2008; Carrer and Opitz, 1999; Pastres and Solidoro, 2012; Brigolin et al., 2012; Bertazzon et al., 2006). Finally, the aquatic food web for Venice lagoon proposed by Micheletti et al. (2008) has been adopted for the case-study (Fig. 4.5).

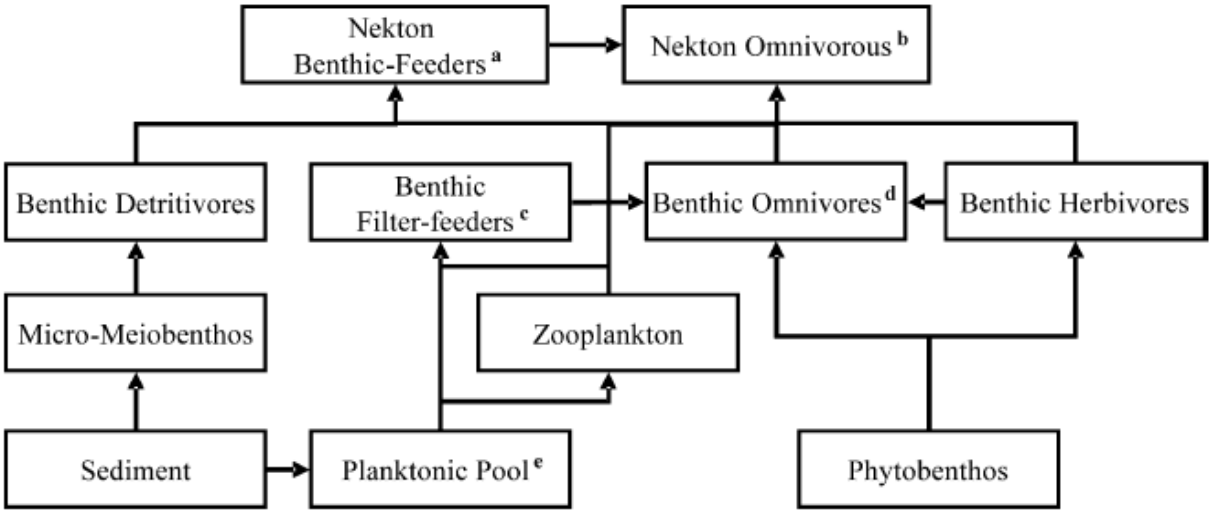

Fig. 4.5 Food web structure by Micheletti et al. (2008).

Two fish species representing edible species of considered lagoon food web are taken under consideration for the application in MERLIN-Expo Fish model: *Chelon labrosus* (mullet and *Zosterisessor ophiocephalus* (goby). These two species are both Nekton Benthic-Feeders (Micheletti et al., 2008). *Chelon labrosus* is a benthic-feeder fish living very closely with the sea bottom and its diet is composed by benthic diatoms, algae, small invertebrates and detritus (Ben-Tuvia, 1986). They migrate occasionally tending to move as temperatures rise. Adults are often found inshore, frequently entering brackish lagoons and freshwater (Billard, 1997). *Zosterisessor ophiocephalus* is a fish more static than mullets, living most in estuaries and lagoons, particularly present in North Adriatic sea (Miller, 1979). Both species are edible and they are very important to economy of fishing in Venice lagoon (Granzotto et al., 2001).

Physiological parameters values about the two selected fishes needed by MERLIN-Expo are described in Chapter 5.

5. MODEL PARAMETERIZATION

The application of the Fish model implemented in the MERLIN-Expo tool to the Venice lagoon case study requires:

- the parameterization of the model in accordance with the specific case-study conditions and target chemicals, and the selection of required input data;
- the definition of simulation settings (e.g. deterministic/probabilistic, simulations time, etc.) and the performance of simulations;
- the analysis and evaluation of model results against real monitoring data.

In this chapter the estimation and selection of input data for model parameters and forcing variables are reported, while the definition of simulation settings and the evaluation of different model outcomes are illustrated in details in the following chapter (Chapter 6).

5.1 CHARACTERIZATION OF CONTAMINATION: CHEMICALS CONCENTRATIONS IN WATER

A time series of concentration of the target chemical dissolved in water is required by the Fish model to simulate the fish uptake through respiration and ingestion and obtain an estimate of time-dependent concentrations in fish (see Chapter 3). Since the final goal of the 4FUN case-study is to assess the lifetime human exposure to PCBs, and available human biomonitoring data date back to 1997-1998, the objective is to dynamically simulate concentrations in fish for some decades before 2000.

However, historical data of PCB emissions suitable to reconstruct the historical development of PCBs contamination in the lagoon of Venice are not available. As an alternative, sediment cores proved to be useful in supporting the reconstruction of temporal trends of environmental contamination, as demonstrated by many authors (Marcomini et al., 1999; Frignani et al.,2004; 2005), also in combination with environmental modelling approaches (Dalla Valle et al., 2005a).

Starting from contaminants' concentrations in dated sediment cores it is possible to estimate the trend of contaminants concentrations in water, to serve as an input to bioaccumulation modelling.

In order to assess the extent to which a compound is associated with solid phases in a given system at equilibrium, the ratio of the compound total equilibrium concentrations in the solids and in the aqueous solution has to be known (Schwarzenbach et al., 2003). The solid/water distribution coefficient, K_d, is defined as (Eq. 5.1):

$$K_d = \frac{C_S}{C_W} \qquad \textit{Eq. 5.1}$$

Then, in order to evaluate the ability of natural organic materials (e.g. sediment) to sorb organic pollutants (e.g. POPs), an organic carbon normalized sorption coefficient, K_{OC} can be defined (Eq. 5.2):

$$K_{OC} = \frac{K_d}{f_{OC}} \qquad \textit{Eq. 5.2}$$

From Equation 5.1 and 5.2, it follows that K_d (Solid/Water Distribution Coefficient) can be described as:

$$K_d = K_{OC} \cdot f_{OC} \qquad \textit{Eq. 5.3}$$

Therefore, concentrations in water can be estimated from concentration in sediments, as shown in Eq. 5.4.

$$C_W = \frac{C_S}{K_{OC} \cdot f_{OC}} \qquad \textit{Eq. 5.4}$$

Where:
C_W= Concentration in water (µg/l)
C_S= Concentration in sediment (µg/kg)
K_{OC} = Water/Organic Carbon Partition Coefficient (l/kg)
f_{OC} = Fraction of Organic Matter in Sediment (%).

Studies focused on the reconstruction of temporal trends of POPs contamination in radio-dated sediment cores from the Venice lagoon have been reviewed (e.g., Marcomini et al., 1999; Frignani et al., 2001, 2004, 2005; Piazza et al., 2003; Pavoni et al., 1987; Dalla Valle et al., 2005b;). The works by Marcomini and colleagues (1999) and Frignani and colleagues (2005) have been selected because they provide individual concentrations for the PCBs congeners of interest (PCB126, PCB169 and PCB180) at specific cores depths (corresponding to specific time period).
Figure 5.1 shows the sampling locations of sediment cores analysed in the studies by Marcomini at al. (1999) and Frignani et al. (2005) in the Venice lagoon.

Fig. 5.1. Sampling locations of sediments cores from Frignani et al. (2005) and Marcomini et al. (1999).

An evaluation of sediment cores data has been performed in order to select the most suitable ones for the purposes of the present application. POPs concentrations in sediment core M3 (salt marsh) seem attributable mainly to atmospheric depositions, since a high correlation coefficient between atmospheric deposition profile and the salt marsh core layer was observed (Dalla Valle et al., 2005b). Therefore M3 core has been excluded because it does not provide integrate information about overall pollution processes (including other main sources, such as industrial waste water).

Frignani and colleagues (2005) report that dating was impossible for canal core I1 due to ambiguous information provided by the used radiotracers.

Sediment cores W (Porto Marghera industrial channel) and R (Venice historical centre) were sampled in areas with elevated sediment mixing, thus they are scarcely suitable for temporal reconstruction (Marcomini et al., 1999).

Sediment cores B (close to Porto Marghera) and E (Campalto), instead, refer to a less perturbed sedimentation area, so that the dating results are more reliable. Moreover, these sediment cores should be more representative of the average contamination trends in the central part of Venice lagoon (Fig. 5.2) and therefore more suitable to reconstruct exposure of edible aquatic species of interest for the present application.

Central lagoon is one of the most influenced areas by pollution because of his proximity to Porto Maghera (Pavoni et al., 1990; Di Domenico et al., 1998; Moret et al., 2001). Next to the industrial area, PCBs in water or sediments reach higher concentrations than the rest of lagoon (Guerzoni et

al., 2007; Moret et al., 2005). Therefore, applying a conservative approach, this area has been selected for the assessment of fish exposure to PCBs as it is one of the most polluted area in the lagoon (Secco et al., 2005).

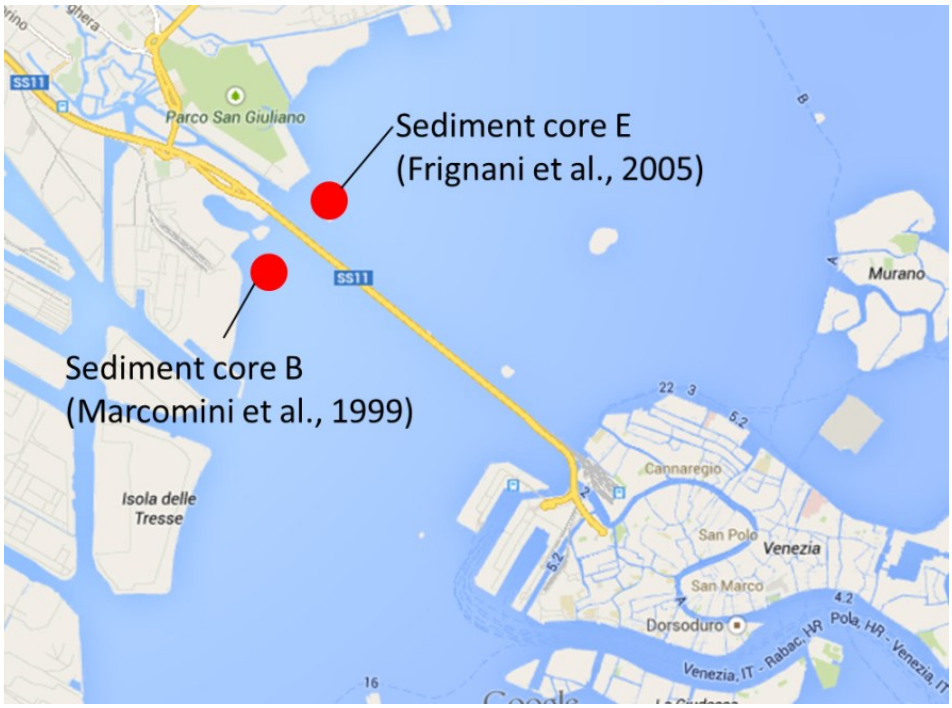

Fig. 5.2. Two sediment cores selected for the historical reconstruction.

The method applied to date sediment cores in Marcomini et al. (1999) and Frignani et al. (2005) studies was very similar. After treating the cores with detergents and solutions, they were prepared for the radiodating process. Radiodating of the core was performed by measuring the ^{210}Pb and ^{137}Cs activities. The HRGC/HRMS analyses were conducted using a HP 5970 and a HP 6890 gas-chromatographs coupled to a Micromass Autospec mass-spectrometer. The quantitative determination of PCB was performed by an isotope dilution method using relative response factors obtained from five standard solutions (EC1668 Cambridge Isotope Laboratories, Woburn, MA), as recommended by US EPA (1999).

In Table 5.1 and Table 5.2 the concentrations of PCB126, PCB169 and PCB180 at different depths in the two selected sediment cores (E and B) are shown, corresponding to different years of the sedimentation process. Concentrations in sediment cores have been considered from 1930s, PCBs producing starting period. PCB126 and PCB169 concentrations resulted to be below the detection limit in many layers of the sediment cores. Comparing the concentrations in radiodated sediment cores with monitored data in Frignani et al. (2001) and Secco et al., (2005), it is described a pick of PCBs concentrations during the '70s, period of maximum factories activity.

Where the concentrations are under the Limit of Detection, half values are considered to calculate water concentrations input (Menichini et al., 2004).

Depth	PCB126	PCB169	PCB180	Year
cm	µg/kg	µg/kg	µg/kg	
(12-15)	<0,01	<0,01	0,92	1940
(9-12)	0,16	<0,01	1,82	1950
(6-9)	0,04	<0,01	1,71	1960
(3-6)	0,04	<0,01	5,78	1975
(0-1,5)	0,02	<0,01	1,92	1995

Table 5.1. Concentrations of PCB126, PCB169 and PCB180 in radiodated sediment core E (Frignani et al., 2005).

Depth	PCB126	PCB169	PCB180	Year
cm	µg/kg	µg/kg	µg/kg	
(17-18)	<1	<1	<1	1935
(11-13)	<1	<1	<1	1954
(7-10)	0,7	2	3	1969
(5-7	<0.5	1	1	1976
(3-5)	<0.5	2	3	1980
(1-2)	<0.5	<1	1	1984
(0-1)	0,5	<1	2	1987

Table 5.2. Concentrations of PCB126, PCB169 and PCB180 in radiodated sediment core B (Marcomini et al., 1999).

In order to reconstruct concentrations in water (Equation 5.4), $\log10K_{OC}$ values for the target chemicals are needed. The selected values, calculated by Barkhard (1984) for selected PCBs congeners are reported in Table 5.3.

Water/Organic Carbon Partition Coefficient	PCB 126	PCB 169	PCB 180
$\log10K_{OC}$	6.18	6.60	6.92

Table 5.3. Log10K_{OC} values for the target chemicals used in MERLIN-Expo.

The sediment organic carbon fraction value (f_{OC}) assumed to solve Eq. 5.4 is 1.5%. It corresponds at the average annual value of organic carbon (Della Valle et al., 2003), measured in one sampling site next to Sediment Core E in the Central Venice Lagoon during Project 2023 by MAV (2000b).

Fraction of Organic Matter in Sediment	Value	Reference
% f_{OC}	1,5	Dalla Valle et al. (2003)

Table 5.4.Fraction of organic matter in sediments assumed to solve Eq. 5.4.

The estimated concentrations of the target PCBs congeners in dissolved water are reported in Table 5.5 for sediment core B (Marcomini et al.,1999) and in Table 5.6 for sediment core E (Frignani et al., 2005).

PCB126	PCB169	PCB180	Year
mg/m^3	mg/m^3	mg/m^3	
4,40E-07	8,37E-08	7,37E-06	1940
7,05E-06	8,37E-08	1,46E-05	1950
1,76E-06	8,37E-08	1,37E-05	1960
1,76E-06	8,37E-08	4,63E-05	1975
8,81E-07	8,37E-08	1,54E-05	1995
2,38E-06	8,37E-08	1,95E-05	**Mean**

Table 5.5. Water concentrations of target PCBs calculated from Sediment Core E (Frignani et al., 2005).

PCB126	PCB169	PCB180	Year
mg/m^3	mg/m^3	mg/m^3	
2,20E-05	8,37E-06	4,01E-06	1935
2,20E-05	8,37E-06	4,01E-06	1954
3,08E-05	3,35E-05	2,46E-05	1969
1,10E-05	1,68E-05	8,02E-06	1976
1,10E-05	3,35E-05	2,41E-05	1980
1,10E-05	8,37E-06	8,02E-06	1984
2,20E-05	8,37E-06	1,60E-05	1987
1,86E-05	1,68E-05	1,26E-05	**Mean**

Table 5.6. Water concentrations of target PCBs calculated from Sediment Core B (Marcomini et al., 1999).

Estimated concentrations in water for PCB180 according to the two selected cores data series are represented in the chart in Figure 5.3. It is evident an increasing trend in water concentrations for the calculated concentrations, up to peak during the '70s corresponding to the maximum activity of chemical industries in Porto Marghera (Frignani et al., 2004).

Nowadays the concentrations in water are lower than three decades ago as described also by Frignani et al. (2004) and Dalla Valle et al. (2005). The calculated decrease could be related to the improvement of the depuration systems of the industrial plants in Porto Marghera, to a natural degradation process or to the increased sediment resuspension that has spread the contaminated sediment far from the most impacted areas to other areas of the lagoon (Secco et al., 2005) but first of all because of their production was been banned from 1983.

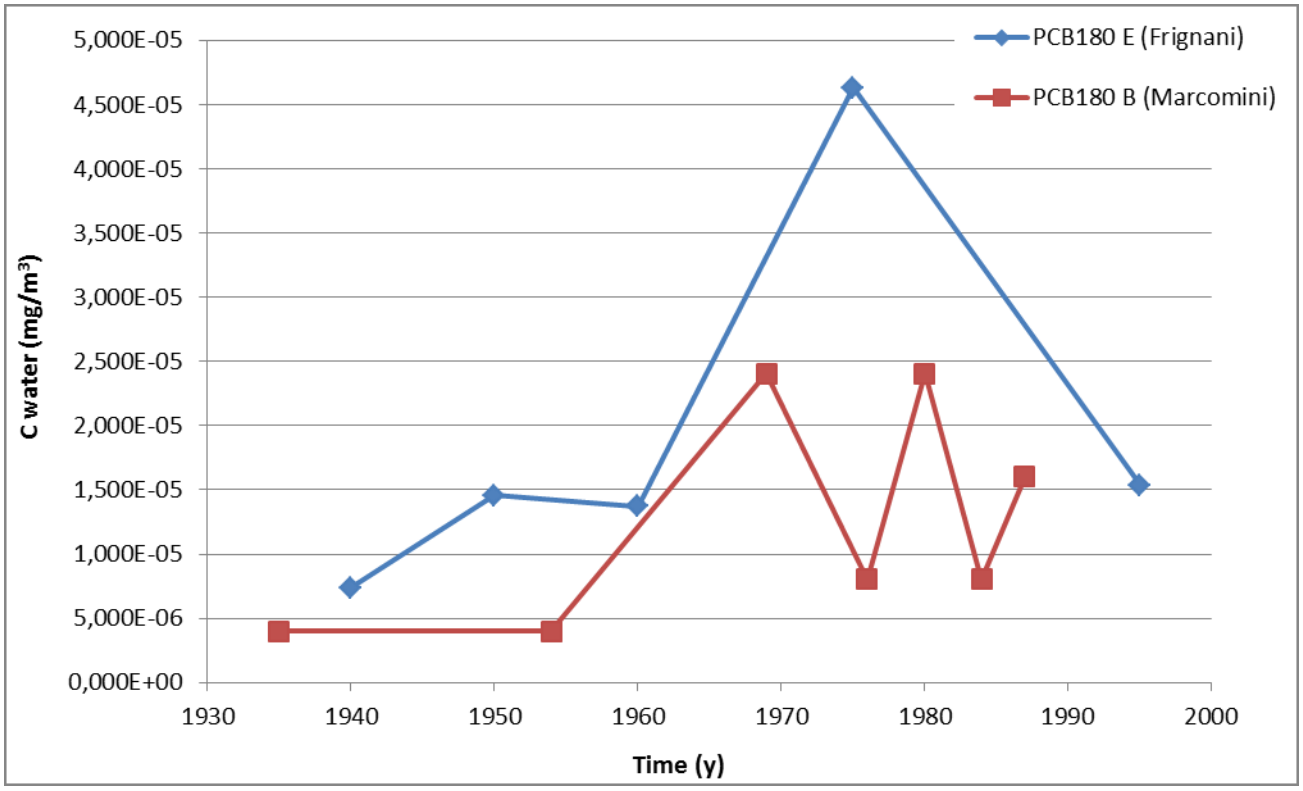

Fig. 5.3. Calculated concentrations of PCB180 in water from sediment data of B and E sediment cores.

5.2 WATER TEMPERATURE

Water temperature is a forcing variable in the Fish Model in MERLIN-Expo. A mean water temperature equal to 15°C is assumed as input value for Venice lagoon water according to Dalla Valle and colleagues (2003) and Lee and co-workers (1998). This value is confirmed by the calculation of mean water temperature from the Samanet database including monitoring data for the last two decades (MAV, 2014).

T_river	Value	Reference
°C	15	Dalla Valle et al., 2003

Table 5.7.Water temperature value assumed as input in MERLIN-Expo applications.

5.3 PHYSICO-CHEMICAL PROPRIETIES OF TARGET PCBs CONGENERS

The Fish model has to be parameterized for the following chemical-specific parameters: octanol-water partition coefficient, bioconcentration factor and metabolic half-life of chemicals. For each selected PCB congener, input values have been calculated using the EpiSuite (software developed by USEPA to estimate chemical-physical proprieties of chemicals and environmental fate). Selected values are reported in Table 5.8.

Parameter	Unit	PCB126	PCB169	PCB180
log10_Kow	Unitless	6.98	7.41	8.27
log10_BCF	L Kg_{fw}^{-1}	4.86	4.51	4.09
HL_metabolic	d^{-1}	288	413	799

Table 5.8. Chemical-physical proprieties values for case-study PCBs congeners.

5.4 PHYSIOLOGICAL PARAMETERS OF SELECTED FISH SPECIES

In order to test the Fish model in the Venice lagoon scenario, two species of fish which play a relevant role in the aquatic food chain have been selected (Micheletti et al., 2008): *Chelon labrosus* and *Zosterisessor ophiocephalus* (Chapter 4). Physiological parameters value for these two fishes have been derived from literature or from available on-line database, as reported in Table 5.9 and Table 5.10.

Physiological parameters of *Chelon labrosus*			
Name	**Abbreviation and unit**	**Value**	**Reference**
Fish age at maturity	time_fishlife (d)	1100	Thomson et al., 1986
Fish length at maturity	L_fish (cm)	30	Ysikliras and Stergiou, 2014
Intercept of weight-length relationship	a_W_fish (unitless)	0.0085	Fishbase.org
Slope of weight-length relationship	b_W_fish (unitless)	3.12	Fishbase.org
Allometric rate exponent	K (unitless)	0.025	Hendriks et al., 2001
Lipid fraction of fish	ρ_water_fish ($kg \cdot d\ kg^{-1}$)	0.068	Micheletti et al., 2008
Fraction of assimilated food	Assimilated_food (unitless)	0,73	Schroeder et al., 1981
Food transport coefficient	γ_food ($kg \cdot kg^{-1} \cdot d^{-1}$)	0,03	Hauck et al, 2011
Lipid-layer permeation resistance	ρ_lipid_layer ($kg \cdot d \cdot kg^{-1}$)	97	Hauck et al, 2011
Water-layer diffusion resistance for uptake of chemicals from water	ρ _water_layer ($kg \cdot d\ kg^{-1}$)	0.0068	Hauck et al, 2011
Water-layer diffusion resistance for uptake of chemicals from food	ρ _water_layer_food ($kg \cdot d\ kg^{-1}$)	0.0002	Hauck et al, 2011

Table 5.9 Physiological parameters of Chelon labrosus.

Physiological parameters of *Zosterisessor ophiocephalus*			
Name	**Abbreviation and unit**	**Value**	**Reference**
Fish age at maturity	time_fishlife (d)	1100	Hajji, 2013
Fish length at maturity	L_fish (cm)	13	Ysikliras and Stergiou 2014
Intercept of weight-length relationship	a_W_fish (unitless)	0.0079	Fishbase.org
Slope of weight-length relationship	b_W_fish (unitless)	3.10	Fishbase.org
Allometric rate exponent	K (unitless)	0.25	Hendriks et al., 2001
Lipid fraction of fish	ρ_water_fish ($kg \cdot d\ kg^{-1}$)	0.1	Micheletti 2008
Fraction of assimilated food	Assimilated_food (unitless)	0,73	Schroeder et al., 1981
Food transport coefficient	γ_food ($kg \cdot kg^{-1} \cdot d^{-1}$)	0,03	Hauck et al, 2011
Lipid-layer permeation resistance	ρ_lipid_layer ($kg \cdot d \cdot kg^{-1}$)	97	Hauck et al, 2011
Water-layer diffusion resistance for uptake of chemicals from water	ρ _water_layer ($kg \cdot d\ kg^{-1}$)	0.0068	Hauck et al, 2011
Water-layer diffusion resistance for uptake of chemicals from food	ρ _water_layer_food ($kg \cdot d\ kg^{-1}$)	0.0002	Hauck et al, 2011

Table 5.10 Physiological parameters of Zosterisessor ophiocephalus.

5.5 DIETARY PREFERENCES AND LIPID CONTENTS OF PREYS

The Fish model implemented in MERLIN-Expo requires the description of the dietary preferences for the target fish.

The diet matrix proposed by Micheletti and colleagues (2008) has been adopted as reference for the selected fish species in the case-study application (*Chelon labrosus* and *Zosterisessor ophiocephalus)*. Accordingly, dietary preferences (expressed as fraction of prey typologies on the overall diet) and corresponding lipid fractions are reported in Table 5.11 and Table 5.12.

Diet preferences and preys lipid contents of *Chelon labrosus*		
DIET	**DIETARY PREFERENCE** **Pref_diet_i** (i=0 to 1)	**LIPID FRACTION** **p_lipid_fi** (unitless)
Sediment	0.45	0
Phytobenthos	0.11	0,0500
Micro-Meiobenthos	0.32	0,0140
Macrobenthos Detritivorous	0.12	0,0140

Table 5.11. Chelon labrosus diet preference (Pref_diet_i) for food item i (i=1 to 10). Matrix diet values goes from 0 to 1 (Micheletti et al., 2008). Lipid fraction values are from Micheletti (2008).

Diet preferences and preys lipid contents of *Zosterisessor ophiocephalus*		
DIET	**DIETARY PREFERENCE** **Pref_diet_i** (i=0 to 1)	**LIPID FRACTION** **p_lipid_fi** (unitless)
Micro-Meiobenthos	0.08	0,0140
Macrobenthos Detritivorous	0.44	0,0140
Marcobenthos Filter Feeders (*Tapes philippinarum*)	0.12	0.0125
Marcobenthos Mixed Feeders	0.23	0.0262
Macrobenthos Omnivorous Predator (*Carcinus mediterraneus*)	0.12	0.0500

Table 5.12. Zosterisessor ophiocephalus diet preference (Pref_diet_i) for food item i (i=1 to 10). Matrix diet values from 0 to 1 (Micheletti et al.,2008). Lipid fraction values are from Micheletti (2008).

5.6 CHEMICAL CONCENTRATIONS IN PREYS

The Fish model requires target chemical concentrations in preys as forcing variables. Concentrations of the selected PCBs in aquatic organisms, which constitute preys of the target fish species, are not available from monitoring campaigns in Venice lagoon. To fill in this data gap, after a literature review, it has been decided to model PCB concentrations in prey according to the equation proposed by Gobas (1993) to assess bioaccumulation in benthonic organisms.
Equation 5.5 has been applied to calculate concentrations of chemicals in each prey organisms included in the dietary preferences of the selected fish species for the case study.

$$\mathrm{CB} = \frac{\mathrm{LB}}{\mathrm{OC}} \cdot \mathrm{CS} \qquad \textit{Eq. 5.5}$$

where,
CB = chemical concentration in benthic organism (µg/kg)
LB = lipid fraction of benthic organism (kg/kg)
OC = organic carbon fraction in sediment (kg/kg)
CS = concentration in sediment (µg/kg).

Concentrations in sediments are the same used for the estimation of the water concentration time series, therefore it is possible to obtain a time-series of estimated concentrations in prey organisms, as reported in Table 5.13.

SEDIMENT CORE	Year	Sediment			Phytobenthos			Micro-Meiobenthos			Macrobenthos Detritivorous		
		PCB 126	PCB 169	PCB 180	PCB 126	PCB 169	PCB 180	PCB 126	PCB 169	PCB 180	PCB 126	PCB 169	PCB 180
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
E	1940	1,000E-05	5,000E-06	9,200E-04	3,333E-05	1,667E-05	3,067E-03	9,333E-06	4,667E-06	8,587E-04	9,333E-06	4,667E-06	8,587E-04
E	1950	1,600E-04	5,000E-06	1,820E-03	5,333E-04	1,667E-05	6,067E-03	1,493E-04	4,667E-06	1,699E-03	1,493E-04	4,667E-06	1,699E-03
E	1960	4,000E-05	5,000E-06	1,710E-03	1,333E-04	1,667E-05	5,700E-03	3,733E-05	4,667E-06	1,596E-03	3,733E-05	4,667E-06	1,596E-03
E	1975	4,000E-05	5,000E-06	5,780E-03	1,333E-04	1,667E-05	1,927E-02	3,733E-05	4,667E-06	5,395E-03	3,733E-05	4,667E-06	5,395E-03
E	1995	2,000E-05	5,000E-06	1,920E-03	6,667E-05	1,667E-05	6,400E-03	1,867E-05	4,667E-06	1,792E-03	1,867E-05	4,667E-06	1,792E-03
B	1935	5,000E-04	5,000E-04	5,000E-04	1,667E-03	1,667E-03	1,667E-03	4,667E-04	4,667E-04	4,667E-04	4,667E-04	4,667E-04	4,667E-04
B	1954	5,000E-04	5,000E-04	5,000E-04	1,667E-03	1,667E-03	1,667E-03	4,667E-04	4,667E-04	4,667E-04	4,667E-04	4,667E-04	4,667E-04
B	1969	7,000E-04	2,000E-03	3,000E-03	2,333E-03	6,667E-03	1,000E-02	6,533E-04	1,867E-03	2,800E-03	6,533E-04	1,867E-03	2,800E-03
B	1976	2,500E-04	1,000E-03	1,000E-03	8,333E-04	3,333E-03	3,333E-03	2,333E-04	9,333E-04	9,333E-04	2,333E-04	9,333E-04	9,333E-04
B	1980	2,500E-04	2,000E-03	3,000E-03	8,333E-04	6,667E-03	1,000E-02	2,333E-04	1,867E-03	2,800E-03	2,333E-04	1,867E-03	2,800E-03
B	1984	2,500E-04	5,000E-04	1,000E-03	8,333E-04	1,667E-03	3,333E-03	2,333E-04	4,667E-04	9,333E-04	2,333E-04	4,667E-04	9,333E-04
B	1987	5,000E-04	5,000E-04	2,000E-03	1,667E-03	1,667E-03	6,667E-03	4,667E-04	4,667E-04	1,867E-03	4,667E-04	4,667E-04	1,867E-03

SEDIMENT CORE	Year	Macrobenthos Filter Feeders			Macrobenthos Mixed Feeder			Macrobenthos Omnivorous Predator		
		PCB 126	PCB 169	PCB 180	PCB 126	PCB 169	PCB 180	PCB 126	PCB 169	PCB 180
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
E	1940	8,333E-06	4,167E-06	7,667E-04	1,747E-05	8,733E-06	1,607E-03	3,333E-05	1,667E-05	3,067E-03
E	1950	1,333E-04	4,167E-06	1,517E-03	2,795E-04	8,733E-06	3,179E-03	5,333E-04	1,667E-05	6,067E-03
E	1960	3,333E-05	4,167E-06	1,425E-03	6,987E-05	8,733E-06	2,987E-03	1,333E-04	1,667E-05	5,700E-03
E	1975	3,333E-05	4,167E-06	4,817E-03	6,987E-05	8,733E-06	1,010E-02	1,333E-04	1,667E-05	1,927E-02
E	1995	1,667E-05	4,167E-06	1,600E-03	3,493E-05	8,733E-06	3,354E-03	6,667E-05	1,667E-05	6,400E-03
B	1935	4,167E-04	4,167E-04	4,167E-04	8,733E-04	8,733E-04	8,733E-04	1,667E-03	1,667E-03	1,667E-03
B	1954	4,167E-04	4,167E-04	4,167E-04	8,733E-04	8,733E-04	8,733E-04	1,667E-03	1,667E-03	1,667E-03
B	1969	5,833E-04	1,667E-03	2,500E-03	1,223E-03	3,493E-03	5,240E-03	2,333E-03	6,667E-03	1,000E-02
B	1976	2,083E-04	8,333E-04	8,333E-04	4,367E-04	1,747E-03	1,747E-03	8,333E-04	3,333E-03	3,333E-03
B	1980	2,083E-04	1,667E-03	2,500E-03	4,367E-04	3,493E-03	5,240E-03	8,333E-04	6,667E-03	1,000E-02
B	1984	2,083E-04	4,167E-04	8,333E-04	4,367E-04	8,733E-04	1,747E-03	8,333E-04	1,667E-03	3,333E-03
B	1987	4,167E-04	4,167E-04	1,667E-03	8,733E-04	8,733E-04	3,493E-03	1,667E-03	1,667E-03	6,667E-03

Table 5.13. Estimated concentrations time trends in diet matrix of mullet and goby fishes after applying the Equation 5.5 from Gobas (1993).

5.7 UNCERTAINLY VALUES USED IN PARAMETRIC SIMULATIONS

As explained in Chapter 3, it is possible to run probabilistic simulations with MERLIN-Expo.
Here in Table 5.14 are reported the Probability Density Functions (PDF) for each input parameters required to Fish model.

Parameter	PDF	Reference
Allometric rate exponent	Normal Mean: 0.25 - SD: 0.11	Hauck et al., 2011
Lipid fraction of fish	Logarithmic Uniform Min: 0.01 - Max: 0.2	Hauck et al., 2011
Lipid fraction of food	Logarithmic Uniform Min: 0.01 - Max: 0.7	Hauck et al., 2011
Fraction of assimilated food	Beta α: 50 - β:18.5	Hauck et al., 2011
Octanol-water partition coefficient PCB180	Normal 5^{th}: 7.91 - 95^{th}: 8.63	Nikolova and Jaworska, 2005
Metabolic half-life of PCB180	Normal 5^{th}: 129.6 - 95^{th}: 3958.6	Arnot et al., 2009
BCF PCB180	Normal 5^{th}: 5.7 - 95^{th}: 6.7	Mackay et al., 1982
Food transport coefficient	Logarithmic Uniform 5^{th}: 0.022 - 95^{th}: 0.041	Hauck et al., 2011
Lipid layer permeation resistence	Lognormal 5^{th}: 32 - 95^{th}: 298	Hauck et al., 2011
Fish age at maturity	Uniform Min: 730 - Max: 1460	Fishbase.org
Intercept of weight-length relationship	Lognormal Mean: 0.01 - SD 1.78	Fishbase.org
Slope of weight-length relationship	Normal Mean: 3.03 - SD: 0.16	Fishbase.org
Water-layer diffusion resistance for uptake of chemicals from food	Lognormal 5^{th}: 3.6E-6 - 95^{th}: 0.011	Hauck et al., 2011
Water-layer diffusion resistance for uptake of chemicals from water	Lognormal 5^{th}: 0.0037 - 95^{th}: 0.013	Hauck et al., 2011

Table 5.14 Input parameters PDF used for probabilistic simulations in Fish model.

6. RESULTS AND DISCUSSION

In order to test the performance of the Fish model on the Venice case-study, deterministic and probabilistic simulations in MERLIN-Expo have been run using the input data described in Chapter 5. Starting from Sediment Core E and B, the concentrations of the three selected congeners in the fish species *Chelon labrosus* (mullet) and *Zosterisessor ophiocephalus* (goby) have been simulated for a period of about 60 years (from 1940 to 2000). In this chapter the final results of model simulations are presented and discussed. Modelling results are compared with monitored fish concentrations in the same species measured in the framework of the "Mapping the pollutants in the lagoon bottom sediment" project by MAV (2000a).

6.1 DETERMINISTIC SIMULATIONS

Bioaccumulation in *Chelon labrosus*

Time-trend concentrations for PCB126, PCB169 and PCB180 in the fish species *Chelon labrosus* (expressed as mg/kg_{fw}) obtained from the application of the MERLIN-Expo Fish model are presented in Figures from 6.1 to 6.6.

Specifically, for each congener, two charts are reported: on the left side (Fig. 6.1, 6.3 and 6.5) the time-trend concentrations in organism obtained by using as input data the water concentrations and dietary concentrations calculated from sediment cores E (Frignani et al., 2005), as explained in Chapter 5, and on the right side (Fig. 6.2, 6.4 and 6.6) the time-trend concentrations in the organisms estimated using water and dietary concentrations calculated from sediment core B (Marcomini et al., 1999).

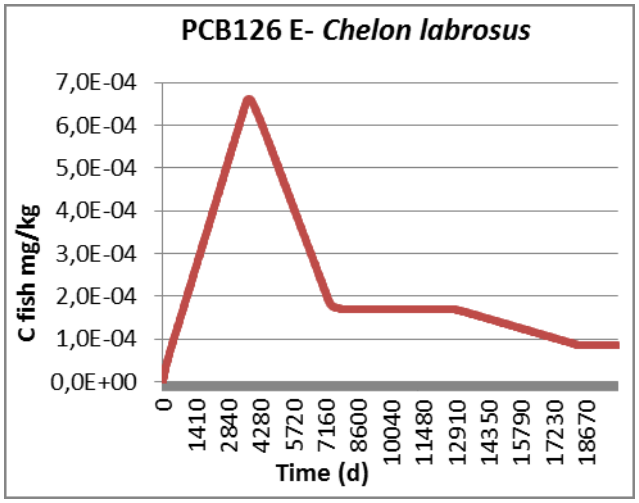

Fig. 6.1. PCB126 time trend concentrations in Chelon labrosus deriving input data from Core E.

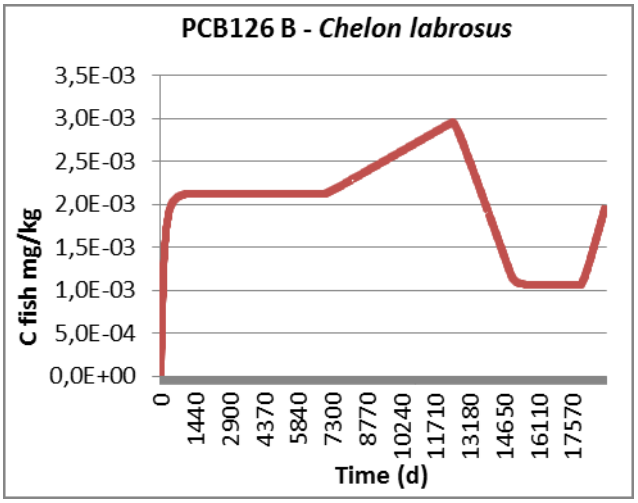

Fig. 6.2. PCB126 trend concentrations in Chelon labrosus deriving water input data from Core B.

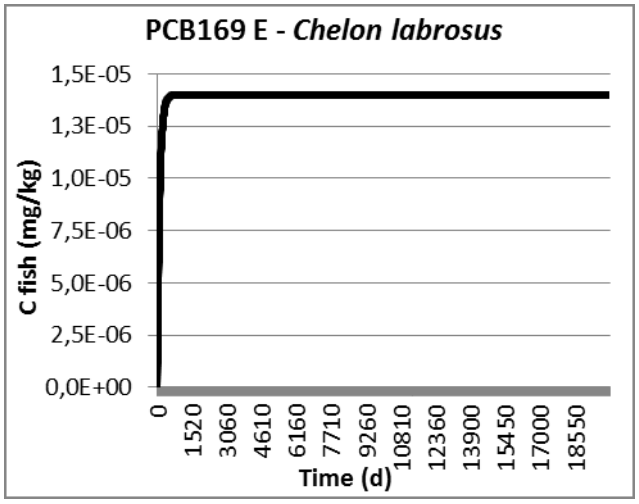

Fig. 6.3. PCB169 trend concentrations in Chelon labrosus deriving input data from Core E.

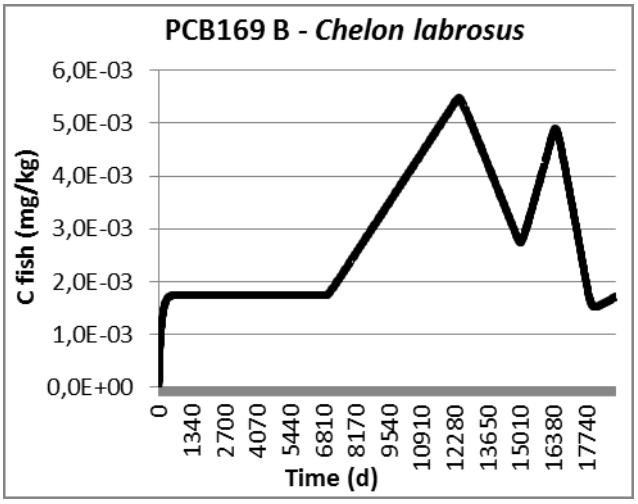

Fig. 6.4. PCB169 trend concentrations in Chelon labrosus deriving input data from Core B.

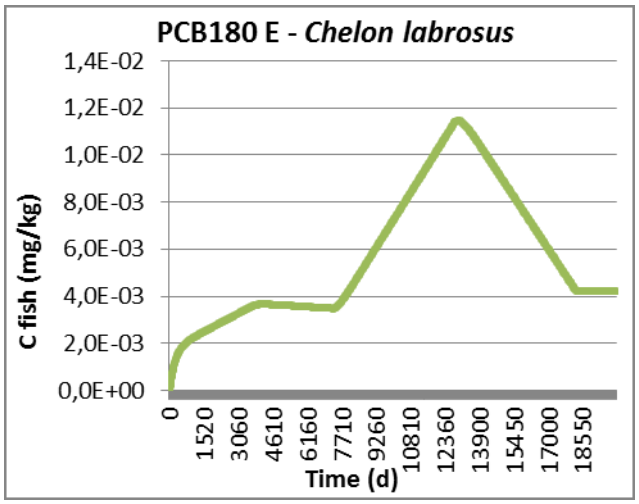

Fig. 6.5. PCB180 trend concentrations in Chelon labrosus deriving input data from Core E.

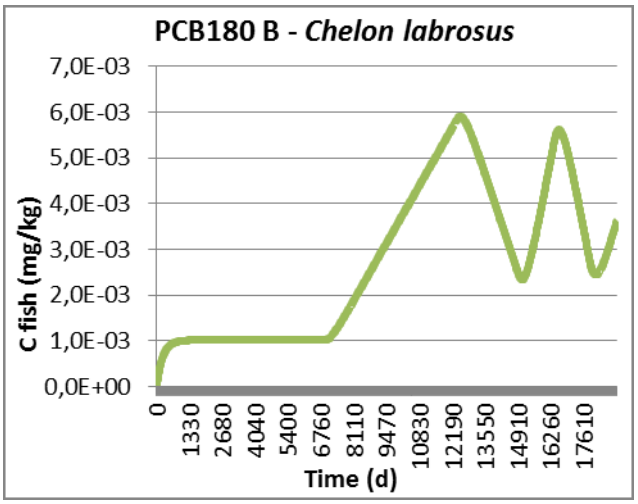

Fig. 6.6. PCB180 trend concentrations in Chelon labrosus deriving input data from Core B.

As can be observed from the charts, at time 0 the chemical concentration in both fish species is equal to zero for all the congeners and then it gradually increases (with different magnitudes). This is related to the fact that in the Fish model it is assumed that the initial chemical concentration in the fish is always null. Then, after the fish has reach its maturity age (3 years in the case of the selected species), the chemical concentration in fish provided by the model at any time for the following periods has to be interpreted as the chemical concentration of a fish at its maturity age (considered as ready to be caught for human diet), depending on the result of all uptake and elimination processes and on the variations in water concentrations.

In general, it can be noticed that, in all the simulations, the trends of modelled concentrations of pollutants in *Chelon labrosus* tend to follow the same trend which characterize chemical concentrations in sediment and water (described in Chapter 5) used as input for the Fish model, but also for calculating the chemical concentrations in preys (Paragraph 5.6). For example, in Figures 6.1 and 6.2 showing calculated concentrations of PCB126 in fish, it is possible to notice that the relatively small peaks in fish concentrations correspond to the period of observed peaks in sediment/water concentrations. Furthermore, in Figures 6.4 (referred to PCB169) and in both Figures 6.5 and 6.6 (referred to PCB180), along the simulated time period there is a trend similar to the water concentrations for the corresponding congeners: in both of them, the concentration peaks can be observed for the period between the '70s and the '80s (about 10 to 12.000 days), consequently to a period of significant industrial activity in Porto Marghera.

For a preliminary validation of the results provided by the Fish model against real monitoring data, the calculated concentrations have been compared with available measured mean concentrations in individuals of *Chelon labrosus* sampled in the Venice lagoon in 1997 (MAV, 2000a).

In Table 6.1, the chemical concentrations in fish estimated by the Fish model (mean concentration over the entire simulation period and concentration corresponding to the most recent year according to available data) and the mean measured concentrations are reported.

PCB	**Sediment Core Input**	**Calculated concentration (mean over the entire simulation period)**	**Calculated concentration (in 1995 for sediment core E; in 1987 for sediment core B)**	**Measured concentration (MAV, 2000a)**
		mg/kg_{fw}	mg/kg_{fw}	mg/kg_{fw}
PCB126	E	2,31E-04	8,72E-05	5,79E-06
	B	2,12E-03	1,99E-03	5,79E-06
PCB169	E	1,40E-05	1,40E-05	5,28E-07
	B	2,96E-03	1,71E-03	5,28E-07
PCB180	E	5,68E-03	4.24E-03	1,01E-03
	B	2,85E-03	3,62E-03	1,01E-03

Table 6.1. PCB126, PCB169 and PCB180 concentrations calculated by the Fish model compared with measured concentration in Chelon labrosus (MAV, 2000a).

Looking at Table 6.1, it can be concluded that for PCB180 there is a quite good agreement (same order of magnitude) between calculated concentrations and empirical values measured in *Chelon labrosus* (MAV, 2000a). This can be observed for both the simulation with data from sediment core B and that with data from sediment core E.

For PCB126 congener, the difference between predicted and measured concentration in fish is larger than for PCB180, being of one order of magnitude for simulation from sediment core E and of three orders for simulation from sediment core B. For PCB169, the discrepancy between predicted and measured concentrations is even more significant (between two and three orders of magnitude). However, it is important to remember this overestimation could be due to the high uncertainty in input data derived from sediment cores concentrations with many values below the detection limit. Moreover, for sediment core E the last available measurement dates back to 1987, so there is a quite relevant time gap to available biota measurements.

Bioaccumulation in *Zosterisessor ophiocephalus*

Time-trend concentrations for PCB126, PCB169 and PCB180 in the *Zosterisessor ophiocephalus* (expressed as mg/kg$_{fw}$) obtained from the application of the MERLIN-Expo Fish model are in Figures from 6.7 to 6.12.

As for *Chelon labrosus,* for each congener, two charts are reported: on the left side (Fig. 6.7, 6.9 and 6.11) the time-trend concentrations in organism obtained by using as input data the water concentrations and dietary concentrations calculated from sediment cores E (Frignani et al., 2005), as explained in Chapter 5, and on the right side (Fig. 6.8, 6.10 and 6.12) the time-trend concentrations in the organisms estimated using water and dietary concentrations calculated from sediment core B (Marcomini et al., 1999).

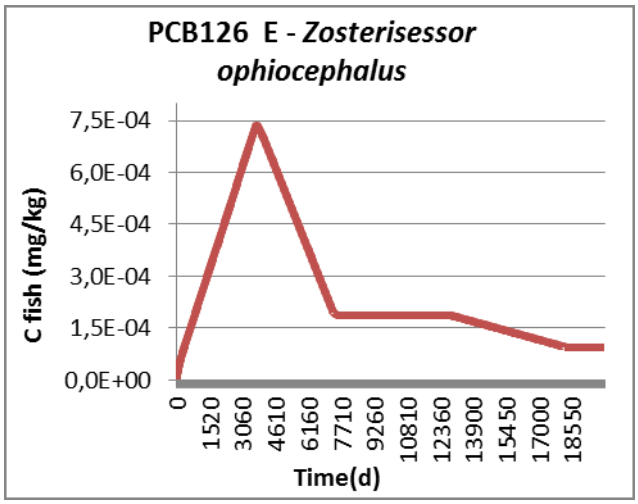

Fig. 6.7. PCB126 trend concentrations in Z. ophiocephalus deriving input data from Core E.

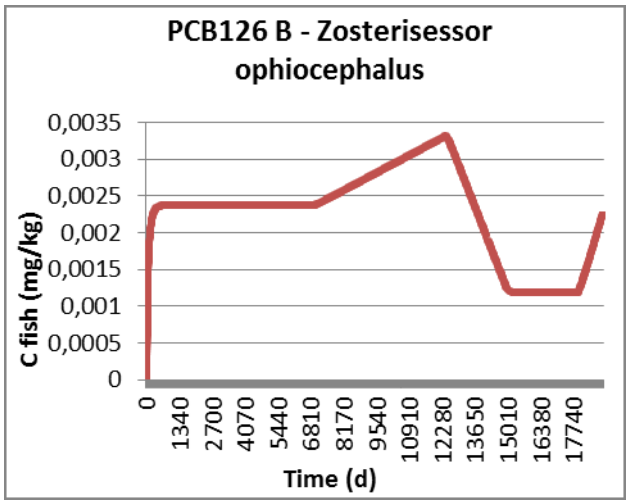

Fig. 6.8. PCB126 trend concentrations in Z. ophiocephalus deriving input data from Core B.

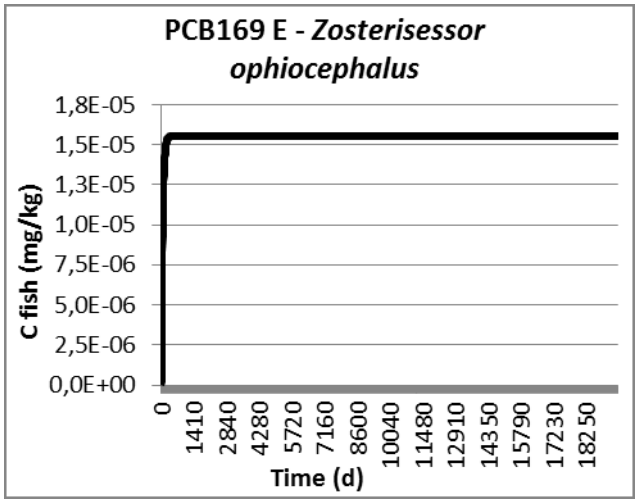

Fig. 6.9. PCB169 trend concentrations in Z. ophiocephalus deriving input data from Core E.

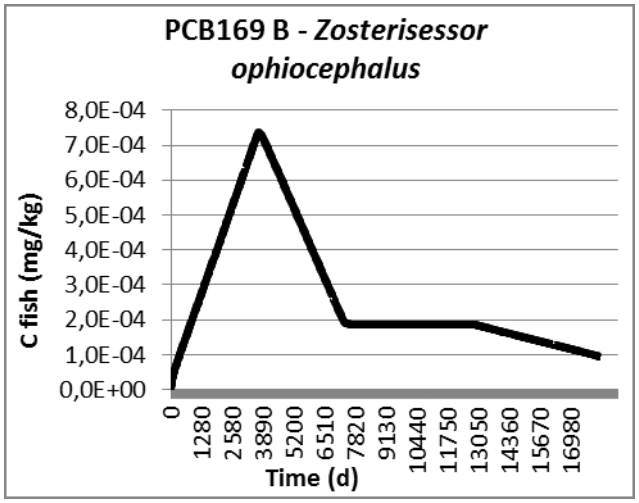

Fig. 6.10. PCB169 trend concentrations in Z. ophiocephalus deriving input data from Core B.

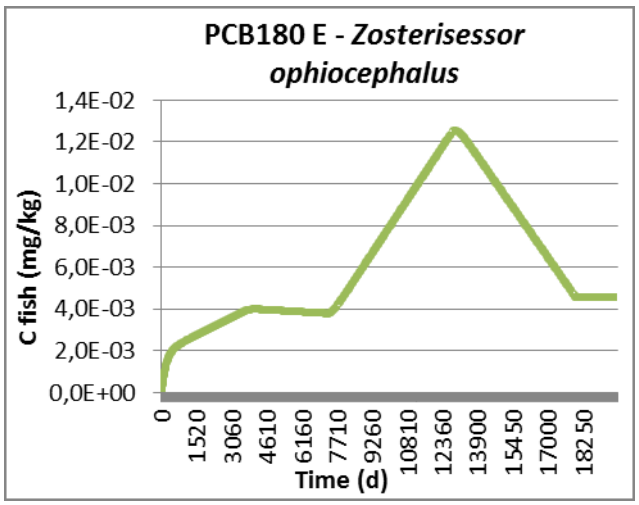

Fig. 6.11. PCB180 trend concentrations in Z. ophiocephalus deriving input data from Core E.

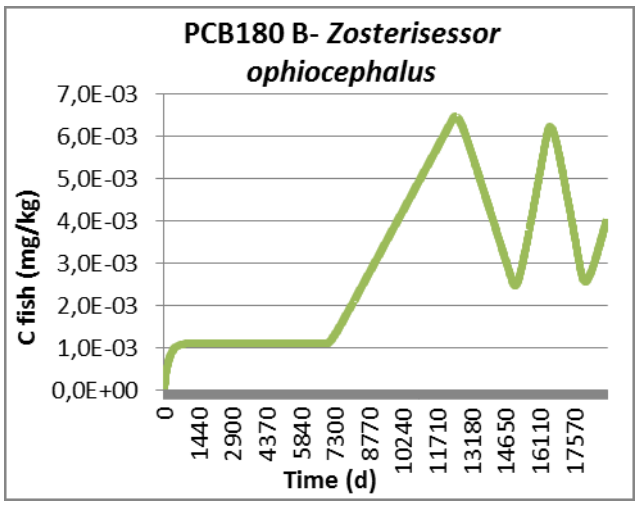

Fig. 6.12. PCB180 trend concentrations in Z. ophiocephalus deriving input data from Core B.

The trend of modelled concentrations in *Zosterisessor ophiocephalus* follows a similar trend to the simulated results in *Chelon labrosus*. In all the simulations, indeed, the concentrations of PCB congeners in *Z. ophiocephalus* turn out to be significantly conditioned by water and sediments concentrations used as input data for model simulations (described in Chapter 5).

By considering the general trend of concentrations of different PCBs congeners, it is possible to see a slightly decrease of calculated concentrations in fish during the last years, after a peak, corresponding to the '70s (i.e., about 12.000 days in model results), particularly marked in PCB180 trend concentrations. As observed in simulations performed for *Chelon labrosus*, PCB169 concentrations predicted by using as input data the concentrations calculated from sediment core E result particularly critical because of the large number of measured concentrations below the LOD.

In Table 6.2 chemicals concentrations values for *Zosterisessor ophiocephalus* at maturity age (mean concentration over the entire simulation period and concentration corresponding to the most recent year according to available data) are compared with measured concentrations by MAV (2000a), expressed both as mean measured in individuals caught in the entire lagoon and as mean of the individuals sampled only in the area of the central lagoon in 1997.

PCB	Sediment Core Input	Calculated concentration (mean over the entire simulation period)	Calculated concentration (1995 for sediment core E; 1987 for sediment core B)	Measured concentration Central Lagoon (MAV, 2000a)	Measured concentration Entire Lagoon (MAV, 2000a)
		mg/kg_{fw}	mg/kg_{fw}	mg/kg_{fw}	mg/kg_{fw}
PCB126	E	2,70E-04	9,47E-05	1,50E-05	1,75E-05
	B	2,27E-03	2,29E-03	1,50E-05	1,75E-05
PCB169	E	1,55E-05	1,55E-05	2,37E-06	4,00E-06
	B	3,05E-03	9.50E-05	2,37E-06	4,00E-06
PCB180	E	6,37E-03	4.54E-03	2,39E-03	3,25E-03
	B	2,94E-03	4,04E-03	2,39E-03	3,25E-03

Table 6.2. PCB126, PCB169 and PCB180 concentrations calculated by the Fish model compared with measured concentration in Zosterisessor ophiocephalus (MAV, 2000a).

Taking into account the concentration of PCB126 calculated in 1995 by the model (from sediment core E), despite a certain overestimation, the value can be considered quite close (same order of magnitude) to measured concentrations in fishes sampled in central and in the entire lagoon of Venice in 1997. The difference between the calculated PCB126 concentrations and PCB169 concentration in 1989 (from sediment B) simulated by the model and the measured concentrations in *Z. ophiocephalus* range from one to three orders of magnitude, i.e. the model seems to overestimate significantly the predicted concentrations in fish tissues. As explain for *Chelon labrosus* results, this difference could be reasonably related to the uncertainty derived from input parameters value for sediment and water concentrations. This issue will be further discussed in the next paragraph.

As for PCB180, calculated concentrations are in quite good agreement with measured concentrations (the same order of magnitude) for both simulations related to data from sediment core E and B, showing a similar result as for *Chelon labrosus*.

Identification of "likely water concentrations" for values below the detection limit

As described in the previous paragraphs, if calculated concentrations of PCB126 and PCB169 in fish are considered, a difference of few orders of magnitude between measured and calculated concentrations can be observed in some cases. This difference can be attributed to the fact that the model input data for these two congeners are characterized in some cases by a high uncertainty due to the presence in the original dataset of many concentrations values in sediment below the detection limit (Paragraph 5.1). As reported in Chapter 5, for these values, the half of the LOD value (LOD/2) has been considered as input concentration in sediment to estimate concentrations in water (Menichini et al., 2004). In this way, however, estimated water concentrations still turn out to be higher than water concentrations values measured in MAV (2000a; 2000b) and Secco et al. (2005).

For congeners PCB126 and PCB169, a set of simulations have been run using as input data a set of concentrations progressively decreasing from LOD/2. It turned out that, in order to get from the model a predicted concentration in fish (*C. labrosus*) quite "close" to the real monitoring data in biota (MAV, 2000a), the corresponding concentrations in water used as input data should be lowered approximately by two orders of magnitude. Table 6.3 present a comparison of concentrations in *Chelon labrosus* calculated first by the model using water values derived from sediment core E, and then by using "decreased" water concentrations (lowered by two orders of magnitude). The results of this "exercise" shows how the proposed modelling approach could be apply to "complement" in some way the available monitoring data, by providing an estimate of the more likely concentrations in water in the case these values are below the limit of detection of the available instruments.

Data Sources	PCB126		PCB169	
	Concentration in Water (mg/m^3)	**Fish Mean Concentrations** (mg/kg_{fw})	**Concentration in Water** (mg/m^3)	**Fish Mean Concentrations** (mg/kg_{fw})
Sediment Core B (Marcomini et al., 1999)	1,86E-05	2,12E-03	1,68E-05	2,96E-03
"Presumed" water concentration	1,86E-07	9,79E-06	1,68E-07	4,42E-07
Biota samples (MAV, 2000a)	-	5,79E-06	-	5,28E-07

Tab. 6.3. PCB *concentrations in Chelon labrosus calculated by the model using water values derived from sediment core B (Marcomini et al., 1999) and the "presumed" water concentrations.*

Comparison of results for *Chelon labrosus* and *Zosterisessor ophiocephalus*

Comparing water input concentrations derived from sediment cores (Tab. 5.3 in Chapter 5) with the simulated time-trend concentrations of target PCBs, it can be observed how both fishes can actually bioaccumulate PCBs reaching quantities of chemicals in their body tissues higher than the environment where they live in. As reported by modelled results, when concentrations of pollutants in water increase (as during the '70s), for both species an increased bioaccumulation can be noticed, leading to increased concentrations of chemicals in the body.

The results of the applications show that bioaccumulation of selected PCBs congeners is affected by species-specific physiological parameters, i.e. lipid content and fish weight.

According to results of the simulations, concentrations of the chemicals in *Chelon labrosus* are lower than those in *Zosterisessor ophiocephalus*. Goby fishes are characterized by a higher lipid fraction than the mullets, therefore they may accumulate more chemicals. This observation is in accordance with available literature, i.e. the lipid content in fish influences heavily the bioaccumulation of organic chemicals (e.g., Arnot and Gobas, 2006).

Fish weight is shown to influence bioaccumulation processes. Values from the database "Fishbase" (www.fishbase.org) are used in the model to calculate fish weight at the age of maturity (about three years for both species). Age of maturity is an important parameter because it corresponds to the time at which fishes are assumed to be caught and then they may enter human diet. *C. labrosus* is an organism heavier than *Z. ophiocephalus* when measured at its maturity age. However *Zosterisessor ophiocephalus* shows to accumulate more than *Chelon labrosus*, implying that low weight organism can be characterized by more significant bioaccumulation than heavier organisms.

Different body weights and lipid contents account for different values of respiratory uptake and excretion rate constants of studied fish species, which are reported in Table 6.4.

Rate Constant / Parameter	**k_respiratory_uptake** ($L\ kg^{-1}\ d^{-1}$)	**k_excretion** (d^{-1})	**Weight at the maturity** (kg)	**Lipid fraction** (-)
Chelon labrosus	1.92E+02	1.56E-02	0.345	0.068
Zosterisessor ophiocephalus	3.80E+02	3.09E-02	0.0225	0.1

Table 6.4. Rate constants, weight and lipid fraction for the two target fish species.

Respiratory uptake and excretion rate constants are compared with the minimum and maximum value calculated in Hendriks el al. (2001) in fishes (Table 6.5). The two rate constants calculated in the MERLIN-Expo applications are in line with the calculated constants by Hendriks.

As described in Hendriks et al. (2001), smaller organisms have faster rates than large ones. In fact, regarding *Chelon labrosus*, being heavier and bigger than *Zosterisessor ophiocephalus*, respiratory uptake and excretion are lower.

Rate Constant / Parameter	**k_respiratory_uptake** ($L\ kg^{-1}\ d^{-1}$)	**k_excretion** (d^{-1})
Chelon labrosus	1.92E+02	1.56E-02
Zosterisessor ophiocephalus	3.80E+02	3.09E-02
Hendriks et al., 2001 (Min weight – Max weight)	1.5E+01 - 1E+04	1.5E-04 - 1E-01

Table 6.5 Rate constants related to the fish species compared with those calculated in Hendriks et al. (2001).

As reported in Chapter 2, the bioaccumulation factor (BAF) is the ratio of the concentration of the chemical in the organism to the chemical concentration in the water, which is a significant indicator of chemical bioaccumulation also applied for regulatory purposes.

It is very interesting to compare BAF values derived from applications of MERLIN-Expo Fish model to the two selected fish species. In table 6.6 mean LogBAF values for the three target congeners of PCBs (from sediment core E) in *Chelon labrosus* and *Zosterisessor ophiocephalus* are shown. Generally, it can be stated that LogBAF values calculated from the simulations result to be in line with the LogBAF calculated in the food chain bioaccumulation study in Venice lagoon by Micheletti et al. (2008).

Usually, pollutants with largely dissimilar K_{ow} values behave differently in bioaccumulation processes. In Venice case-study, K_{ow} values referred to the three target PCBs congeners (Table 4.2, Chapter 4) do not show a clear correlation with bioaccumulation trend results obtained by model simulations.

As reported in Arnot and Gobas (2006), taking into account meanly lipophilic chemicals (characterized by K_{ow} value between 3 and 6), bioaccumulation process increase when chemical K_{ow} value increases. However, considering chemicals with high K_{ow} value (i.e., higher than 7), as is the case of the three target PCBs in this study, it can be observed that bioaccumulation process is not more heavily influenced by the differences in their octanol-water partition coefficients.

Therefore, it can be argued that it is appropriate to assume that higher and faster bioaccumulation process observed in *Zosterisessor ophiocephalus* in comparison with *Chelon labrosus* (confirmed by the differences in the corresponding logBAF) can be attributed mainly to different physiological characteristics of the considered fish species.

LogBAF	PCB126	PCB169	PCB180
Chelon labrosus **Mean logBAF**	4,98	5,22	5,40
Zosterisessor ophiocephalus **Mean logBAF**	5,02	5,26	5,44
Episuite	6,80	6,96	6,85

Tab. 6.6. LogBAF mean values derived from Chelon labrosus and Zosterisessor ophiocephalus applications in MERLIN-Expo Fish model (from sediment core E, Frignani et al., 2005) and calculated for a generic fish by Episuite software.

6.2 PROBABILISTIC SIMULATIONS

Probabilistic simulations (see Chapter 3) with Fish model have been run to assess concentrations in *Chelon labrosus* and *Zosterisessor ophiocephalus* only for PCB180, considering input data from both sediment core E and B. The probabilistic distribution functions used in MERLIN-Expo Fish model for each input parameter are showed in Table 5.14 in Chapter 5.

For this calculation, Monte Carlo approach randomly draws samples from the PDFs of parameters selected (Table 5.14) and generates the integrated distributions of specified outputs through 1.000 times of runs. Figures from 6.13 to 6.16 illustrate the results of probabilistic simulations for PCB180 for the two considered species. Specifically, the mean, the 5^{th} and the 95^{th} percentile concentrations in fish tissues are represented by the red, the blue and the green line respectively.

The figures highlight that concentration trends in fishes reflect the overall trend of input water concentrations (Table 5.3, Chapter 5), but this not as evident as in the deterministic applications. The overall trends of mean concentrations (red line) are smoother than those related to deterministic applications results, without very relevant concentration peaks. However the mean values obtained by using the probabilistic approach are in the same range of magnitude of deterministic applications results. The range between the mean values for 5^{th} and 95^{th} percentiles (blue and green lines respectively) provides an estimate of the overall uncertainty associable to model predictions.

Also the difference between the concentrations in the two fish species turns out to be smoothed in the probabilistic applications in comparison with the differences shown by the deterministic results.

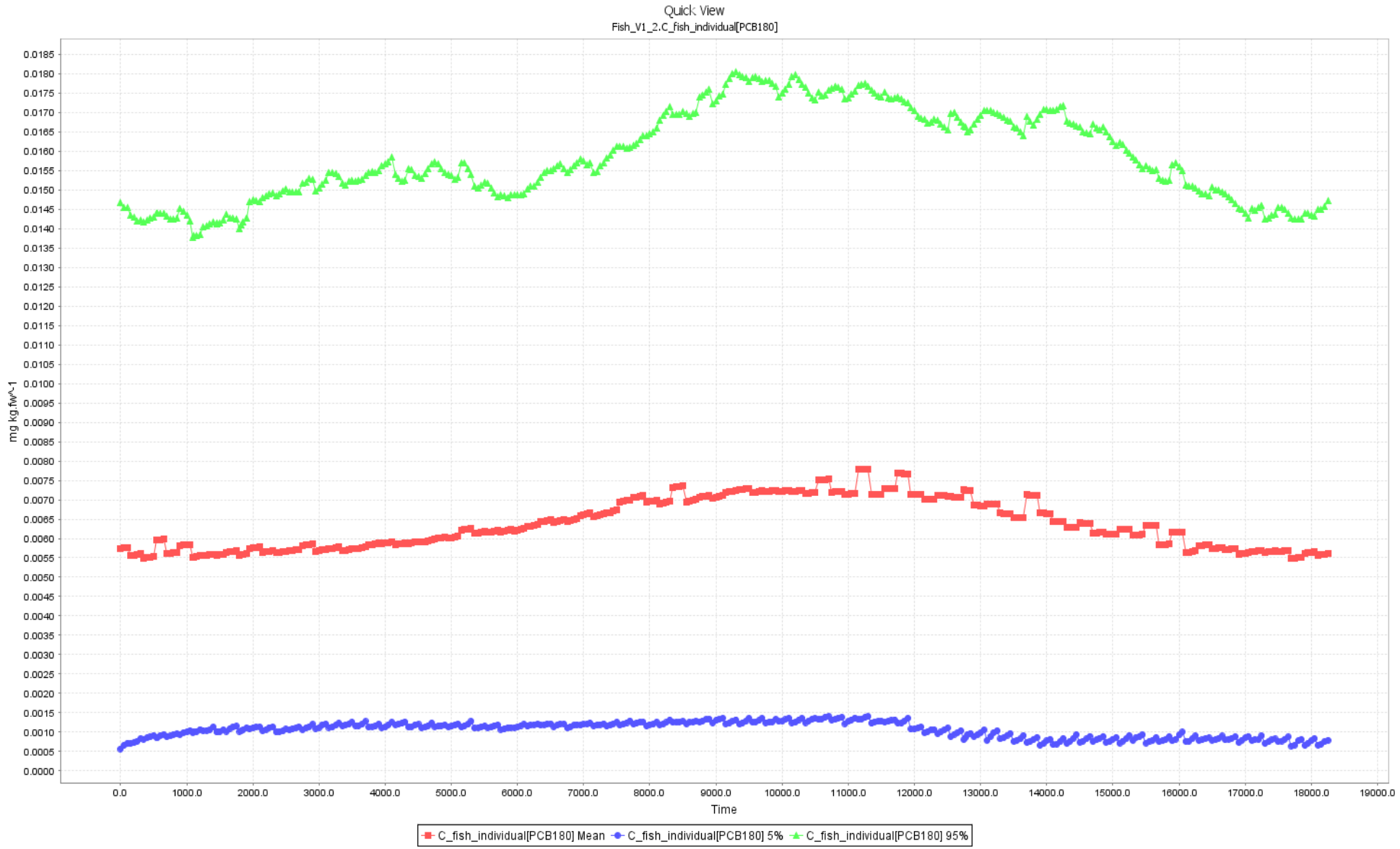

Fig. 6.13. Probabilistic simulation results for PCB180 in Chelon labrosus (input values derived from Sediment Core E).

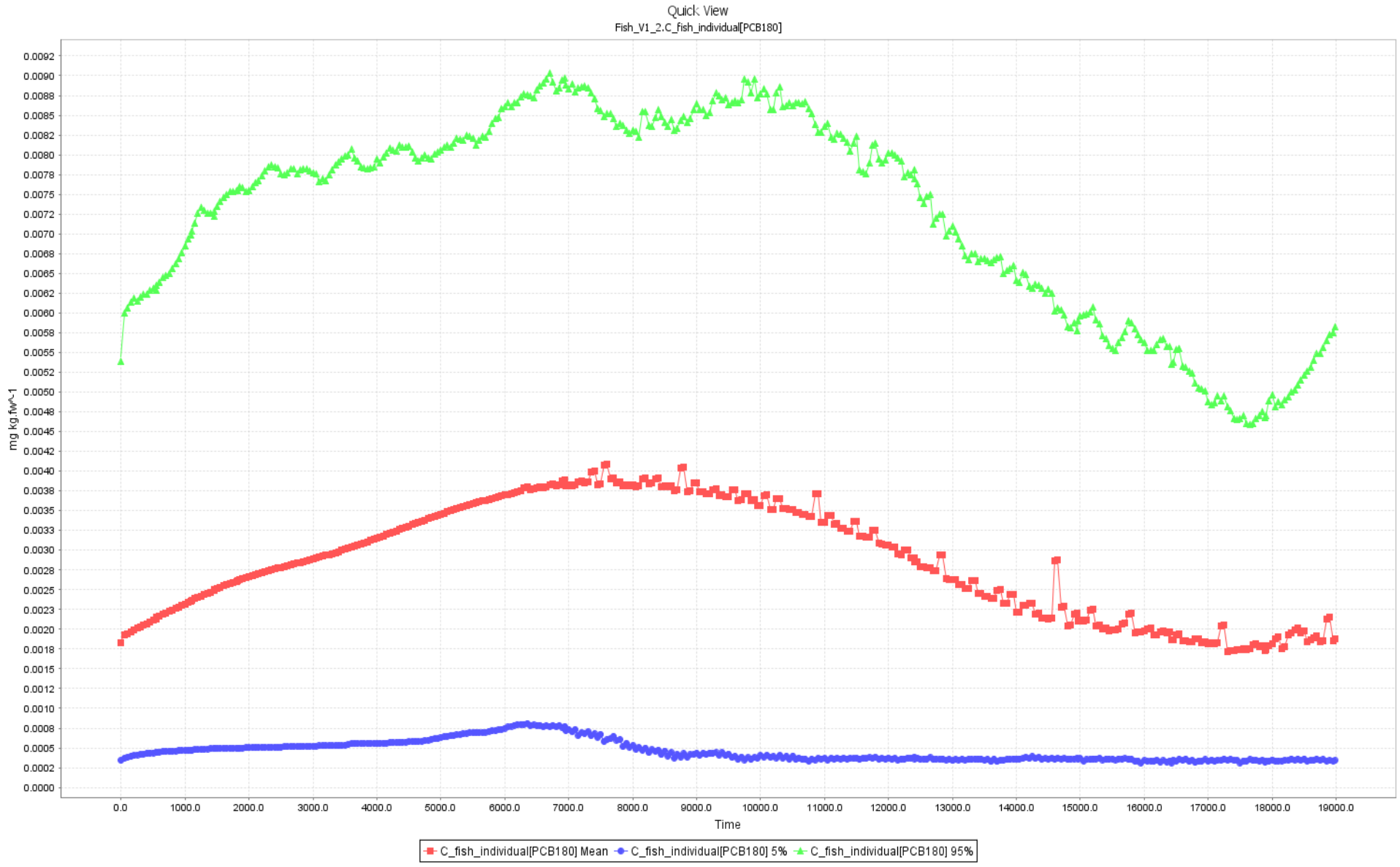

Fig. 6.14. Probabilistic simulation results for PCB180 in Chelon labrosus (input values derived from Sediment Core B).

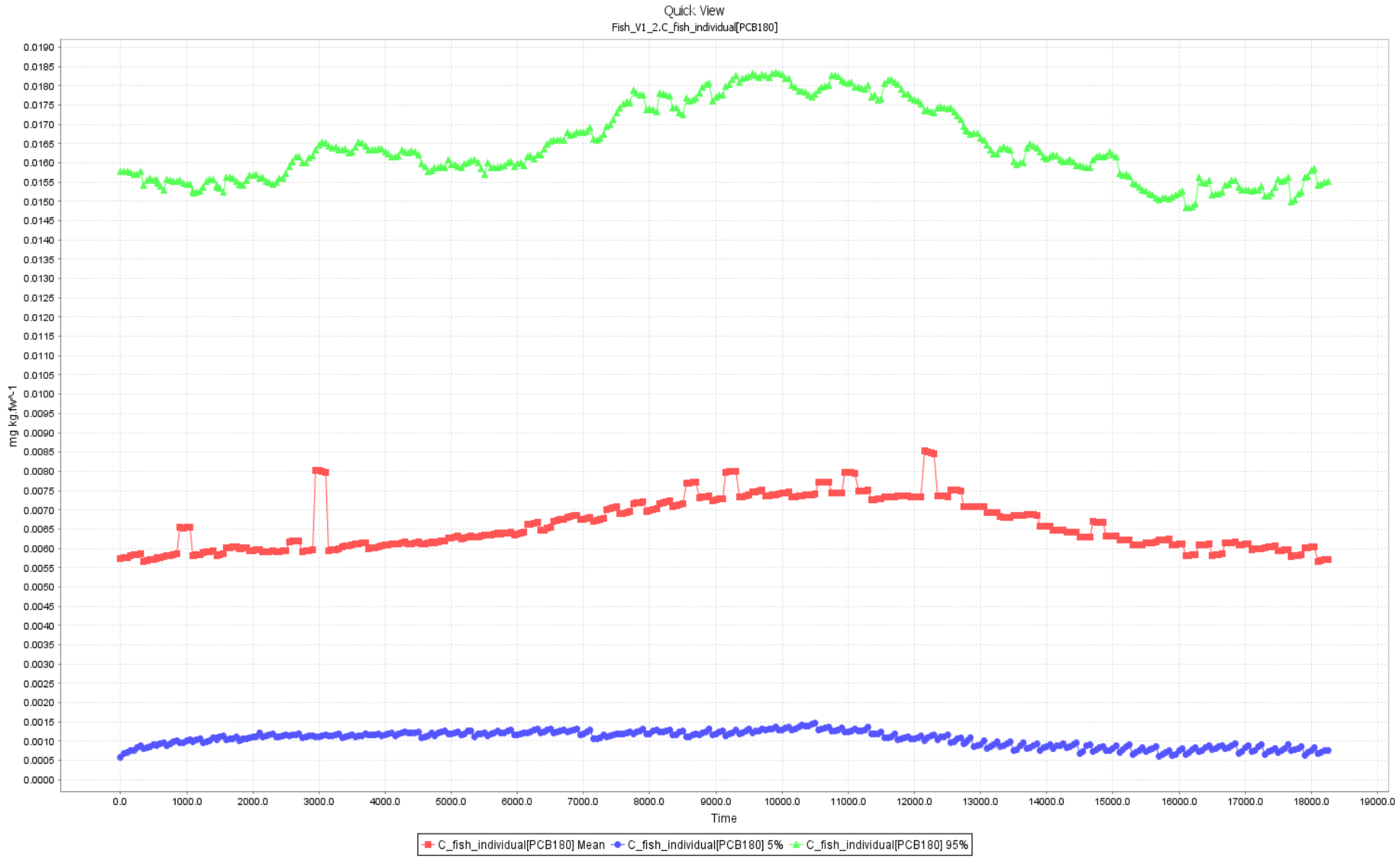

Fig. 6.15. Probabilistic simulation results for PCB180 in Zosterisessor ophiocephalus (input values derived from Sediment Core E).

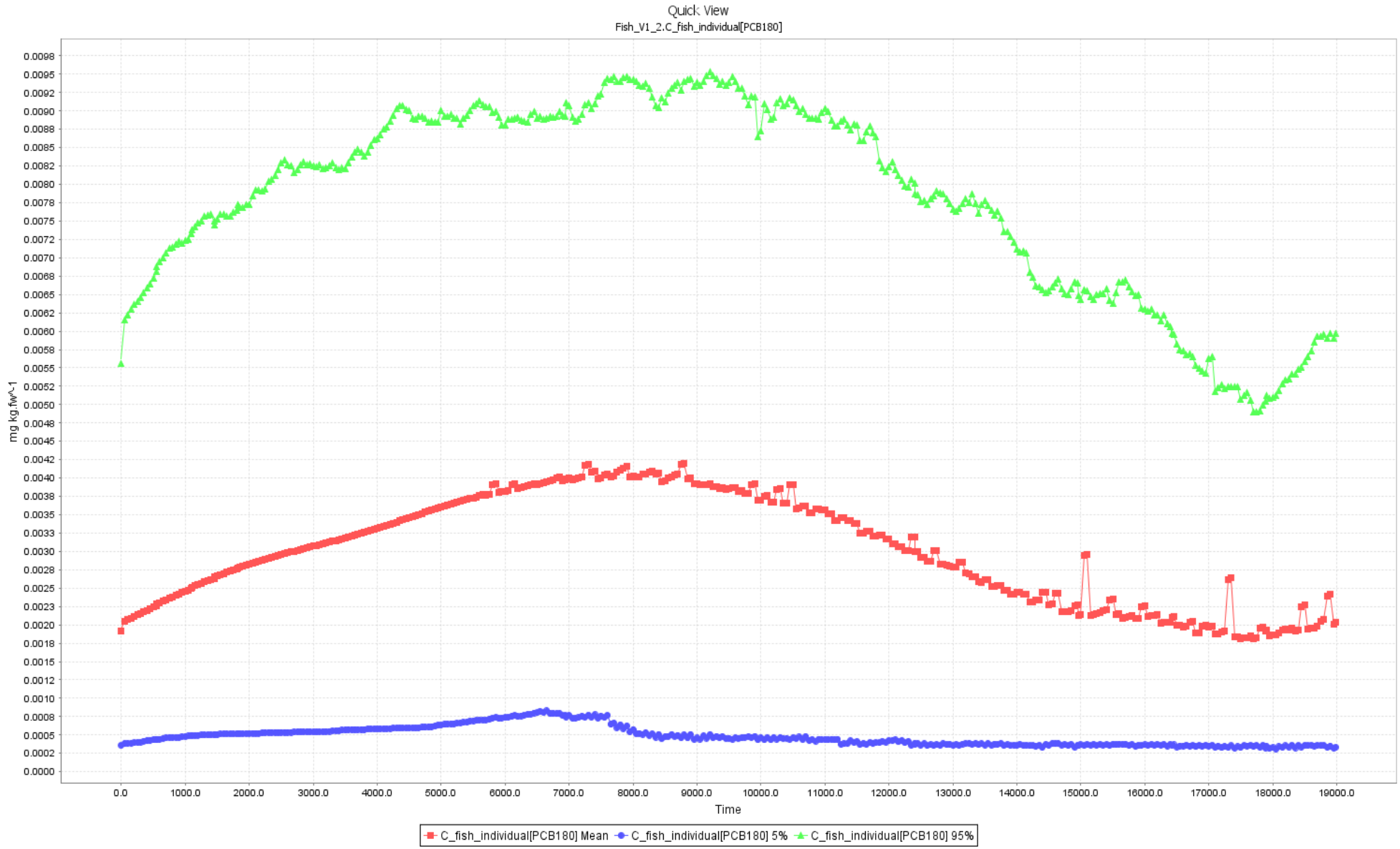

Fig. 6.26. Probabilistic simulation results for PCB180 in Zosterisessor ophiocephalus (input values derived from Sediment Core B).

Hereunder in Table 6.7 mean values for rate constants for respiratory uptake and excretion calculated according to the probabilistic simulation results are reported. As resulted in the deterministic approach, *Chelon labrosus* is shown to have slower physiological processes than *Zosterisessor ophiocephalus*. Bioaccumulation process in goby fishes is more significant than in mullets, but the differences between the two species according to probabilistic applications are not as remarkable as in the results of deterministic simulations.

Sed. Core	Rate Constant	Fish	Unit	Mean Calculated	Hendriks et al., 2001 (Min – Max weight)
E	k_excretion	*Chelon labrosus*	d^{-1}	1.58E-04	1.5E-04 - 1E-01
B	k_excretion	*Chelon labrosus*	d^{-1}	1.56E-04	1.5E-04 - 1E-01
E	k_excretion	*Zosterisessor ophiocephalus*	d^{-1}	1.61E-04	1.5E-04 – 1E-01
B	k_excretion	*Zosterisessor ophiocephalus*	d^{-1}	1.6E-04	1.5E-04 - 1E-01
E	k_respiratory_uptake	*Chelon labrosus*	L Kg d^{-1}	2.00E+02	1.5E+01 - 1E+04
B	k_respiratory_uptake	*Chelon labrosus*	L Kg d^{-1}	1.96E+02	1.5E+01 - 1E+04
E	k_respiratory_uptake	*Zosterisessor ophiocephalus*	L Kg d^{-1}	2.01E+02	1.5E+01 - 1E+04
B	k_respiratory_uptake	*Zosterisessor ophiocephalus*	L Kg d^{-1}	2.01E+02	1.5E+01 - 1E+04

Table 6.7. Rate constants and weight related to the two fishes in probabilistic applications.

7. CONCLUSIONS

The main objective of this thesis was the application of Fish model (recently implemented into the MERLIN-Expo tool for exposure assessment) to the case-study of the Venice lagoon for the assessment of PCBs bioaccumulation in two fish species, in order to test the model applicability and evaluate its performance against real monitoring data.

To reach this objective, the following tasks were accomplished:

- the parameterization of the model according to the specific case-study conditions and characteristic of three target PCBs congeners (PCB126, PCB169, PCB180) and the selection of required input data;
- the definition of simulation settings and the performance of simulations (deterministic and probabilistic approaches);
- the analysis and evaluation of model results against real monitoring data.

The MERLIN-Expo Fish model, which allows dynamic simulation of bioaccumulation in different fish species, was firstly analysed in detail, also through the comparison with other existing predictive models for bioaccumulation in aquatic organisms. The literature review also supported the collection of input data needed for model parameterization, included the selection of appropriate probability density functions for a full probabilistic application of the model. QSAR models were used to derive chemical-specific input values, while temporal series of PCB concentrations in water were reconstructed starting from chemical concentrations in two dated sediment cores collected in the central area of the Venice lagoon.

The Fish model was successfully applied to the selected case-study and the results of deterministic simulations demonstrate the feasibility of predicting time-dependent concentrations in the selected target fish species (Chelon labrosus and Zosterisessor ophiocephalus).

By comparing modelled results with measured data in fish samples (MAV, 2000a), it is demonstrated that Fish model predictions reasonably approximate measured PCB180 concentrations accumulated in fishes (i.e., the same order of magnitude). Model calculated concentrations in both species in 1995 are indeed in quite good agreement with measured values in fishes caught in the same period (MAV, 2000a).

Model predictions are less satisfying for PCB126 and PCB169 concentrations, with a difference of one or two order of magnitude with real monitoring data. However, this difference can be at least partially explained by considering that the model input data for these two congeners are characterized in some cases by a high uncertainty due to the presence in the original dataset of many concentrations values in sediment below the detection limit (substituted by values equal to LOD/2 as input data). This result suggested to reconstruct more “likely” PCB126 and PCB169

water concentrations, by identifying possible concentrations which could explain the measured real concentrations in fish. These concentrations turned out to be two order of magnitude lower that the actual LOD. This “reverse modelling” approach suggests that the Fish model could be a suitable tool to derive scenarios of historical pollution possibly closer to the real ones in case of undetected chemical concentrations.

The model results show how different physiological parameters (i.e. lipid fraction and weight of fishes) may influence bioaccumulation processes, as reported also in literature (e.g., Arnot and Gobas, 2006). This is confirmed by the analysis of respiratory uptake and excretion rate constants for both fishes. As already described in Hendriks et al. (2001), smaller organisms have faster bioaccumulation rates than large ones: in *Chelon labrosus*, being heavier and bigger than *Zosterisessor ophiocephalus*, respiratory uptake and excretion calculated values turned out to be lower. BAF values derived by the modelled results are in line with those described in literature for the same compounds and similar species (e.g., Micheletti et al., 2008; Arnot and Gobas, 2006).

The applicability of a full probabilistic approach with MERLIN-Expo Fish model was demonstrated for PCB180 through simulations of time-dependent concentrations in both target species, using probabilistic density functions for input parameters derived from literature. The probabilistic approach allow to identify and visualize the range of uncertainty associated with model results when considering the full range of possible values for the input parameters data (describing their inherent uncertainty and/or variability).

As a further step, it is surely advisable to perform a sensitivity analysis of the Fish model, in order to identify which is the contribution of each parameter to the final model outcomes. This would allow to focus further efforts in the description of the most relevant parameters, through a more detailed collection of suitable and representative input data.

Moreover, even if the results of the preliminary application presented in this thesis are encouraging, it will be beneficial to apply MERLIN-Expo Fish model to other classes of organic chemicals, such as PAHs and PCDD/Fs, to test the performance of the model on a wider datasets.

The study presented in this thesis has been influenced by the availability of monitoring data in biota samples. On one side, the availability of larger datasets including more fish species (with different ecological roles, and thus different diets) and more samples in time would allow to improve the performance testing through the comparison of predicted data with an extended datasets. On the other side, the availability of more biomonitoring data for potential preys (e.g. molluscs, crustaceans, etc.) would allow to substitute modelled prey concentrations with real monitoring data.

Furthermore, it can be appropriate to apply MERLIN-Expo tool considering the full aquatic food chain in order to better understand the transfer of bioaccumulative chemicals between different trophic levels. For this purpose, specific models for Phytoplankton and Aquatic Invertebrates are currently under development within 4FUN project.

In conclusion, it is possible to confirm that the model parameterization and testing application presented and discussed in this thesis has been useful in improving the understanding of the model and support its final implementation in MERLIN-Expo tool. The overall evaluation of model performance can be considered in general as satisfying, taking into account the high uncertainty associated to the measurement and assessment of individual congeners of PCB in environmental matrices and biota.

The case-study application demonstrated the feasibility of historical time-trend reconstruction of bioaccumulation processes in aquatic organisms, which could be beneficial for estimating lifetime human exposure to persistent, bioaccumulative contaminants.

Once the process of validation of individual models of MERLIN-Expo tool will be completed, human intake and PBPK modules could be coupled to environmental and bioaccumulation models to integrate ecological and human health exposure assessment.

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Acknowledgements

I would like to thank some people
for all their effort, motivation, and encouragement in this year of master thesis.

First of all, thanks to Prof. Antonio Marcomini of Ca' Foscari University of Venice,
my thesis supervisor.

My sincere gratitude goes to Elisa,
for her fundamental help and essential suggestions
during this entire year of thesis development.

I would like to thank Artur,
for generously spending his time helping me to better understand the subject of thesis.

Thanks to all the research group of Prof. Marcomini,
for having contributed in many different ways to make this experience unforgettable.

Finally, I would like to thank my parents
for their support and encouragement in all these years of study.