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Integration of ecological and human PBPK exposure models and their application in exposure assessment to POPs in Venice lagoon

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ABSTRACT

Industrial and urban emissions over several decades left a legacy of contamination by persistent organic pollutants in the sediments of Venice lagoon (Italy), which might still represent a hazard for the health of ecosystems and population. The dissertation addresses the problem of exposure to persistent organic contaminants in the Lagoon of Venice throughout: i) the development and implementation of three predictive exposure models to simulate chemical bioaccumulation in aquatic species and food webs (namely, Phytoplankton, Invertebrate and Fish models), ii) performing deterministic and probabilistic integrated ecological and human exposure modelling exercise for the selected chemicals, iii) study of the uncertainty and sensitivity of the exposure modelling outputs. The PhD project was developed within the EU-funded 4FUN project and supported the improvement of the tool MERLIN-Expo developed therein.

In a nutshell, the MERLIN-Expo platform was applied to simulate internal exposure of selected aquatic organisms and human population to PCBs and dioxins, by setting up chain of models consisting of environmental fate models and a human physiologically-based pharmaco-kinetic (PBPK) model to represent the real life exposure scenario of the Lagoon of Venice.

As the part of the PhD project, the Phytoplankton, Invertebrate and Fish models were first developed in Ecolego, a software enabling creation of dynamic models (http://ecolego.facilia.se/ecolego/show/HomePage), and then implemented in MERLIN-Expo library. Then the models were used to create an aquatic food web specific to the lagoon environment and to dynamically simulate bioaccumulation of dioxins and PCBs. Subsequently, such estimated concentrations in selected edible aquatic species were used to estimate daily human intake through the consumption of local seafood. Finally, the the PBPK model was applied to explore the accumulation of the two contaminants 2,3,7,8-TCDD and PCB126 in human tissues for several decades.

Simulated chemical concentrations in biota were then evaluated against monitoring data for four aquatic species, finding an appreciable agreement, with some differences depending on the species and target chemicals. Likewise, estimated chemical concentrations in human blood were compared to human biomonitoring data measured in adult men. Despite several assumptions included in the assessment framework, simulated concentrations resulted close to measured data (the same order of magnitude or one order of difference). Eventually a preliminary ecological and human health risk assessment was performed using the results

from deterministic simulation for the selected chemicals by evaluating the exposure estimates against benchmark values available in literature.

Next, the study of uncertainty and sensitivity of the exposure assessment output was performed in order to support evaluation of large exposure models where a significant number of parameters and complex exposure scenario is involved. Probabilistic analysis was applied to simulated concentrations of PCB 126 and 2,3,7,8-TCDD in biota and human blood obtained from the integrated models. Uncertainty in model output was further apportioned between parameters by applying sensitivity analysis tools. In this part, uncertainty has been extensively addressed in the probabilistic distribution functions (PDFs) to describe the data input and the effect on model results by applying sensitivity analysis techniques (screening Morris method, regression analysis, and variance-based method EFAST). In the exposure scenario developed for the Lagoon of Venice, the concentrations of 2,3,7,8-TCDD and PCB 126 in human blood turned out to be mainly influenced by a combination of parameters (half-lives of the chemicals, body weight variability, lipid fraction, food assimilation efficiency), physiological processes (uptake/elimination rates), environmental exposure concentrations (sediment, water, food) and eating behaviours (amount of food eaten).

LIST OF CONTRIBUTIONS

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EXTENDED ABSTRACTS

A. Radomyski, E. Giubilato, A. Critto, P. Ciffroy, C. Brochot, A. Marcomini*, Modelling ecological and human exposure to Persistent Organic Pollutants in the Venice lagoon through the application of the MERLIN-Expo tool,* 6/2017, ICCA-LRI and JRC 2017 workshop, Como, Italy,

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CHAPTER 1 INTRODUCTION

1.1. MOTIVATION AND OBJECTIVES

The production volume of chemicals (both hazardous and non-hazardous) in the European Union (EU-28) amounted to about 319.5 million tonnes in 2016 of which 200.7 million tonnes is hazardous to health and 117.8 million tonnes hazardous to the environment (Eurostat: http://ec.europa.eu/eurostat/product?code=env_chmhaz&language=en&mode=view). The production of industrial chemicals was largely concentrated in Western Europe: Germany was the largest producer in the EU-28, followed by France, Italy and the United Kingdom.

Historically, human health and environmental exposure assessment methodologies have generally developed independently (Munns et al., 2003). Regulatory agencies often use a chemical-by-chemical approach, focusing on a single medium, a single source, and a single toxic endpoint. Moreover, current exposure assessment is recognised as a weak point in ecological and human health risk assessment due to a lack of integrated approaches for combined stressors, widespread use of over-conservative 'worst-case' scenarios, estimation of only external and not internal exposures, and a lack of uncertainty and sensitivity analysis for the identification of key exposure drivers. Since exposure assessment and hazard characterization are the pillars of risk assessment, integrating Environmental Exposure assessment (EEA) and Human Exposure assessment (HEA) is a major component of an IRA framework. To this purpose there is a need of better exploitation of all currently existing data, experimental approaches and modeling tools created for both EEA and HEA.

Many international and national organizations have expressed a need for an integrated, holistic approach to exposure assessment that addresses real life situations of multichemical, multimedia, multiroute, and multispecies exposures (International Program on Chemical Safety (IPCS) of the World Health Organization (WHO), the European Commission (EC), the Organization for Economic Cooperation and Development (OECD) and the US Environmental Protection Agency (US EPA) (WHO, 2001)). Constructing and applying ecological and human exposure models help in a number of ways, for instance in evaluating experiments design and results under different hypotheses, guiding data collection, predicting exposures, underlying key uncertainties and influential parameters. Models better our understanding of the complex interactions among various human and nonhuman components of ecosystems. Therefore, a need to integrate human and ecological

domains are emerging to support a more harmonised framework for assessing the fate and effects of industrially produced and naturally occurring pollutants.

The project main objective is the integration of multimedia models (MM) simulating the fate of chemicals in environmental media, and of physiologically based pharmacokinetic (PBPK) models simulating the fate of chemicals in human body using MERLIN-Expo in order to determine internal effective chemical concentrations integrating uncertainty, variability and sensitivity analysis to allow complete realistic exposure analysis.

The PhD project focuses on the transitional ecosystem of the Lagoon of Venice, affected by catchment densely populated area, industrial settlings, oil refining plants, wastewaters and waste incineration plants. The pollution sources have been affecting different environmental compartments, through the release of range of environmental contaminants to the lagoon including organic (e.g. PCBs, dioxin-like PCBs, PCDD/Fs, PAHs) and inorganic (e.g. Cd, Pb, As, Cr, Zn, Ni) chemicals (Micheletti et al., 2007, 2008). Persistent organic pollutants tend to accumulate and magnify in aquatic organisms, causing a potential significant long term human dietary exposure (Von Stackelberg et al., 2002). No previous studies assessed ecological and human exposure in an integrated way for long term scenarios.

The specific objectives of the PhD project were:

1. reviewing and selecting bioaccumulation modelling approaches for aquatic organisms;
2. parameterising bioaccumulation and human PBPK models for the Venice lagoon case-study, addressing uncertainty and variability of input parameters and exposure factors;
3. evaluating model outputs by comparing the model outcomes with actual chemical concentrations in animal tissues and human biomonitoring data;
4. analysing variance in exposure modelling outcome through uncertainty and sensitivity analysis.

1.2. OUTLINE OF THE THESIS

CHAPTER 2

Chapter 2 adresses the concept of holistic approaches to risk assessment (RA) for better informing decision making by integrating environmental risk assessment (ERA) and human health risk assessment (HHRA) into Integrated risk assessment (IRA) as a solution to current scientific, societal and policy needs. Then it describes integrating Environmental Exposure assessment (EEA) and Human Exposure assessment (HEA) as a major component of an IRA framework. Finally, the use of exposure modelling in the integrated exposure assessment is outlined with focus on bioaccumulation models.

CHAPTER 3

The objective of the chapter 3 is to present the development of food web models implemented in the already existing MERLIN-Expo tool and to clarify that this development was based on the existing modelling approaches and the features required by MERLIN-Expo platform i.e. possibility to be parameterized for probabilistic assessment, the modular structure.

CHAPTER 4

Chapter 4 describes the case study of the lagoon of Venice and its conceptualisation in the form of exposure scenario. Both general and site-specific input data are described (full details in the APPENDIX) separately for ecological and human modelling parts, followed up by details on deterministic and probabilistic simulation settings. Finally, sensitivity analysis set up is laid out.

CHAPTER 5

Chapter 5 includes the results from ecological and human deterministic and probabilistic exposure simulations. It concludes with three step sensitivity analysis of the input variables on the concentration of PCB126 and 2,3,7,8-TCDD in human blood.

CHAPTER 6

The final chapter 6 highlights the PhD project outcomes, the perspectives for possible improvements and way further for the carried out work.

1.3. FUN PROJECTS AND MERLIN-EXPO TOOL

The FP6 project 2-FUN (Full-chain and Uncertainty approaches for assessing health risks in FUture eNvironmental scenarios) produced a prototype software containing a library of models for exposure assessment, coupling environmental multimedia and pharmacokinetic

models. The main objectives of the 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 standardised solution) was to further improve and standardise the 2-FUN tool in order to guarantee its long-term technical and economic viability. The 2-FUN tool was subject to rigorous standardisation, which include benchmarking, documentation and demonstration. To demonstrate the reliability of modelling estimations and the feasibility of building complex realistic scenarios, three case studies based on actual datasets were performed, one placed in the Venice Lagoon. The improved and standardised 2-FUN software, named MERLIN-Expo, was delivered, together with supporting documentation and training courses. The training events were organised in such a way as to reach out to as many people as possible in different locations across the EU and promote the tool. The aim was also to raise the skill level of regulators and enable a community of users to develop.

All models within the tool are implemented on the same platform, that is Ecolego (http://ecolego.facilia.se/ecolego/show/HomePage), to facilitate integrated full-chain assessments for combined exposures. During the project, a standard framework for exposure model documentation was developed in conjunction with the European Committee for Standardisation (CEN) and was for the description of all MERLIN-Expo models, with the aim of ensuring the rigorous formulation of exposure models, comparability between the different exposure models and transparency and ease of understanding for the users of the tool.

1.3.1. MERLIN-Expo

The MERLIN-Expo (Modelling Exposure to chemicals for Risk assessment: a comprehensive Library of multimedia and PBPK models for Integration, Prediction, uNcertainty and Sensitivity analysis) tool was developed within the EU-funded 4FUN project (2012-2015) and aims to provide decision-makers with state of the art tools to analyse the current and future trends in environmental conditions and pressures that may lead to health problems. The software structure was primarly developed by Facilia AB (Sweden) while the models for soil, freshwater, atmosphere, tubers, fruit tree, leaf and root crops by Electricité de France S.A. – France (EDF), the human PBPK by the Institut National de l'Environnement Industriel et des Risques – France (INERIS), the mammals (ruminants) model by EDF and Università Ca' Foscari Venezia, and finally, bioaccumulation models for aquatic organisms (phytoplankton, invertebrates and fishes) developed whithin the framework of this PhD

project by Università Ca' Foscari Venezia in collaboration with EDF.

MERLIN-Expo is a software structure aimed at performing exposure assessment for both organic and inorganic chemicals for environment, biota and humans. It includes a library of environmental and human exposure models which can be flexibly combined to recreate several exposure scenarios, including different exposure pathways, to explore the evolution of ecological and human exposure (up to internal exposure in target organs/tissues) to chemicals over time. MERLIN-Expo allows the users to perform both deterministic and probabilistic dynamic simulations of exposure estimates and incorporate a set of functionalities for applying different methods of sensitivity analysis.

The software is an advanced tool for higher tiers in exposure assessment; it includes dynamic modelling, a wide range of chemicals, uncertainty and sensitivity analysis, probabilistic simulations and a combination of health exposure assessment and environmental exposure assessment. Equipped with a flexible modular format, pharmacokinetic considerations, and uncertainty and sensitivity analysis functionality, MERLIN-Expo can enable robust, regulatory-relevant environmental and exposure assessments with ease and transparency. The main challenges in exposure modelling that MERLIN-Expo tackles are: (i) the integration of multimedia models (MM) simulating the fate of chemicals in environmental media, and of physiologically based pharmacokinetic (PBPK) models simulating the fate of chemicals in human body. MERLIN-Expo thus allows the users to determine internal effective chemical concentrations; (ii) the incorporation of a set of functionalities for uncertainty/sensitivity analysis, from screening to variance-based approaches. The availability of such tools for uncertainty and sensitivity analysis aimed to facilitate the incorporation of such issues in future decision making; (iii) the integration of human and wildlife biota targets with common fate modelling in the environment. MERLIN-Expo is composed of a library of fate models dedicated to non biological receptor media (surface waters, soils, outdoor air), biological media of concern for humans (plants, mammals, milk, edible aquatic organisms), as well as wildlife biota and humans. These models can be linked together to create flexible scenarios relevant for both human and wildlife biota exposure. Standardized documentation for each model and training material were prepared to support an accurate use of the tool by end-users.

One of the objectives of the 4FUN project was to provide standard documentation for each of the models included in the library, guaranteeing their transparency and the long-term technical viability of the tool. Contrary to analytical 'simple' models, the multimedia and PBPK models that are included in the MERLIN-Expo library involve a large set of entities,

i.e. a large set of compartments, state variables, forcing variables, parameters, equations, variables, and several regulatory outputs. They are then difficult to communicate in a comprehensive, unambiguous and accessible way. This situation could be shared with other complex models, but despite this background, it can be observed that no standard documentation protocol has been followed so far for describing large multimedia and/or PBPK models. In the 4FUN project, transparency was identified as a key criterion for evaluating models included in the MERLIN-Expo tool. In this context, an action was undertaken in the frame of the project in coordination with CEN (European Committee for Standardisation) to propose a standard documentation protocol (SDP) for exposure models. The SDP can be defined as a generic format and a standard structure by which all MM models could be documented.

CHAPTER 2 INTEGRATION EXPOSURE ASSESSMENT

2.1. INTEGRATED RISK ASSESSMENT

The National Research Council (NRC) defined risk assessment as "the use of the factual base to define the health effects of exposure of individuals or populations to hazardous materials and situations" (NRC 1983). It describes the assessment of risks from chemicals to the environment and human health as a four steps common paradigm consisting of: hazard identification, hazard characterization, exposure assessment and risk characterization (Figure 1).

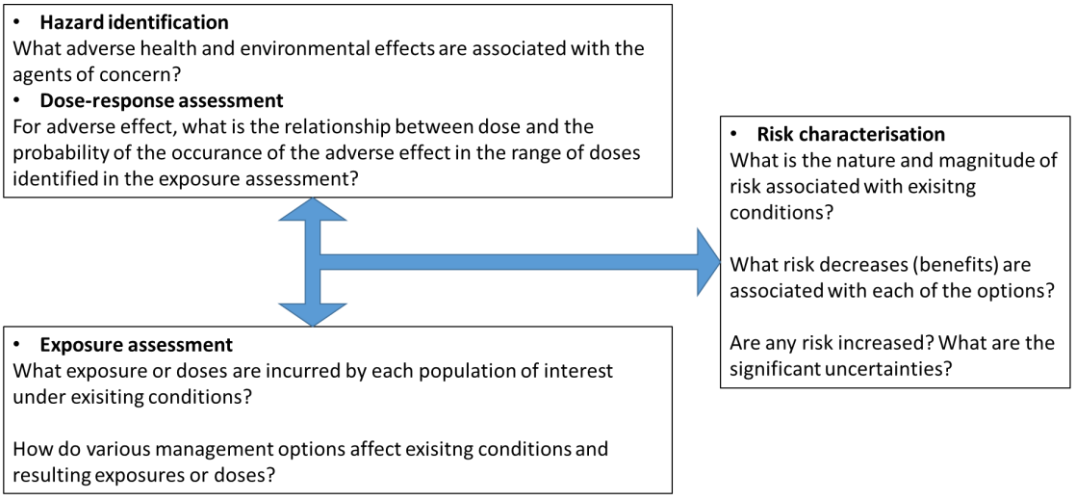


Figure 1 The risk-assessment process as defined by its four elements: hazard identification, dose–response assessment, exposure assessment, and risk characterization. Source: Adapted from NRC 2009.

Human health and ecological risk assessment practice developed independently mainly from the health risk assessment framework developed by National Research Council (1983),

and ecological risk assessment framework developed by US EPA (1992). These frameworks differ as to how the assessment process is carried out, various type of data, models and assumptions.

Narrow focus of the assessment of the risks to contaminants may lead to inadequate management decision. It has become apparent that the risk assessment should be broadened by involving more disciplines (Munns et al., 2003). One way to achieve it is to integrate human health and ecological risk assessments (WHO, 2001; Suter II et al., 2005). Integrated Risk Assessment (IRA) has been defined in a recent white paper (Wilks et al., 2015) as "the mutual exploitation of Environmental Risk Assessment for Human Health Risk Assessment and vice versa in order to coherently and more efficiently characterize an overall risk to humans and the environment for better informing the risk analysis process". A central feature to IRA is the connection of independent sources of information, such as toxicological and ecotoxicological data, that are usually kept separate, to enable a more comprehensive, efficient and informative RA (Bridges, 2003; Suter et al., 2005; Andersen et al., 2016). Despite the fact that HHRA and ERA target different receptors, human and nonhuman respectively, both frameworks can benefit from sharing information between them. The rationale behind integrating human health and ecological risk assessments is indeed to improve exchange of information between the two risk assessment domains in order to provide more complete inputs to decision making process.

Some of the benefits of integrated risk assessment (Bridges, 2003):

1. involvement of both the decision makers and stakeholders from both domains,
2. information sharing in order to define common sources, stressors, system at risk and effects on the receptor,
3. common analysis fostering the development of models (multimedia fate models, toxicokinetic models) for all receptors and pooling the knowledge necessary to feed such models,
4. providing holistic integrated risk characterisation, including expression of variability and uncertainty for all endpoints.

Indeed, with increased recognition of the need to protect both humans and the environment more effectively, an integrated approach to risk assessment that holistically addresses situations of multichemical, multimedia, multiroute, and multispecies exposures is needed. In fact the risk assessment has been evolving towards holistic assessment mainly due to available tools allowing to increase the speed at which information from various fields is obtained and thus expanding the amount of data available (Committee on Incorporating 21st

Century Science into Risk-Based Evaluations et al., 2017).

The importance of IRA was recognised by the European Commission and highlighted as a key element of future action in its European Environment and Health Strategy (EC, 2003), fostering the development of the IRA concept as new EU research projects under the 6th/7th Framework Programmes (FP6/7) (e.g. HEIMTSA (http://cordis.europa.eu/project/rcn/81281_en.html), INTARESE (http://www.intarese.org/), NoMiracle (http://nomiracle.jrc.ec.europa.eu/), OSIRIS (http://www.ufz.de/osiris/), 2-FUN (http://www.2-fun.org/)), ENVIRISK http://envirisk.nilu.no/, HEROIC http://www.heroic-fp7.eu/.

Within the EU chemicals are risk assessed within different regulatory frameworks depending on their intended use (Ågerstrand and Beronius, 2016). For different compound, even though similar risks to human and environmental health can be expected, the risk assessment process depends on the considered policy. Chemical risk assessment is included in several EU regulatory frameworks: Regulation (EC) No. 1907/2006 (REACH) (Industrial chemicals), Regulation (EC) No. 1107/2009 (Plant protection products), Regulation (EU) No. 528/2012 (Biocides), Regulation (EC) No. 1223/2009 (Cosmetics), Directive 2001/83/EC (Human pharmaceuticals in the environment), Directive 2001/82/EC (Veterinary pharmaceuticals in the environment), Regulation (EC) No. 178/2002 (Contaminants in food), Directive 2000/60/EC (Water framework directive), Regulation 1272/2008/EC (Classification, labelling and packaging).

The fact that changes in ecosystem condition can influence the health and well-being of people both directly and indirectly is recognised in the ecosystem services (ES) concept (Maes et al., 2016). ES has been coined as a “common currency” potentially enabling integration of human health and ecological risk assessment into environmental decision making process (Munns et al., 2016, 2017; Selck et al., 2017). The concept was recently demonstrated by assessing risk for four ecosystem services (human health, water quality, recreation, and the recreational fishery) using a multiple stressor–multiple endpoint approach with Bayesian networks relative risk model where ecological services were incorporated as ecological and human health risk assessment endpoints (Harris et al., 2017; Landis et al., 2017). Another way for informing decision making using both ecosystem and human health data can be through integrating them as lines of evidence into a weight of evidence approach (Caeiro et al., 2017).

Since exposure assessment and hazard characterization are the pillars of risk assessment, integrating Environmental Exposure assessment (EEA) (i.e. exposure of biota) and Human

Exposure assessment (HEA) can be a major component to be included in the IRA framework (Ciffroy et al., 2015). That is, in both HHRA and ERA frameworks the exposure assessment stage can be seen as a common denominator in a sense that both human and nonhuman targets are exposed to the same chemicals released to the same media where they undergo the same transport and transformation processes.

There are other types of integration that could be used to support even further environmental decision making such as integration of exposure and effects, multiple endpoints, multiple receptors, multiple spatial and temporal scales, a product's life cycle, management alternatives, and socioeconomics with risk assessment (Suter II et al., 2003a). However, to avoid "paralysis by analysis", caused by unnecessary integration of too many aspects into the risk assessment process, the extent of integration should be decided during the problem formulation step (Suter II et al., 2003b).

So far however, EEA and HEA have generally used and developed their own models parallelly, lacking appropriate linkage between them.

2.2. INTEGRATED HUMAN AND ECOLOGICAL EXPOSURE ASSESSMENT

The exposure assessment is the most important phase in influencing the risk characterization since exposures can be controlled, whereas hazard identification and dose-response assessments describe inherent features of the agent (US EPA, 2016). In general, exposure assessment involves the process of estimating or measuring the magnitude, frequency and duration of exposure to chemicals, along with the number and characteristics of the population exposed. An application of this field of science, has been crucial in helping to forecast, prevent, and mitigate exposure events that lead to adverse human or ecological health, to identify and protect highly exposed populations, and to assess and manage human health and ecosystem risks. The field of exposure science evolves as a result of advances in the tools and approaches such as personal monitors that will enable the measurement of individuals' exposure over time or remote sensing and GIS-based spatial methods (Su et al., 2015, Vrijheid et al., 2014). The developments in infrastructure supporting exposure-data acquisition, collection, organization, and access lead to the improvement of the accuracy, completeness, efficiency, and transparency of exposure assessment and modelling (Glasgow et al., 2016). This also facilitated broadening the reach of exposure science from a traditional focus on discrete exposures to an integrated approach which considers exposures from source to dose, on multiple levels of integration (including time, space, and biological scale), multiple stressors, and scaled from molecular systems to

individuals, populations, and ecosystems (NRC 2012) (Figure 2).

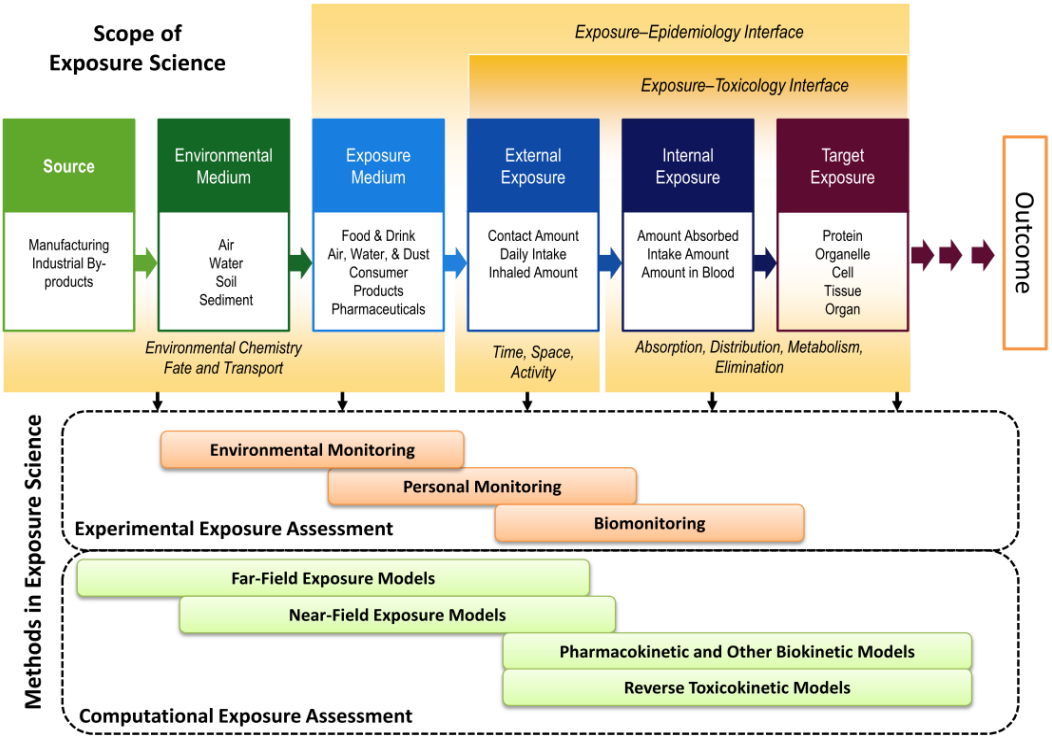


Figure 2 Conceptual overview of the scope of and common methods for exposure science.

The ultimate goal of integrating exposure assessments is to have a reliable, validated, integrated source-to-receptor exposure modelling tool capable of generating realistic predictions of exposure to chemicals, which enables the determination of the proportion of contaminant exposures over different routes and sources (Vrijheid, 2014). The challenging concept of the exposome, encompassing all environmental exposures from the conception onwards, was defined in 2005 (Wild, 2005) in order to draw attention to the need for the developments in exposure assessment. On-going exposome projects (http://www.projecthelix.eu/, www.exposomicsproject.eu/) are trying to overcome technical and statistical challenges the concept is currently facing such as untangling from a wide range of exposures those that truly affect health outcomes (Siroux et al., 2016).

Exposure assessment is generally considered as a weak point in risk assessment due to a lack of data and the inherent natural variability in exposure levels, leading to uncertainties in the estimates (Bundesinstitut für Risikobewertung, 2015). At the same time exposure characterisation is critical in allocating limited resources while managing risks to chemicals (Bogen et al., 2009). From a regulatory point of view exposure estimation in general must face new challenges because of high uncertainties throughout the health risk assessment chain (Glorennec et al., 2005). Such uncertainties are recognized but rarely explicitly

quantified and integrated over the full impact assessment pathway (Bennett et al., 1999).

There is a need to distinguish between uncertainty due to lack of knowledge and uncertainty due to variability (Hoffman and Hammonds, 1994). The uncertainty in parameters can result from measurement uncertainty, insufficient data, or incomplete knowledge of the processes and mechanisms that give rise to these parameters. The variability in parameters can result from human behavioural and physiological differences and can vary from individual to individual at the same location. The data used in exposure assessment (e.g. contamination levels in environmental media, population distribution) can exhibit significant temporal and spatial variability at various scales depending on the exposure target, and may differ between EEA and HEA.

The committee of National Academies of Science, Engineering and Medicine emphasizes that insufficient attention has been given to analysis, interpretation, and integration of various data streams from exposure science, toxicology, and epidemiology (Committee on Incorporating 21st Century Science into Risk-Based Evaluations et al., 2017). Integrating exposure information such as data on environmental media, biomonitoring samples, conventional samples, and emerging matrices constitute a scientific, engineering, and big-data challenge. This in fact is a key step in developing coherent exposure science, the challenge here however is to evaluate data homogeneity, and to determine confidence in an exposure assessment.

Recognition of common challenges, limitations and shortcomings in terms of data quality, availability, accessibility, sharing and harmonization in the IEA framework point at areas where possible improvements can be made: (i) better exploitation, collection and sharing of existing exposure data; (ii) creation of integrated databases; (iii) harmonization and sharing of sampling design; (iv) better use of metrics. In a broader perspective this is also crucial in developing coherent exposure science (Exposure Science in the 21st Century, 2012). Actually, data allowing a better exploitation of exposure models sometimes exist, but not always in a format allowing them to be readily collected in a homogenous form. A way to better integrate monitoring data that were initially generated independently for EEA and HEA purposes could be to merge such data, even if sporadically available, with modeling results and/or expert judgment through e.g. Bayesian techniques (McNally et al., 2014). Exposure modelling results, eventually in conjunction with other existing knowledge gained e.g. from expert judgment, can form prior estimates of exposure (Quick et al., 2017).

2.3. INTEGRATED EXPOSURE MODELLING

In assessing the risks to chemicals tiered approaches are common. In order to screen out risks, first, low tier assessments using cautious assumptions and conservative worst-case exposure scenarios supported by simple deterministic models are used, whereas higher tiers involving typically complex scenarios and advanced models supported by the analysis of uncertainties are applied when potential risks cannot be ruled out by the low tier assessment.

There are many sources and many routes by which human and biota are exposed to stressors. Exposure models integrating multi-source and multi-routes of exposure seem promising in characterisation of the full impact of chemicals on the receptor (Wania and Mackay, 1999). Specifically, such models show the capacity of: 1) integrating of exposure pathways by translating exposure into internal doses, 2) include tools and data for probabilistic assessments, 3) implementing appropriate mechanisms to underpin exposure routes and pathways, and 4) including tools for reverse dosimetry modelling, and hence allowing the identification of main exposure sources. Exposure data on many agents are often not available, but recent advances in computational tools for exposure science are expected to play a crucial role in most aspects of exposure estimation for risk assessments, not just high-throughput applications.

The exposure to chemicals through multiple pathways is typically estimated by the so-called ‘multimedia models’ (MM models) that calculate the distribution of chemicals over environmental media (MacLeod et al., 2010, Mackay and Arnot, 2011). Among the targeted environmental media which could be of interest for integrated exposure are for instance inhaled air, drinking water, and foodstuffs (Cousins et al., 2002, Bennett et al., 2002). When combined with data describing human behavioural exposure factors (diet preferences, time activity pattern, etc.) MM models would provide an estimation of the daily dose inhaled or ingested by the population of interest (Pennington et al., 2005). Fate models used in EEA and HEA for predicting the distribution of chemicals among physical and biological media are essentially based on properties of environmental compartments (soil, plants, etc.) and on common properties of chemicals. Species that are assessed in the frame of EEA can also form part of the human food chain (e.g. fish).

In conclusion, to assess exposure of humans and biota with similar pathways of exposure a common modelling framework integrating environmental fate of chemicals with both human and environmental targets would be useful (Arnot et al., 2010a).

2.3.1. Exposure Models

There has been growing amount of resources and tools dedicated to exposure assessment (https://www.epa.gov/expobox, OECD 2012). In this paragraph only selected exposure models are shortly described highlighting their main features: INTEGRA, MERLIN-Expo, EUSES, USEtox, CoZMoMAN (ACC-HUMAN), RAIDAR and SHEDS-multimedia. EUSES, RAIDAR and USEtox, which is used in the domain of Life Cycle Assessment, are examples of the models used for screening assessments rather than for higher realistic assessment tiers. In general ACC-HUMAN, SHEDS-multimedia, MERLIN-Expo and INTEGRA can be seen as high tier exposure models, acknowledging the scientific complexity of the nature, and routes of exposures. All four models have clear advantages in terms of their ability to address exposures to chemicals from multiple sources and via multiple routes compared to the lower tier tools. The CoZMoMAN is a dynamic model which combines the multimedia fate and transport model CoZMo-POP2 with the bioaccumulation model ACC-HUMAN. A detailed description of the physical model is provided in Wania et al., (2006). The CoZMoMAN model links these two models, using the environmental concentrations from CoZMo-POP2 to drive the bioaccumulation model ACC-HUMAN (Breivik et al., 2010). INTEGRA is a computational platform that integrates multimedia environmental and micro-environmental fate, exposure and internal dose within a dynamic framework in time (Sarigiannis et al., 2014). INTEGRA was initially developed to address consumer exposure (Sarigiannis et al., 2016). This is reflected in the fact that INTEGRA provides the option to set up detailed consumer-oriented exposure scenarios, and accounts for dermal, oral and inhalation pathways. The MERLIN-Expo is an exposure assessment software tool that was developed over the course of two successive EU funded projects, 2FUN (FP6) and 4FUN (FP7) (Van Holderbeke et al., 2016). The software allows the users to carry out lifetime exposure assessments at the individual or population level, integrating exposure through multiple pathways. Benchmarking MERLIN-Expo against EUSES revealed that both models are comparable in their exposure predictions (Suciu et al., 2016). SHEDS-multimedia has been developed within the US EPA Policy, Regulation and Risk Assessment context, to support EPA in performing cumulative and aggregate assessments for multiple chemicals (Isaacs et al., 2014). The model gives equal importance and tiered level to dietary exposure as well as to residential exposure, i.e. non dietary exposure, including exposure via hand-mouth contact. The model is not intended to be used as a full chain source-to-receptor model since the model does not include an environmental fate model. Moreover, the model is

based on records on food monitoring data in the US only.

Models such as MERLIN-Expo and INTEGRA seem to be very flexible when compared to other exposure models, however this can be also a drawback, especially because setting up exposure scenario and parameter values may be time and effort consuming. Another downside to complex exposure models is their validation which appears to be very challenging and would require gathering of a huge number of environmental and person-oriented records for dietary exposure, human activities, and monitoring levels in environmental media. There are however ongoing efforts aimed at collecting such chemical and non-chemical exposure factors on human exposure such as IPCHEM (https://ipchem.jrc.ec.europa.eu/RDSIdiscovery/ipchem/index.html#), FACET (Oldring et al., 2009, 2014) or ExpoFacts (http://expofacts.jrc.ec.europa.eu/).

Among other models often used to assess human and environmental exposure are for instance CalTox (www.dtsc.ca.gov/AssessingRisk/caltox.cfm), and AQUATOX (water.epa.gov/scitech/datait/models/aquatox/); some models are dedicated to a given class of chemicals e.g. PEARL (www.pearl.pesticidemodels.eu/) and PRZM (http://www.epa.gov/oppefed1/models/water/#przm); another ones are dedicated to a given environmental media e.g. CLEA (www.gov.uk/government/publications/updated-technical-background-to-the-clea-model) and CSOIL (www.rivm.nl/bibliotheek/rapporten/711701054.html).

2.3.2. Bioaccumulation Models

The bioaccumulation of chemicals can be generally described as a phenomenon leading to increase of the concentration of chemicals in a biotic compartment with respect to the surrounding medium (Arnot and Quinn, 2015; US EPA, 2012). Accumulation processes in living organisms can be classified on the basis of the uptake of chemicals: i) uptake from the environment media together with dietary uptake is described as bioaccumulation, ii) bioconcentration accounts for accumulated contaminant resulting solely from uptake through respiratory surfaces (body surface, tracheal tubes, gills, lungs), and iii) biomagnification is distinguished from other modes because it includes transfer and accumulation of contaminants along trophic levels in a food web (Arnot and Gobas, 2006; Van Leuwen and Vermeire, 2007).

The accumulation of a xenobiotics in biota has to be carefully evaluated in ecological risk assessment, because the knowledge of average concentrations in the whole organism or in

some specific tissues can help in estimating the likelihood of adverse effects resulting from partitioning between contacting media and food web transfers, or in assessing the influence of large masses of biota on overall chemicals fluxes in the environment (Schwarzenbach et al., 2003; Müller and Nendza, 2007). The internal concentration level reached in aquatic or terrestrial organisms over long term exposures may cause adverse effect, either unspecific (e.g., narcosis) or specific (e.g., neurotoxic effects), after reaching a critical threshold, referred to as critical body burden. Since humans consume food originating from both the aquatic and terrestrial environment and represent the top consumers of food webs, bioaccumulation/biomagnification processes are relevant also for human health risk assessment (McLachlan et al., 2011) and measurement or modelling of bioaccumulative substances in edible plants and animals have to be performed to complete the assessment of chemical exposure through other routes (e.g., inhalation, dermal contact, soil/dust ingestion).

The awareness that bioaccumulation/biomagnification can be phenomena lasting over decades and inducing effects (often irreversible) long after the environmental release of chemicals encouraged to include bioaccumulation assessment in many national and international legislative frameworks. Stockholm Convention (2004; Annex D and E) requires information on bioaccumulation as one of the screening criteria used in identifying persistent organic pollutants and in evaluating chemical risk profile. Bioaccumulation is also listed by Basel Convention among hazardous characteristics of toxic wastes or substances that are subject to transboundary movement, as possible threat to the environment (Basel Convention, Annex III). In the European Union, the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation (Regulation (EC) No 1907/2006) asks for the identification of chemicals with hazardous properties of concern, in particular substances which are persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) have to be included in REACH Annex XIV (Authorisation list), meaning that after the “sunset date” they are banned for all uses except those specifically authorised by the European Commission (EC). REACH indicates that information on bioaccumulation in aquatic species is required for substances manufactured and imported in quantities of 100 tons per year or more (ECHA, 2014a). The EU Regulation No 528/2012 on Biocidal Products states that information on bioconcentration is relevant to the assessment of the ecotoxicological profile of the biocidal active substance and that evaluation of aquatic bioconcentration should include an estimate of the bioconcentration factor (ECHA, 2014b). The European Food Safety Authority, following the EC Regulation

No 1107/2009 on authorisation of Plant Protection Products, recommends in its Guidance Document on Aquatic Ecotoxicology to further elaborate a risk assessment methodology to better address biomagnification in the future, and proposes to consider food-chain modelling as an option for higher tier assessment (EFSA PPR Panel, 2013). The importance of monitoring bioaccumulation in aquatic organisms is stressed by the European Commission also in the EU Directive 2013/39/EU. It states that for very hydrophobic substances which accumulate in biota and are hardly detectable in water, Environmental Quality Standards (EQS) should be set for biota (EC, 2011). For dioxins and dioxins-like compounds, EQS values refer to the concentration in fish, crustaceans, and molluscs (Schäfer et al., 2015).

Bioaccumulation results from net competing uptake and elimination processes, or, more precisely, is the result of absorption, distribution, metabolism and excretion (ADME) of a substance in an organism. The degree to which bioaccumulation occurs can be expressed by different metrics (Mackay et al., 2013), which may also have a regulatory relevance and can be used for priority setting in chemicals management. The Bioaccumulation Factor (BAF) can be expressed as the steady-state (equilibrium) ratio of the substance concentration in an organism to the concentration in the surrounding medium. For sediment-dwelling organisms, the BAF is often expressed as the ratio of the concentrations in the organism and the sediment, in which case the term is referred to as Biota Sediment Accumulation Factor (BSAF). In absence of dietary exposure, the Bioconcentration Factor (BCF) can be estimated, while the Biomagnification Factor (BMF) is the steady state ratio of chemical concentration between an organism and its food.

Several approaches exist to estimate bioaccumulation metrics. Bioaccumulation data can be obtained from laboratory tests. A number of standardised test guidelines exist for estimating bioconcentration potential of organic compounds using laboratory experiments, such as Bioaccumulation in Fish - Aqueous and Dietary Exposure (TG 305; OECD, 2012), Bioaccumulation in Sediment-dwelling Benthic Oligochaetes (TG 305; OECD, 2008), and Bioaccumulation in Terrestrial Oligochaetes (TG 317; OECD, 2010).

BAF are rarely estimated from laboratory tests, more commonly field data are used for this purpose. The advantage of these measurements is that they include environmentally relevant processes, however field measurements and thus bioaccumulation data are characterized by high variability, which provide challenges to the collection, interpretation and regulatory use of field bioaccumulation data (Nichols et al., 2015).

Bioaccumulation metrics can be also predicted by Quantitative Structure-Activity Relationship (QSAR) models. The most common QSAR models are based on the relation

between BCF and chemicals hydrophobicity (K_{ow}). The relationship can be explained by the analogy between n-octanol - water partitioning to that of tissue lipids - water partitioning. Common BCF/K_{ow} QSARs for non-polar, hydrophobic organic chemicals include models by Veith et al., (1979), Mackay and Paterson (1982), Meylan et al., (1999), and Dimitrov et al., (2002). Linear correlation (Mackay and Paterson, 1982; Veith et al., 1979) is a good approximation of the BCF for non-ionic compounds, however this relation fails with more hydrophobic chemicals, overestimating BCF values due to reduced bioavailability of highly hydrophobic compounds, slow membrane transfer of large molecules, and processes affecting bioaccumulation such as growth of the organism, or metabolism (Schüürmann et al., 2007). Higher degree polynomial QSAR models (Dimitrov et al., 2002; Meylan et al., 1999; Bintein, 1993; Connell and Hawker, 1988) show better performance than linear models in predicting BCF of highly hydrophobic chemicals.

Furthermore, chemical bioaccumulation process can be simulated by mechanistic mass balance models for specific organisms, based on one or more body compartments (Korsman et al., 2015; Hauck et al., 2011; Hendriks et al., 2001; Gobas et al., 1993). In these models, different uptake processes (respiration, dietary uptake) as well as elimination processes (excretion, egestion, metabolism, growth dilution, reproductive losses) can be considered and described, each one characterized by a specific kinetic rate constant (Hendriks, 1995; Hendriks et al., 2001). Information on the target chemical and the organism, such as chemical partition coefficients or respiration and feeding rates and absorption efficiencies are needed by such kind of models (Mackay and Fraser, 2000).

Mass-balance models for individual organisms (e.g., fish), can be incorporated into larger descriptions of food webs, to simulate chemical bioaccumulation resulting both from exposure to environmental media (air, water, soil) and diet. Several food web bioaccumulation models are available, both for aquatic (e.g., Arnot and Gobas, 2004; Hendriks et al., 2001; Campfens and Mackay, 1997; Barber, 1991) and terrestrial food webs (e.g., Kelly and Gobas, 2003; Ardestani et al., 2014). The great advantage of these models is that food webs of any dimension can be described, with as many food sources as needed, and concentrations in all species can be calculated simultaneously.

There has been substantial increase in the awareness of key parameters that influence all of the current metrics used to assess chemical bioaccumulation. Metabolic biotransformation rates of organic chemical have been identified as the most important source of uncertainty in modelling bioaccumulation (Papa et al., 2014, Webster and Ellis, 2012). Complementing human exposure assessment to chemicals through various

pathways with food web bioaccumulation process such as bioconcentration, biomagnification, and biotransformation will allow better risk management decisions. Some commonly used models, such as EUSES, do not include mechanistic knowledge on uptake and elimination processes in biota, therefore they cannot provide substance and organism specific human exposure estimates (Undeman and McLachlan, 2011). Particularly, the food web biotransformation is shown to play an important role in human exposure estimation, and neglecting this process in multimedia model calculations is expected to result in substantial errors (Arnot et al., 2010).

There is a strong drive towards the development of methods that could reduce and/or replace use of animals for testing (https://eurl-ecvam.jrc.ec.europa.eu/). Physiologically based pharmacokinetic (PBPK) models are dedicated tools that satisfy this need because they describe the fate of chemicals (and mixtures of chemicals) in the body of an organism and can predict their levels in the internal tissues. They consist of a series of mathematical equations with parameters based on the specific physiology of animal.

Two mechanistic models were considered for application in the PhD project: Optimal Modeling for EcotoxicoloGical Applications (OMEGA) proposed by Hendriks and colleagues (Hendriks et al., 2001; Hendriks and Heikens, 2001) and Aquaweb by Arnot&Gobas 2004 (Arnot et al., 2004, Gobas et al., 1993, 1988, 1986). The accumulation kinetics in the Aquaweb model were compared with those proposed in OMEGA. Although both approaches can represent exchange processes of contaminants between target organisms and its surrounding environment (i.e. overlying water, other biota systems representing its food) dynamically (Lopes et al., 2012, Infantino et al., 2013, Viaene et al., 2014, Ng et al., 2009), due to better availability of parameter values and their specific probability density functions (PDFs) (Hauck et al., 2011, de Leander et al., 2009, 2010), the selected model for implementation in the project is OMEGA. The model proposed by Arnot&Gobas 2004 requires numerous organism specific parameters to describe accumulation rate constants making it hard to provide these parameters with right PDFs (Ciavatta et al., 2009), which are required for uncertainty analysis. On the other hand, the allometric scaling applied by Hendriks describes uptake and elimination kinetics of different species groups and trophic levels providing extensive information on physiological, and ecological parameters (Hendriks 1995, 1999, 2007). Moreover, OMEGA model and its derivatives have been recently used in a number of studies dealing with dynamic simulations and uncertainty analysis (Taffi et al., 2014, de Hoop et al., 2013, Foekema et al., 2012, Hauck et al., 2011). OMEGA features characteristics of the biodynamic approach, it describes accumulation

rates of contaminants, it is mechanistically based, and reflects variability of organisms and contaminants by considering both biological and physico-chemical specific parameters. A biokinetic approach is used rather than an equilibrium-based approach to simulate the accumulation of both organic and inorganic contaminants by aquatic animals (Mackay and Fraser 2000). Dynamic modelling has the capacity to predict temporal patterns of bioaccumulation by taking into account biological and ecological process, providing an alternative to bioaccumulation models based on common assumption that organisms would achieve equilibrium state after rates of uptake and elimination of organic contaminant reach balance whithin the organism's lifespan (McLeod et al., 2016). Apart from biological and ecological factors which affect bioaccumulation of chemicals in time also physico-chemical paramters were noted to prevent achieving equilibrium as in the case of highly hydrophobic chemicals which do not follow the steady state assumption (Arnot et al., 2006; Jonker and van der Heijden, 2007). The explanation is that the passive diffusion of organic contaminants through biological membranes slows down with increasing hydrophobicity and that the exchange rates between organism and environment show seasonal and temporal variability. Overall, using rates to express accumulation processes allows models to be flexible and predictive under changing conditions (Wallace and Blersch 2015). Additional strengths of the biodynamic model are the aspect of taking into account all uptake routes and thus capturing the biological processes that bioaccumulation phenomenon depends on. In case of metals, however, bioaccumulation is highly variable due to differences among species, the environmental chemistry or bioavailability of metals, and the complexities of the cycling of metals in aquatic ecosystems, therefore modelled results should be treated with caution (Luoma et al., 2005, Owsianiak et al., 2014, Ardestani et al., 2014, Groenenberg et al., 2014). While processes involved in bioaccumualtion were assumed to be similar for fish and invertebrate species, for phytoplankton a simpler assumption was made to describe the accumulation of contaminant (Hendriks et al., 2001). Thus, only the uptake from water is considered since phytoplankton consist of autotrophic species, and dietary uptake can be disregarded (Frouin et al., 2013, Swackhamer and Skoglund 1993).

CHAPTER 3 MODEL DEVELOPMENT AND IMPLEMENTATION

3.1. ECOLEGO

Within the present PhD project, the aquatic food web models (i.e. Phytoplankton, Invertebrate, Fish), drawing from previously existing modelling approaches were coded in Ecolego. A detailed model documentation is available on MERLIN-Expo website (http://merlin-expo.eu/learn/documentation/model-documentation/).

Original models were first introduced in Ecolego (MATLAB toolbox for modelling dynamic systems) before transferring to MERLIN-Expo (Avilaa et al., 2003). Implementing models in Ecolego was done by adopting them to project's assumptions such as probabilistic parameterisation of modelling approach for uncertainty and sensitivity analysis following CEN (European Committee for Standardisation) standard documentation protocol (SDP) for exposure models. The SDP can be defined as a generic format and a standard structure by which all multimedia models can be documented (ftp://ftp.cenorm.be/CWA/CEN/MERLIN_EXPO/CWA_to_public_commenting.pdf).

The initial idea of Ecolego was to facilitate the creation of large and complex models and to be able to solve difficult numerical problems. With the purpose to make complicated models with many interconnections easier to overview, the models in Ecolego are represented with the help of interaction matrices instead of the traditional flow diagrams. Combined with hierarchical containers (sub-systems), the interaction matrix greatly facilitates construction and documentation of large and complex models. Objects can be assigned comments, images, units, and hyper links to other documents or Ecolego objects. Ecolego can also create reports that contain everything from interaction matrices, to parameter values, equations, decay chains, plots and tables. In order to increase the flexibility for the user, Ecolego has no restrictions on the order of creation – for instance, a parameter can be used in equations before it is defined. A real-time validation engine reports problems to the user, such as not-yet-defined objects, objects lacking values or having invalid equations. The typical Ecolego model is a compartmental model which requires a solver of differential equations. There is a wide array of numerical solvers to choose from. Some are optimized for stiff and numerically difficult models, others for trivial models. With an extensive list of probability density functions, together with Monte Carlo and Latin hypercube sampling and parameter correlation settings, Ecolego holds all the required tools to perform advanced probabilistic analysis.

3.2. GENERAL DESCRIPTION OF BIOACCUMULATION PROCESSES IN AQUATIC FOOD WEBS

The assessment of bioaccumulation processes plays a significant role in the evaluation of chemical risks. The awareness of long lasting and often irreversible effects of bioaccumulative chemicals on ecological and human targets encouraged the inclusion of bioaccumulation assessment in many national and international legislative frameworks. At the same time, various experimental and modelling approaches have been developed to estimate bioaccumulation metrics such as the bioaccumulation factor (BAF) or the biomagnification factor (BMF).

The main objective of this pragraph is to describe the development of bioaccumulation models for organic and inorganic contaminants in aquatic environment implemented in MERLIN-Expo, taking into account recent progresses in description of the bioaccumulation of contaminants along food webs including phytoplankton, invertebrate and fish species, inlcuding the main processes governing bioaccumulation phenomena in selected groups of aquatic organisms.

A prerequisite for developing a model describing accumulation of chemicals in an aquatic food web is a mechanistic understanding of bioaccumulation phenomena of organics and metals. The model is thus based on the description of main exchange processes between water and organism compartment, i.e. uptake via respiratory route, uptake via dietary route, elimination via respiratory route (excretion), elimination via gastro intestinal track (egestion), metabolism, and growth (Lim et al., 2011; van Leeuwen and Vermeire, 2007; MacLeod et al., 2010). The ‘Fish’ and ‘Invertebrate’ models should then include two compartments that correspond to two input/output pathways for chemical accumulation in organism, i.e. the respiratory system and the gastro intestinal tract (GIT) system. The media considered are represented in Figure 3.

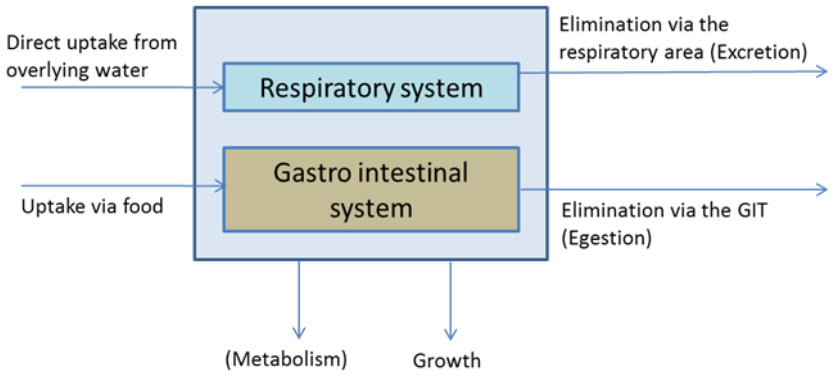


Figure 3 Main bioaccumulation processes included 'Fish' and 'Invertebrate' model. Metabolism is not considered for metals.

While processes involved in bioaccumulation may be assumed to be similar for fish and invertebrate species (Hendriks et al., 2001), for phytoplankton a simpler assumption can be made. The 'Phytoplankton' model can be represented by one compartment and only uptake from water is considered (Figure 4) since phytoplankton includes autotrophic species, and dietary uptake can be disregarded (Frouin et al., 2013).

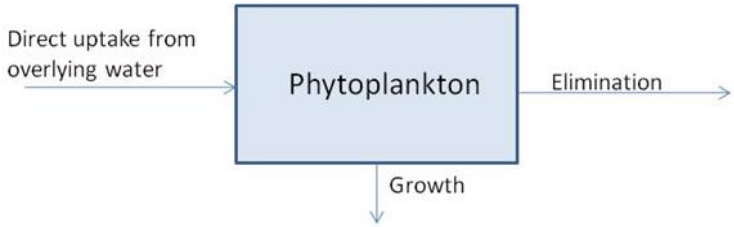


Figure 4 Main bioaccumulation processes included in 'Phytoplankton' model.

3.3. RESPIRATORY UPTAKE AND EXCRETION OF CHEMICALS

3.3.1. Process Description

Bioconcentration in invertebrates and fish partially results from chemical uptake via the respiratory surface (fish's gill) of the organism. A variety of models addressing uptake of chemicals via respiratory route exist (Arnot and Gobas, 2004; Barber, 2008; Hendriks et al., 2001). All these models can be based on different mathematical formulations and parameter names but they actually are consistent and consider common assumptions. In particular, they consider that uptake is governed by an assimilation rate that depends on biological attributes of the fish (e.g. water assimilation varies between freshwater and marine organism as a result of their distinct requirements for osmoregulatory balance), but also on chemical structure of the investigated substance and its affinity with lipids. Assimilation efficiency (AE_{gill}) thus determines the amount of chemicals that fish extracts from the volume of water flowing across the gill membrane. Assimilation efficiency is generally related to the Octanol-

Water partition coefficient (K_{ow}) according to a relationship in the form (Equation 1):

Equation 1

$$AE_{gill} \propto \frac{1}{A + B / K_{ow}}$$

where AE_{gill} (dimensionless) is the assimilation efficiency; K_{ow} (dimensionless) is the octanol-water partition coefficient; A and B (dimensionless) are calibration parameters (see below). Several parameterizations based on empirical observations were proposed for this relationship (Gobas et al., 1988; Arnot et al., 2004; Barber, 2008). Some of them propose piecewise functions, i.e. different A and B values for several $logK_{ow}$ ranges. For example, Barber (2008) observed a positive correlation with K_{ow} for hydrophilic and moderately hydrophobic chemicals and a negative correlation with K_{ow} for extremely hydrophobic chemicals. The decreasing of Assimilation Efficiency for these latter compounds can be caused by the increasing water phase resistance. The applicability domain of these relationships has also to be checked before application. For example, the solution proposed by Gobas et al., (1988) is based on reliable data (i.e., number of observations, confidence intervals), but only chemicals with $logK_{ow} > 4$ were studied.

The Hendrik's model (Hendriks et al., 2001) gives a mechanistic interpretation for the A and B coefficients, i.e. they represent resistances in water and lipid membranes respectively. The resistances for diffusion through water layers can be considered to be the same for different chemicals because molecular weights and volumes are in the same order of magnitude (the use of the constant A derives from this assumption). The resistance during permeation through lipid layers was considered to decrease with the hydrophobicity of the compound (Gobas et al., 1986; Flynn and Yalkowsky, 1972; the use of the expression B/K_{OW} derives from this assumption). In addition to resistances in lipid and water layers, fluxes can also be limited by delays in the flow of water through organisms. However, the delay imposed by the water flow can be ignored for aquatic species because ventilation and filtration are sufficiently fast in these organisms (Hendriks et al., 2001).

Uptake of chemicals through the gill membrane also depends on the volume of water flowing across the gill membrane, i.e. on the ventilation rate. In some models, ventilation rates of fish (expressed in $L.s^{-1}$) are assumed to be directly associated to its needs in oxygen. It is then estimated by the three variables that governs oxygen uptake, i.e. the fish rate of oxygen consumption (expressed in $mgO_2.s^{-1}$), the fish oxygen assimilation efficiency and the

dissolved oxygen concentration of the ambient water (in $mgO_2.L^{-1}$). Assuming that ventilation rates essentially depend on organism body weight, they can also be estimated by allometric scaling.

In conclusion, Hendrik's model (Hendriks et al., 2001) considers that respiratory uptake rate of a chemical depends on K_{ow}, body weight W, and resistances ρ_{water_layer} and ρ_{lipid_layer} that substances encounter in lipid and water layers of the organism (Equation 2):

Equation 2

$$k_{uptake\_resp} = \frac{W^{-\kappa}}{\rho_{water\_layer} + \frac{\rho_{lipid\_layer}}{K_{ow}}}$$

where k_{uptake_resp} ($L.kg^{-1}_{fw}.d^{-1}$) is the respiratory uptake rate; W (kg_{fw}) is the organism weight; κ (dimensionless) is the allometric factor; ρ_{water_layer} ($kg.d.kg^{-1}$) is the water layer diffusion resistance for uptake of chemicals from water; ρ_{lipid_layer} ($kg.d.kg^{-1}$) is the lipid layer permeation resistance; K_{ow} (dimensionless) is the octanol-water partition coefficient.

Such model is expected to accurately predict uptake rate and an uncertainty analysis was already performed by Hauck et al., (2011).

QSAR uptake models are another alternative for estimating uptake across gill. Although such empirical models can provide useful conceptual insight, their utility for actual prediction must be carefully evaluated due to limited databases from which they were calculated and also their implicit assumption that biological determinants of uptake are either insignificant or constant across species or body size.

The respiratory pathway includes chemical uptake, as described above, but also chemical excretion. Excretion can be seen as a release of chemicals from fish's water compartment via respiratory route. Chemical uptake via the respiratory surface (fish's gill) of the organism is indeed associated to chemical excretion associated to the outflux of water via the respiratory surface. Both processes are influenced by the same factors connected with respiration. Many experiments were conducted under controlled laboratory conditions where dietary uptake is considered negligible. Under such conditions, equilibrium between concentration of the chemical in fish tissues and surrounding water can be reached. The bioconcentration factor (BCF), defined as the ratio at equilibrium of biota concentration of the substance to water concentration, can then be defined. As equilibrium condition is assumed to be reached, BCF also represents the ratio between respiratory uptake rate and

excretion rate. BCF can then be used in combination with the respiratory uptake rate k_{uptake_resp} to estimate the excretion rate $k_{excretion\_resp}$ and to reflect affinity of the substance for staying in the lipid compartment of the organism (Mackay and Fraser, 2000). The BCF concept was originally developed for hydrophobic organic substances and several QSAR techniques were proposed to predict BCF from chemical descriptors of hydrophobicity like octanol-water partition coefficients.

3.3.1.1. Allometric scaling

As described above, some models simulating uptake rate by respiration are based on allometric scaling. This is also the case for dietary uptake of fish or absorption rate of mammals. A short introduction to allometric scaling is then proposed here, and it is also valid for other parameters presented in other sections of this chapter.

Allometric relationships provide body-size specific parameters instead of values that are arbitrary or taken from a well-known species. Allometry, or the biology of scaling, is the study of size and its consequences. It has become a useful tool for comparative physiology. There are several empirical allometric equations that relate body size to many parameters, including ingestion rate, lifespan, inhalation rate, mortality, age at maturity, maximum density, territory size, rate constants, etc. Even if these relationships were originally derived from empirical observations, there is a growing body of evidence that these relationships have their origins in the dynamics of energy transport mechanisms. From a meta-analysis based on 230 relationships, slopes of allometric regressions were shown to be mutually consistent with rate constants, and generally decrease with organism mass at a constant exponent (Hendriks, 2007). Slope of allometric regression κ is a component of each rate constant used in fish bioaccumulation model. The range of the slope of allometric regression is derived from reviews of empirical studies that have shown that the exponent is usually within the range of 0.25 to 0.33, theoretically explained by food web networks and surface-volume relationships (Hendriks et al., 2001).

3.4. DIETARY UPTAKE AND EGESTION OF CHEMICALS

The rate at which chemicals are assimilated from the diet via the gastro intestinal tract (GIT) is expressed by the dietary uptake rate constant ($kg.kg^{-1}.d^{-1}$), and the rate at which chemicals are eliminated from the organism body via the gastro intestinal tract (GIT) is expressed by the egestion rate constant ($kg.kg^{-1}.d^{-1}$). Although direct aqueous uptake is the

dominant route of accumulation for moderately hydrophobic chemicals, dietary uptake can be the dominant pathway for extremely hydrophobic chemicals (Barber 2008). Due to the fact that water is not a significant contributor to the storage capacity of highly hydrophobic organic chemicals, its value has a negligible impact on the mechanism of biomagnification for these chemicals. Once the dietary exposure pathway becomes dominant, an actual concentration of accumulated chemical can exceed those predicted by thermodynamic partitioning. This is because of fish's decreasing ability to excrete highly hydrophobic chemicals across its gills, and also due to its ability to maintain high dietary diffusion gradients (Barber, 2008). The high diffusion gradient makes assimilation of digestion products more rapid than hydrophobic chemicals, leading to increase concentration of these chemicals in guts.

In complement to what proposed for the respiratory pathway, Hendriks et al., (2001) proposed formulations to simulate uptake and egestion of chemicals via diet ingestion. It is assumed that chemical exchanges across the gastro intestinal tract (GIT) are driven by diffusion gradients, i.e. the concentration differences between phases within the fish and its food/feces. These exchanges are assumed to be mainly simple molecular diffusions. Although alternative diffusion mechanisms were proposed (e.g., lipid micelle-mediated diffusion model), their role in controlling dietary exchanges of fish is not well established (Barber 2008).

The distribution of food between digested and undigested fractions respectively can be represented by an Assimilation Fraction AF_{food}. Assimilated food may be allocated to production of somatic or gonadal biomass. Dietary assimilation efficiencies reflect dietary matrix (e.g., organic matter quality and quantity), and digestive physiology of the organism (e.g., feeding rates and gut retention time). As the chemical can be both transported in food water and/or food lipids, the assimilation fraction of the chemical $AF_{chemical}$ can be different of those applied for food AF_{food}. For this purpose, food is assumed to be composed of lipids and water only, in respective proportions p_{lipid_food} and (1- p_{lipid_food}). The assimilation fraction of the chemical contained in water is directly related to the assimilation fraction of food AF_{food}; the assimilation fraction of the chemical contained in lipids is assumed to be related to the assimilation fraction of food AF_{food} corrected by the octanol-water partition coefficient K_{ow}.

Similar to what assumed for Hendrik's respiratory uptake model, the dietary uptake is limited by two resistances in series reflecting transport across the water GIT layer and the lipid GIT membrane respectively. As for respiratory uptake, the resistance for diffusion through water

layer is considered to be the same for different chemicals, while partial resistance from lipid layer (encountered to and from food) is inversely proportional to K_{ow}. A flow delay of food and feces, depending on the fraction of undigested chemical contained in lipids, can also be considered. The mathematical formulation respecting these assumptions can be found in Hendriks et al., (2001), i.e.

Equation 3

$$k_{uptake\_diet} = \frac{AF_{food}}{1 - AF_{food}} \cdot \frac{1}{p_{lipid\_food}(K_{ow} - 1) + 1} \cdot \frac{W^{-\kappa}}{\rho_{water\_layer\_food} + \frac{\rho_{lipid\_layer}}{K_{ow}} + \frac{1}{p_{lipid\_food} . K_{ow} . (1 - AF_{food}) . \gamma_{food}}}$$

where k_{uptake_diet} (kg fw.kg^{-1} fw.d^{-1}) is the dietary uptake rate; AF_{food} (dimensionless) is the food assimilation efficiency; $p_{lipid,food}$ (dimensionless) is the proportion of lipids in food; W (kg fw) is the organism weight; κ (dimensionless) is the allometric factor; $\rho_{water\_layer\_food}$ (kg.d.kg^{-1}) is the water layer diffusion resistance for uptake of chemicals from food; ρ_{lipid_layer} (kg.d.kg^{-1}) is the lipid layer permeation resistance; K_{ow} (dimensionless) is the octanol-water partition coefficient; γ_{food} (dimensionless) is the food transport coefficient (that represents delay in advective transport of chemical substances through organism due to limited supply of new food).

Alternatively, bioaccumulation models employ dietary uptake formulations based on assimilation efficiencies at equilibrium. Models applying assimilation implicitly assume that chemical equilibrium assimilation efficiencies describe the net chemical exchange between fish and their food. These models describe a fish's chemical elimination either as a single, lumped parameter process that is independent of the fish's egestion rate or as a process that does not require an explicit fecal egestion term. Chemical assimilation efficiencies via food often have been considered only as functions of K_{ow}, similarly to what is established for respiratory uptake. Moreover, as far as the fish's dietary assimilation efficiencies are concerned, Thomann et al., (1992) concluded that a fish's dietary and gill assimilation efficiencies could be estimated with the same empirical function of K_{ow}. These relations are presented often as hyperbolic functions (i.e., $AE_{diet} = (a_0 + a_1 K_{ow})^{-1}$). One example of this approach is described by Gobas et al., (1988).

3.5. METABOLIC BIOTRANSFORMATION

One of the routes of chemical elimination in fish is metabolic transformation (or biotransformation), defined as a change of the parent substance to another molecule or a conjugated form of the parent substance. Negligible biotransformation rates are often assumed for screening level hazard and risk assessment, thus resulting in overestimates of bioaccumulation, exposure, and risk for chemicals that undergo biotransformation processes. Biotransformation can however lead to reduction in bioconcentration of some non-ionic substances due to reactions associated with certain functional groups. Metabolic biotransformation has a larger influence on bioaccumulation factor for more hydrophobic chemicals. This is because the rates of chemical elimination by gill respiration become slower with increasing hydrophobicity of contaminants. Schüürmann et al., (2007) states that for predictive BCF assessment, factors such as biotransformation should be taken into account.

Biotransformation results in formation of a more hydrophilic compound which is more easily excreted that a parent compound. The organ that is most commonly involved in the biotransformation of xenobiotics is the liver. The main processes involved in metabolism of chemical in fish body are categorised in three phases (van der Oost et al., 2003): Phase I of metabolism involves such process as oxidation, reduction, or hydrolysis to unmask or add reactive functional groups to xenobiotic compound; Phase II of metabolism involves enzymes catalyzing conjugation of the xenobiotic or its metabolite with an endogenous ligand (e.g. glutathione, glucuronic acid), thus facilitating the excretion of chemical; Phase III involves enzymes (i.e. peptidases, hydrolases, and β-lyase) that catalyse metabolites to form products easily removable from the organism body.

Models that include biotransformation generally assume first-order processes and do not estimate biotransformation rates that may occur under non-first-order conditions, such as enzyme saturation. Biotransformation half-lives and rate constants ($\lambda_{metabolism}$) for organic chemicals in fish can be derived from QSAR models (under applicability domain limitations) (Arnot et al., 2009). QSAR predictions of metabolic biotransformation are functions of structural properties, and can be presented as normalised values (e.g., to 0.01 kg fish and 15°C). In this latter case, for comparison of models' estimates and use in mass balance models, it is recommended to convert QSAR predicted values to body weight and temperature specific values. Alternatively, biotransformation rate constant can be calculated as the difference between two quantities, a measured bioconcentration factor or elimination

rate constant, and a model-derived bioconcentration factor or elimination rate constant estimated assuming no biotransformation.

3.6. BIOCONCENTRATION FACTOR FOR METALS

Bioaccumulation of metals via the respiratory pathway can occur across body surfaces, such as gill, and is generally described by the bioconcentration factor (BCF), defined as the ratio at equilibrium of the substance concentration in fish tissue to water concentration in water. The BCF concept was originally developed for hydrophobic organic substances. Simple passive diffusion across the lipid biomembranes is believed to be the key process for the accumulation of neutral hydrophobic substances in biota, which ensures BCF is independent of exposure concentrations (McGeer et al., 2003). In the case of metal, however, the assumption of BCF being independent of exposure concentrations is controversial. As a result of complex physiological processes such as sequestration, detoxification, storage, branchial elimination, biota is often actively able to regulate metal bioconcentration via dynamic reaction systems that respond to environmental loading and maintain homeostasis (Chapman et al., 1996; Hamilton et al., 1986). In addition, Deforest et al., (2007) hypothesized the trend in which metal uptake increases at lower exposure concentrations, according to the basis that organisms actively uptake essential metals at low concentrations to satisfy metabolic requirements. Non-essential metals would also be regulated because the mechanisms for regulating essential metals are not metal-specific (Phillips and Rainbow, 1989).

Based on the factors influencing metal uptake and accumulation described above, it can be assumed BCF values for metals are not independent of exposure. BCF can then assumed to be related to the metal concentration in water. Several authors observed an inverse linear relationship between BCF and the total metal concentration in water in log units (Hendriks et al., 2001; McGeer et al., 2003; Deforest et al., 2007; Tanaka et al., 2010), i.e.

Equation 4

$$\ln(BCF) = a_{BCF} + b_{BCF} \cdot \ln(C_{tot\_water})$$

where BCF (L.kg^{-1} fw) is the Bioconcentration factor; C_{tot_water} (mg.L^{-1}) is the total concentration of the chemical in water; a_{BCF} and b_{BCF} (dimensionless) are calibration parameters.

For example, Tanaka et al., (2010) built a database containing BCF, as well as

concentrations in water, for five metals (Cd, Cu, Zn, Pb and As). Data were extracted from ECETOX and US EPA databases, as well as from data available in McGeer et al., (2003) or other more recent papers. Only chronic exposure conditions (> 28 days) were considered for deriving BCFs, because they are assumed to be relevant for equilibrium situations. As a result of the data selection, an estimation of the a_{BCF} and b_{BCF} parameters was thus obtained with confidence intervals. McGeer et al., (2003) also fitted BCF-Concentration relationships for Ag and Ni.

An example the media and processes considered and their matrix representation is shown in Figure 5.

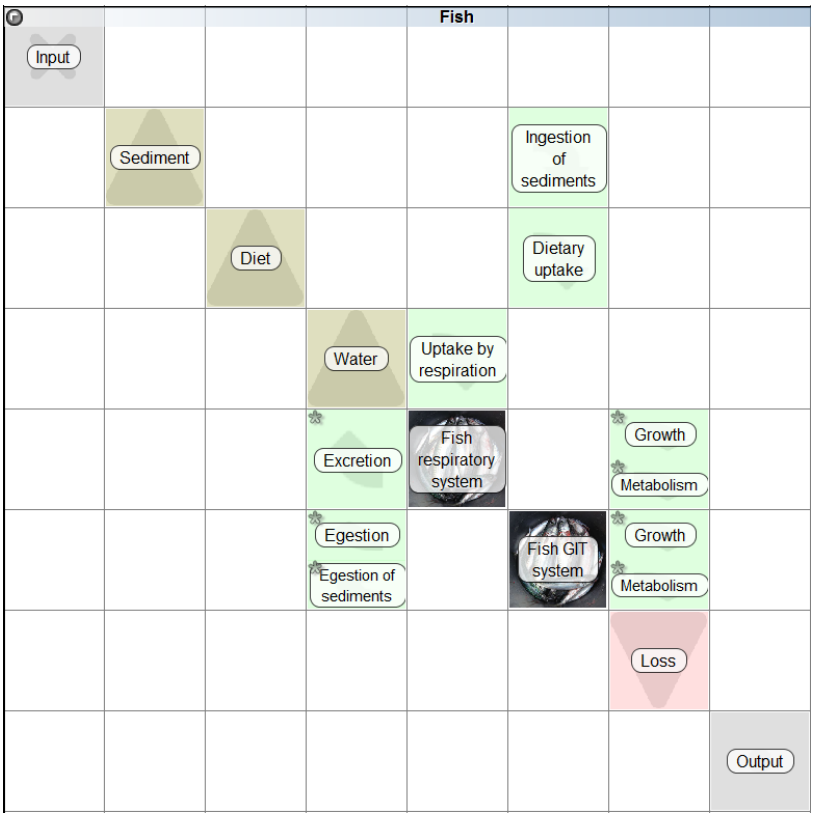


Figure 5 Processes included in estimation of bioaccumulation in 'Fish'

CHAPTER 4 INTEGRATED EXPOSURE MODELLING IN THE LAGOON OF VENICE

4.1. VENICE CASE STUDY

Increasing the confidence in the applicability of the integrated exposure assessment and the MERLIN-Expo can be achieved through testing on complex realistic case studies. The case study can be seen as reference case that provides credibility in the integrated exposure assessment and potential guidance to future users on how to apply the models in different situations and how to interpret the results from the assessments.

The lagoon of Venice can be divided into three main basins: southern, central and northern lagoon. For the integrated exposure assessment, the central lagoon has been selected as target area: it is close to Porto Marghera industrial area and has been strongly influenced by input of contaminants associated to industrial activities. Many studies on superficial sediments showed that the concentrations of persistent organic pollutants such as dioxins and PCBs in the central basin were higher than other areas (Frignani et al., 2001; Marcomini et al., 1997; Secco et al., 2005; Venice Water Authority, 2000a, 2000b, 1999). At the same time, central lagoon became the most relevant area for local shellfish industry. The biological resources of the lagoon have been exploited since centuries by traditional fishing and farming activities. After the introduction and the extensive diffusion of the bivalve Manila clam (*Tapes philippinarum,* which rapidly replaced the autochthonous species *T. decussatus*), since the early 90's the mechanical clam harvesting became the most important activity in the fishing sector, with an annual production of clams up to 40.000 tons. Dredging grounds are mainly concentrated in the central basin of the lagoon, where high nutrient, organic matter, phytoplankton and microphytobenthos concentrations created the optimal conditions for clam growth (Pranovi et al., 2003; Sfriso et al., 2003, 2005). Even after the prohibition of fishing activities in the areas close to Porto Marghera (and the identification of suitable areas for clam harvesting), illegal fishing continued in the most contaminated areas (Boscolo et al., 2007). Therefore, according to a conservative approach, the selection of central lagoon as target area for exposure modelling made possible to consider the worst-case scenario for both ecological and human exposure assessments.

For human exposure assessment, it is worth remembering that the overall body burden of PCBs and dioxins (reflected in blood concentrations) depends on toxico-kinetic processes, governed by age-dependent human physiology and by physico-chemical properties of chemical, as well as on external environmental exposure. Environmental burdens of PCBs

and dioxins have changed over the last 70 years, as demonstrated by retrospective studies. For PCBs, a peak of exposure in the 70's has been identified, followed by a decrease since the 80's as consequence of chemical use and emission regulation (e.g., Fensterheim, 1993). It is therefore necessary to reconstruct possible past exposure scenarios to perform lifetime human exposure assessment. Emission data suitable to retrace the historical development of PCBs and dioxins contamination in Venice lagoon area are not available. As an alternative, sediment cores proved to be useful in reconstructing temporal contamination trends in Venice area (Frignani et al., 2005; Marcomini et al., 1999), also in combination with modelling approaches (Dalla Valle et al., 2005). In general, a significant increase of organic pollutants in lagoon sediment has been observed since the 1940's, the maximum inputs of contaminants are on average associated with the period '60-70's, then an appreciable decrease was observed. However relative abundance and behaviour of individual substances might follow a different trend depending on emission patterns and chemical characteristics. Concentrations of PCBs and dioxins in different layers of a dated sediment core from central lagoon measured by Frignani et al., (2005) have been selected for the purposes of this study.

With the aim of testing the accuracy of MERLIN-Expo models in predicting ecological and human exposure, measurements of chemical concentrations in biota and human tissues were required. Unfortunately, only few human biomonitoring studies have been performed in the Venice area. The selected study was conducted in 1998, funded by Venice municipality, and it involved 41 volunteers (adult males resident in the municipality of Venice) (Frangipane, 1999; Raccanelli et al., 2007). Concentrations of TCDD/Fs and PCBs were analysed in serum extracted by an isotope dilution method using a relative response factors previously obtained from five standard solutions injections, according to USEPA recommendations (USEPA methods 1613B/94 and 1668A/99). The lipid content of serum was analytically determined for normalization of chemical levels to serum fat content. The volunteers were divided into two groups according to their diet: 22 consumers of large amounts of locally caught fish and shellfish (at least 3 times a week) and 19 persons consuming little quantities of fish of any kind (less than 2 times a week). For the purpose of the present study, data related to high fish consumers were selected.

For the same time period as human biomonitoring data, measurements of bioaccumulation of PCBs and dioxins were available for four aquatic species, namely *Tapes philippinarum* (Manila clam), *Carcinus mediterraneus* (Green crab), *Zosterisessor ophiocephalus* (Grass goby), and *Chelon labrosus* (Grey mullet) (Venice Water Authority, 1999).

According to the availability and suitability of chemical measurements in environmental, biota and human samples, PCB77, PCB126, PCB167, PCB169, PCB170, PCB180, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HCDD were selected for the ecological exposure assessment, while the full chain assessment (i.e., up to human internal exposure) focused on PCB126 and 2,3,7,8-TCDD.

4.2. EXPOSURE SCENARIO

Five models (i.e., Phytoplankton, Invertebrate, Fish, Human Intake and Man (PBPK) models) have been selected among those available in MERLIN-Expo library and coupled to recreate the target exposure scenario, as represented in Figure 6. All models were implemented in the library during 4FUN project. Specifically, within the present PhD project, the aquatic food web models (i.e. Phytoplankton, Invertebrate, Fish) drawing from previously existing modelling approaches were finally coded in Ecolego. A detailed model documentation is available on MERLIN-Expo website (http://merlin-expo.eu/learn/documentation/model-documentation/). Figure 6 illustrates the linkages between the selected models, with grey arrows in the matrix representing output(s) of one model used as input(s) to another one. To recreate the required exposure scenario, all the models can be coupled in a model chain in MERLIN-Expo, by defining output(s) of one model as input(s) to another one. This can be done using Graph or Matrix design. Here the scenario is built as chain of models using the matrix feature, where models selected from the software library are pictured as boxes placed on matrix diagonal and connected by off-diagonal 'Connectors'. Aquatic food web models (i.e., Phytoplankton, Invertebrate and Fish) are connected based on the prey-predator relationships in the food web: this means that the estimated chemical concentration in each aquatic species and its lipid fraction are used by the subsequent model to estimate chemical dietary intake for the predator organism. Some of the organisms included in the Venice lagoon food web are edible organisms, which are commonly caught or harvested in the lagoon. In order to simulate the consumption of specific fish and shellfish species by local population and estimate human internal exposure to POPs associated with the diet, the aquatic food web models were linked to the Human Intake and to the Man model. For the case at hand, only dietary intake (i.e., ingestion of contaminated fish and seafood) is considered for human exposure. Inhalation is a recognized exposure route for many persistent organic compounds, but its relative contribution to the overall exposure can be considered to be small when compared with dietary exposure (Alcock et al., 2000). Significant dermal contact can usually be restricted

to few occupational exposure scenarios. Therefore, these two exposure routes are not further taken into account in human exposure modelling for the Venice lagoon case study.

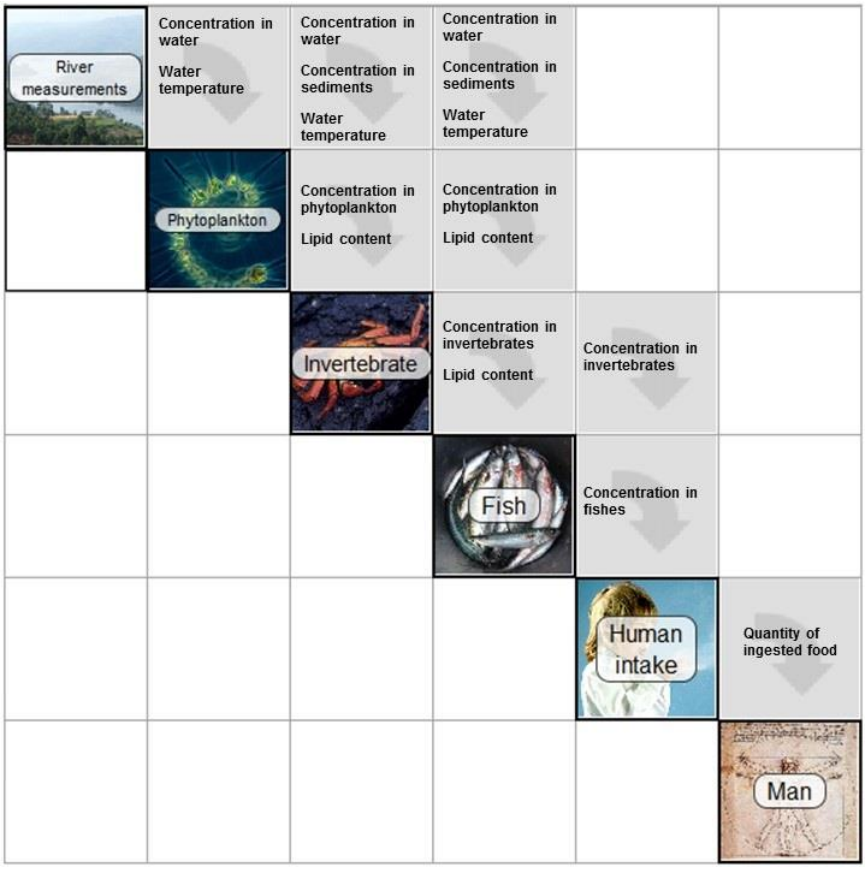


Figure 6 Models selected for the integrated exposure assessment in Venice lagoon visualized in the MERLIN-Expo matrix interface.

As specified in more detail in the Chapter 3 Phytoplankton, Invertebrate and Fish models were used to dynamically simulate the bioaccumulation of chemicals in aquatic organisms and can be linked to recreate an aquatic food web of various dimensions and complexity. They are based on the “Optimal Modelling for EcotoxicoloGical Applications” (OMEGA) modelling approach proposed by Hendriks and colleagues (Hendriks et al., 2001; Hendriks and Heikens, 2001), with some adaptation needed to fit MERLIN-Expo requirements (e.g., population renewal in time). The Fish and Invertebrate models include two compartments corresponding to two input/output pathways for chemical accumulation, namely the respiratory system and the gastrointestinal tract (GIT) system, while Phytoplankton is represented by a single-compartment model. The main processes simulated by the models are: chemical uptake through respiration, chemical uptake through ingestion of food (preys) or sediment, elimination through respiratory excretion, elimination through egestion, growth and metabolism (Equations A1, A2). For Phytoplankton, uptake from water, elimination and

growth are considered (Equation A3).

The Human Intake model estimates chemical daily intakes for human targets through different exposure pathways. This can be considered a kind of “connection model”, which has been created *ad hoc* to link environmental and human models in MERLIN-Expo. It is composed of a set of equations combining chemical concentrations estimated by other MERLIN-Expo models in environmental matrices (water, soil, dust, atmosphere, etc.) or food items (fish or aquatic invertebrates, grain, leafy vegetables, potato or root) with human daily intake rates (ingestion or inhalation rates) and human activity patterns (time spent indoor/outdoor) to derive the total quantity of chemical(s) ingested or inhaled per day by each individual.

The Man model is a PBPK model composed of 22 compartments representing organs connected through blood floow (Beaudouin et al., 2010) and it is aimed at simulating the evolution of the amounts or concentrations of chemical compounds in tissues and organs of the human body over lifetime. Using as input the amount of inhaled or ingested contaminant, the Man model can predict internal dosimetry of the compound, as concentrations in target tissues that can be linked to toxic effect or in the form of biomarkers of exposure (such as concentration in blood or urine) that can be compared to appropriate reference or guidance threshold values (e.g. biomonitoring equivalents). The model accounts for the following processes: uptake processes (absorption of contaminant by ingestion and inhalation), distribution of the compound in body organs, metabolism by enzymatic processes and excretion from the body. Moreover, it takes into account the evolution of the anatomy and physiology over the lifetime of individuals, simulating all the physiological or biochemical changes arising during the development and growth from birth onwards.

The Man model (PBPK model) is applied to predict time-dependent concentrations of organics in the blood of individual human males. The blood is represented in the model by two compartments: the arterial and venous blood. The arterial blood is distributed into all tissue compartments and the venous blood collects blood at the exit of most of the tissue compartments. Mass balance equations for calculating concentration in blood are described by Equations A4, A5.

4.3. INPUT DATA

4.3.1. Environmental Exposure

In order to simulate the accumulation of target chemicals in aquatic organisms of Venice lagoon, the definition of a site-specific food web structure is required. A food web describes the pattern of trophic relationships among selected species in an ecosystem and provides a simplified representation of biomass and energy flows. Feeding relationships not only expose organisms to contaminants, but also represent a critical process of pollutants transfer, resulting in biomagnification phenomena as the consequence of dietary uptake (Kelly et al., 2007; Mackay and Fraser, 2000). The characterization of predator-prey interactions is pivotal to understand contamination patterns and associated adverse effects when moving from individuals to the ecosystem level (Rohr et al., 2006). A site-specific food web for the bioaccumulation assessment in Venice lagoon has been proposed by Micheletti et al., (2008) based on extended literature on Venice lagoon ecosystems assessment and modelling (e.g., Carrer and Opitz, 1999; Libralato et al., 2002; Pranovi et al., 2003). This food web has been slightly adapted for the application of MERLIN-Expo and it includes 17 species plus the sediment compartment, which constitutes part of the diet for some benthic organisms. For some species (*Tapes phillipinarum*, *Chelon labrosus*, *Sparus aurata*, *Dicentrarchus labrax*), adult and juvenile individuals are considered as two separate components in the network, to account for differences in their metabolism, feeding habitat and internal tissue composition. The proposed food web includes species which have been selected to cover specific trophic roles (primary producers, top predators, etc.) and/or play an important role for fishing activity and can therefore be part of the human diet (i.e., they are also relevant in the perspective of human exposure assessment).

The food web includes two planktonic groups, eight benthonic species/groups, eight nektonic species/groups (19 elements in total plus the sediment compartment). A diagram representing the Venice lagoon food web is presented in Figure 7 (only main trophic relationships are reported).

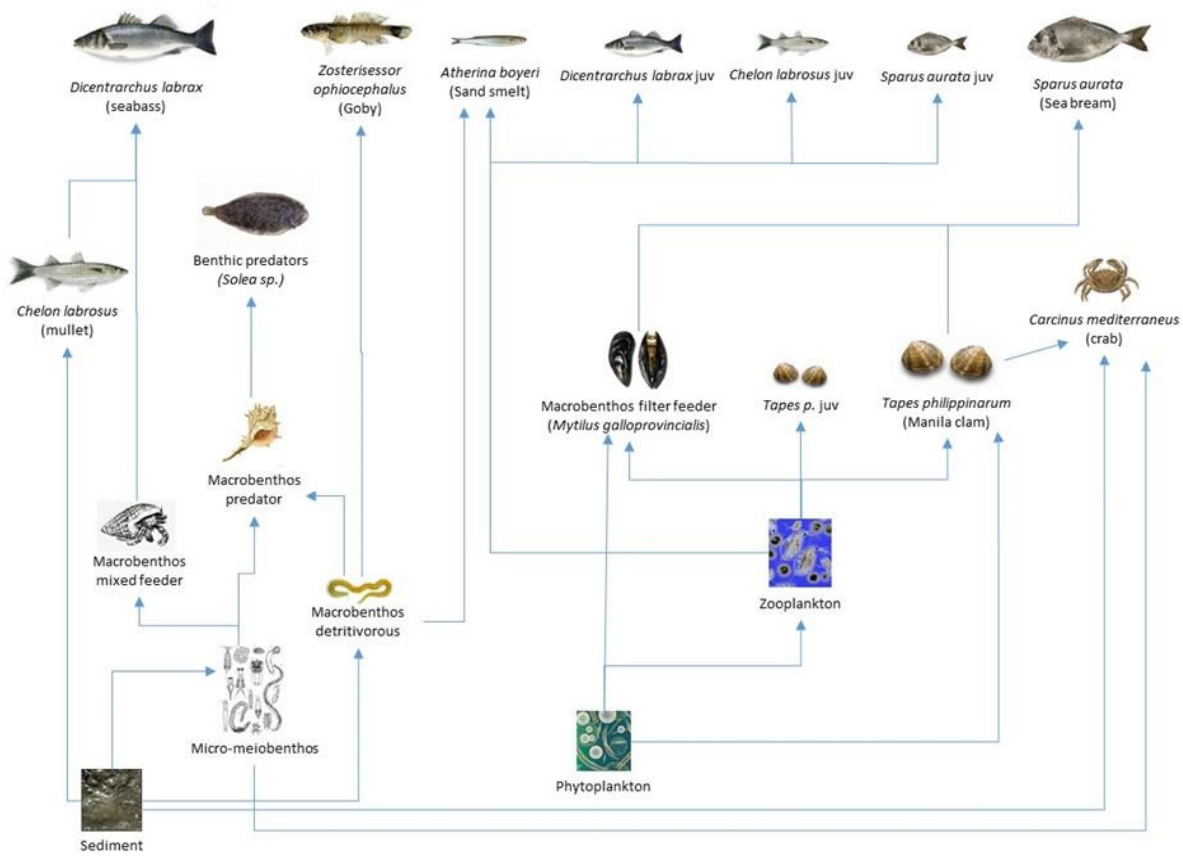


Figure 7 Aquatic food web for Venice lagoon. Adapted from Micheletti et al., (2008) and Libralato et al., (2002).

Input data required by MERLIN-Expo models are grouped as:

a) “parameters”, which are constant over each simulation and can be classified as chemical related parameters (e.g., physico-chemical properties of target chemicals) and biota related parameters (e.g., diet preferences, physiological parameters of selected species);

b) “time series”, which are time-dependent environmental data (e.g. concentrations in environmental media such as water or sediment, water temperature).

Phytoplankton, Invertebrate and Fish models require the same chemical-related parameters (i.e., octanol-water partition coefficient, bio-concentration factor, metabolic half-life of chemicals); moreover, Phytoplankton model requires also the water-organic carbon partition coefficient. Full list of input parameters used in bioaccumulation models along with their description is provided in the Tables A1-A3. Contaminants specific parameter values were derived using QSAR models implemented in EPI Suite software (USEPA, 2012a): metabolic half-life of chemicals for organics (Arnot et al.,2008, 2009), bioconcentration factor for organics (Arnot and Gobas, 2003, 2006), water-organic carbon partition coefficient (Schüürmannet al., 2007), and octanol/water partition coefficient (Meylan and Howard,

1995). All input values for the selected substances are reported in Table 1.

Table 1 Input values for chemical related parameters for aquatic food web models.

Parameter	**Octanol-water partition coefficient** (log K_{ow})	**Water-organic carbon partition coefficient** (logK_{oc})	**Bioconcentration factor** (BCF)	**Metabolic half-life of chemical** ($\lambda_{metabolism}$)
	unitless	unitless	unitless	d^{-1}
PCB77	6.34	474	5.03	2254
PCB126	6.8	4.93	5.08	288.4
PCB167	7.5	5.22	4.75	271.64
PCB169	7.46	5.17	5.64	413
PCB170	8.27	5.64	3.69	799.83
PCB180	8.27	5.29	6.20	716.2
2,3,7,8-TCDD	6.92	4.83	3.54	16
1,2,3,7,8-PeCDD	7.56	4.74	3.74	27.3
1,2,3,7,8-HxCDD	8.21	5.38	3.64	46.5

Input values for biological parameters for the species included in the Venice food web have been derived from available literature and free databases and are reported in Table 2 and Table 3.

Table 2 Input values for biological parameters of Phytoplankton model.

Parameter	**Unit**	**Value**	**Reference**
Allometric rate exponent	unitless	0.25	Hauck et al., 2011
Intercept of phytoplankton growth rate	unitless	0.22	Marañón et al., 2013
Slope of phytoplankton growth rate	unitless	0.15	Marañón et al., 2013
Lipid fraction of phytoplankton	unitless	0.02	Skoglund et al., 1999; Olenina et al., 2006
Lipid layer permeation resistance	kg.d /kg	97	Hauck et al., 2011
Lipid layer resistance exponent	unitless	0.41	Hauck et al. 2011
Organic carbon fraction of phytoplankton	unitless	0.29	Skoglund et al., 1999; Olenina et al., 2006
Phytoplankton cell volume	μm^3	7.68	Olenina et al., 2006
Water layer diffusion resistance for uptake of chemicals from water	kg.d / kg	0.0068	Hauck et al., 2011

Table 3 Input values for biological parameters of Invertebrate and Fish models.

Parameter	Allometric exponent	Lipid fraction of invertebrate	Food transport coefficient	Fraction of assimilated food	Lipid layer permeation coefficient	Water layer diffusion resistance for uptake of chemicals from food	Water layer diffusion resistance for uptake of chemicals from water	Age at maturity	Weight at maturity	Fish lenght at maturity	Intercept of lenght-weight relationship	Slope of lenght-weigth relationship
Units / Species	unitless	unitless	kg_{fw} / kg_{fw} d	unitless	kg.d / kg	kg.d / kg	kg.d / kg	d	kg_{fw}	cm	unitless	unitless
INVERTEBRATE MODEL												
Zooplankton	0.25	0.05	0.03	0.73	97	0.0002	0.0068	20	3.42E-05			
Micro-meiobenthos		0.014	0.03	0.73	97	0.0002	0.0068	20	1.00E-04			
Macrobenthos Detritivorous		0.014	0.03	0.73	97	0.0002	0.0068	90	3.20E-04			
Macrobenthos Omnivorous Filter Feeder		0.012	0.03	0.73	97	0.0002	0.0068	548	6.71E-03			
Tapes philippinarum juv		0.0125	0.03	0.73	97	0.0002	0.0068	90	1.00E-03			
Tapes philippinarum		0.0125	0.03	0.73	97	0.0002	0.0068	910	7.00E-03			
Macrobenthos Omnivorous Mixed Feeder		0.0262	0.03	0.73	97	0.0002	0.0068	90	1.41E-03			
Carcinus mediterraneus		0.05	0.03	0.73	97	0.0002	0.0068	730	1.02E-02			
Macrobenthos Omnivorous Predator		0.05	0.03	0.73	97	0.0002	0.0068	545	1.57E-03			
FISH MODEL												
Atherina boyeri	0.25	0.096	0.03	0.73	97	0.0002	0.0068	1778.5		10.5	0.00603	3.07
Chelon labrosus		0.068	0.03	0.73	97	0.0002	0.0068	4745		30.3	0.00794	3.12
Chelon labrosus juv		0.068	0.03	0.73	97	0.0002	0.0068	730		3	0.0091	3.02
Dicentrarcus labrax		0.1338	0.03	0.73	97	0.0002	0.0068	1095		35.9	0.00891	3.05
Dicentrarcus labrax juv		0.0076	0.03	0.73	97	0.0002	0.0068	75		3	0.0076	3.2

Nekton carnivorous benthic feeder		0.08	0.03	0.73	97	0.0002	0.0068	1058.5		32.5	0.0123	2.96
Sparus aurata		0.0973	0.03	0.73	97	0.0002	0.0068	949		30	0.01259	3.03
Sparus aurata juv		0.0973	0.03	0.73	97	0.0002	0.0068	365		3	0.00923	3.28
Zosterisessor ophiocephalus		0.1	0.03	0.73	97	0.0002	0.0068	1659.5		16.3	0.00813	3.07
REFERENCES	Hauck ., 2011	R. Froese et al., 2014; M. Hauck et al., 2011; C. Micheletti et al., 2008; http://www.fishbase.org/	Hauck et al., 2011	Hauck et al., 2011	Hauck et al., 2011	Hauck et al., 2011	Hauck et al., 2011	R. Froese et al., 2014 http://www.fishbase.org/	Micheletti et al., 2008; Durbin and Durbin, 1978; P. Palmerini et al., 1994; Robinson et al., 2010	R. Froese et al., 2014 http://www.fishbase.org/	R. Froese et al., 2014 http://www.fishbase.org/	R. Froese et al., 2014 http://www.fishbase.org/

Description of the probability density functions is fully defined for all parameters in the documentation supporting developed models (http://merlin-expo.eu/learn/documentation/model-documentation/).

Fish and Invertebrate models require the user to define the diet preferences of each species included in the simulation. Components of organisms' diet can be either other aquatic organisms, such as invertebrate, fish or phytoplankton species (in this case the parameter to be informed is “Diet preference for food item”) or the organic fraction of sediment (in this case the parameter to be informed is “Diet preference for sediments”). In order to better clarify the trophic relationships between the considered species, these data are included all together in

the so called “diet matrix”, which reports, for each target species, the fraction of each prey/food item over the total dietary intake (in the interval [0; 1]). Diet preferences for the Venice lagoon organisms have been defined according to available literature data and adapting the diet matrix proposed by Micheletti et al., (2008). The diet matrix is reported in Table 4.

Table 4 Dietary preference for all aquatic species included in the Venice lagoon foodweb (adapted from Micheletti et al., 2008).

	Sediment	Phytoplankton	Zooplankton	Micro-meiobenthos	Macrobenthos detritivorous	Macrobenthos Omnivorous Filter Feeder	*Tapes philippinarum* juv	*Tapes philippinarum*	Macrobenthos Omnivorous Mixed Feeder	*Carcinus mediterraneus*	Macrobenthos Omnivorous Predator	*Chelon labrosus* juv	*Chelon labrosus*	*Atherina boyeri*	*Zosterisessor ophiocephalus*	Nekton carnivorous benthic feeder	*Sparus aurata* juv	*Sparus aurata*	*Dicentrarcus labrax* juv	*Dicentrarcus labrax*
Phytoplankton																				
Zooplankton	0.50	0.50																		
Micro-meiobenthos	1.00																			
Macrobenthos detritivorous	0.66			0.34																
Macrobenthos Omnivorous Filter Feeder	0.20	0.56	0.24																	
Tapes philippinarum juv	0.52	0.26	0.22																	
Tapes philippinarum	0.83	0.07	0.10																	
Macrobenthos Omnivorous Mixed Feeder	0.34	0.25	0.28		0.09	0.04														
Carcinus mediterraneus	0.25	0.15		0.20	0.10	0.04	0.06	0.10	0.10											
Macrobenthos Omnivorous Predator				0.25	0.20	0.04	0.20	0.16	0.15											
Chelon labrosus juv			0.69		0.31															
Chelon labrosus	0.45	0.11		0.32	0.12															

Atherina boyeri			0.38	0.12	0.28				0.15	0.01	0.06								
Zosterisessor ophiocephalus				0.08	0.44		0.12		0.23	0.01				0.12					
Nekton carnivorous benthic feeder			0.04	0.41	0.18	0.15			0.15	0.01	0.06								
Sparus aurata juv			0.52	0.24	0.24														
Sparus aurata					0.20	0.22	0.26	0.21	0.09	0.005				0.005	0.005	0.005			
Dicentrarcus labrax juv	0.12		0.48		0.18				0.16	0.01				0.05					
Dicentrarcus labrax	0.03	0.05	0.10						0.52	0.05				0.05	0.05	0.08	0.07		

Time dependent input values required by aquatic food web models include water temperature, chemical concentrations in dissolved water, and chemical concentrations in sediment. Water temperature affects organisms' uptake and excretion processes. A constant temperature of 15 °C is assumed for the Venice lagoon. Time series of concentrations of the target chemicals in sediment and in water are required by the food web models to simulate chemical uptake and obtain an estimate of time-dependent chemical concentrations in phytoplankton, invertebrate and fish species. In order to cover the temporal scenario of several decades required by the human exposure assessment, concentration of individual congeners in different layers of a dated sediment core collected in central lagoon (sediment core named “E” in Frignani et al., 2005; Venice Water Authority, 2000b) were used to reconstruct historical trends of sediment contamination. Values between the measured points have been interpolated in order to reconstruct continuous temporal trends. Chemical concentrations dissolved in water (reported in Table 5) were calculated starting from chemical concentrations in sediment following the approach described below:

Table 5 Temporal trend of reconstructed chemical concentrations in sediment and water

Year	Sediment depth (cm)	PCB77	PCB126	PCB167	PCB169	PCB180	2,3,7,8-TCDD	1,2,3,7,8-PCDD	1,2,3,4,7,8-HCDD
SEDIMENT (mg/g $_{dw}$)									
1920	21-18	3.00E-08	5.00E-09	2.00E-08	5.00E-09*	1.00E-07	2.50E-10	2.00E-10	4.00E-10
1935	18-15	1.40E-07	2.40E-07	1.20E-07	5.00E-09*	6.40E-07	3.00E-10	6.00E-10	1.30E-09
1940	12-15	4.50E-07	1.00E-08	4.00E-07	5.00E-09*	9.20E-07	7.00E-10	9.00E-10	1.70E-09
1950	9-12	4.60E-07	1.60E-07	4.40E-07	5.00E-09*	1.82E-06	7.00E-10	1.70E-09	4.10E-09
1960	6-9	5.20E-07	4.00E-08	7.00E-07	5.00E-09*	1.71E-06	2.50E-10*	2.30E-09	3.40E-09
1975	3-6	3.50E-07	4.00E-08	2.61E-06	5.00E-09*	5.78E-06	2.50E-10*	1.50E-09	2.00E-09
1998	1.5-3	2.30E-07	2.00E-08	7.80E-07	5.00E-09*	1.92E-06	4.00E-10	1.30E-09	2.00E-09
WATER (mg/m^3)									
1920	21-18	7.12E-07	2.01E-08	3.88E-09	2.19E-09	5.84E-10	6.11E-10	2.96E-11	3.08E-12
1935	18-15	3.32E-06	9.65E-07	2.33E-08	1.35E-09	3.74E-09	7.33E-10	8.88E-11	1.00E-11
1940	12-15	7.96E-06	2.88E-08	5.39E-08	8.06E-10	3.71E-09	1.21E-09	9.25E-11	9.02E-12
1950	9-12	5.36E-06	2.88E-07	3.59E-08	4.88E-10	4.40E-09	7.52E-10	1.06E-10	1.31E-11
1960	6-9	9.62E-06	1.21E-07	9.96E-08	8.52E-10	7.29E-09	4.57E-10	2.50E-10	1.91E-11
1975	3-6	6.69E-06	1.26E-07	3.86E-07	8.86E-10	2.56E-08	4.74E-10	1.69E-10	1.17E-11
1998	1.5-3	4.62E-06	6.64E-08	1.23E-07	9.42E-10	9.07E-09	8.03E-10	1.56E-10	1.24E-11

Notes: * original value below the detection limit (<LOD); a value of LOD/2 has been used.

Taking into account the partitioning process of chemicals between the aqueous and the solid phase, the total concentration of chemical in water ($C_{w,t}$ in g/L) can be calculated according to Equation 5.

Equation 5

$$C_{w,t} = \frac{Cs}{K_{sw}}$$

where C_s (g/kg) is the concentration in sediment and K_{sw} the partition coefficient between bottom sediment and water.

K_{sw} can be estimated according to the relationship to the octanol-water partition coefficient (K_{ow}) proposed by Seth and colleague (1999) (Equation 6):

Equation 6

$$K_{sw} = f_{oc} \cdot 0.35 \cdot K_{ow} \cdot d_{bs}$$

where foc is the fraction of organic carbon in the sediment and dbs (kg/L) is the bottom

sediment density.

Since the aquatic food web models require as input data the dissolved concentrations of chemicals in water, these have been derived according to the equation proposed by Gobas (1993) (Equation 7).

Equation 7

$$C_{w,d} = \frac{Cw,t}{1+\left(\frac{K_{OW} \cdot M_{OM}}{d_{OM}}\right)}$$

where M_{OM} is the organic matter concentration in water (kg/L), and d_{OM} is the organic matter density (kg/L). M_{OM} can be estimated as the product of suspended solids concentration in water (kg/L) by the organic carbon fraction in suspended solids (assumed to be the same as sediment). The selected site-specific parameters for the Venice lagoon used for the calculation of dissolved concentration of pollutants in water are reported in Table 6.

Table 6 Site specific parameters used for the calculation of dissolved concentration in water.

Parameter	Unit	Value		Reference
Sediment density (d_{bs})	kg/L	1.71		Venice Water Authority, 1999
Suspended solids	kg/L	3.60E-05		Venice Water Authority, 2000
Density of organic matter (d_{OM})	kg/L	0.9		Gobas et al., 2003
Fraction of organic carbon (f_{OC})	%	Year	f_{OC} %	Frignani et al., 2006
		1940	1.72	
		1950	2.23	
		1960	1.67	
		1975	1.64	
		1995	1.59	

4.3.2. Human Exposure

Parameterization of the PBPK model. The Man model in MERLIN-Expo was parameterized for the two target chemicals selected for the human exposure assessment, namely 2,3,7,8-TCDD and PCB126. List of parameters used in the PBPK model is available in Table A3.

Although chemicals absorbed from gut lumen enter the liver first, ingested 2,3,7,8-TCDD and PCB126 were set to enter the blood flow directly in this model, assuming that they pass liver fast enough to avoid accumulation or first pass effects such as metabolic elimination. The option “Ingestion via the liver” available in MERLIN-Expo

for this purpose was then used. The absorption rate was obtained by Mclachlan, (1993). Only one elimination route was considered in the liver via biliary excretion, since urinary excretion of dioxins and PCBs can be neglected. The excretion rates were set to the values provided by Milbrath et al., (2009) and Ogura (2004). The most up to date elimination values derived by Ritter et al., (2011) are not available for the organic chemicals selected for this study.

Tissue-blood partition coefficients of liver, kidney, fat, muscle and richly perfused tissue were calculated using dioxin concentration data in human tissues (Iida et al., 1999), or determined based on structural information of the chemicals (Parham et al., 1997). The fat-blood partition coefficients were estimated using a quantitative structure-activity relationship (QSAR) specific to PCBs (Parham et al., 1997). The other tissue-blood partition coefficients were obtained by multiplying the fat-blood partition coefficients by a factor related to the tissue composition. Input values for PBPK model parameters are reported in Table 7.

Table 7 Input values for PBPK model parameters for 2,3,7,8-TCDD and PCB126

Parameters	**2,3,7,8-TCDD**	**PCB 126**
Absorption rate		
Oral	0.97	1
Excretion and metabolism		
Excretion rate in liver ($min^{-1}.kg^{-1}$)	4.257×10^{-7}	-
Clearance in liver ($L.min^{-1}.kg^{-1}$)	-	5×10^{-5}
Partition coefficients		
Adipose	247	152
Adrenal	9.8	20.7
Blood	1	1
Blood_Arterial	1	1
Blood_Venous	1	1
Bones	9.8	7.6
Bones_NP	1	1
Brain	4.1	18.2
Breast	17	101.8
Gut	9.8	10.5
Gut_Lumen	1	1
Heart	9.8	9.3
Kidneys	3.1	7.9
Liver	9.8	7.7
Lungs	4.1	1.6
Marrow	1	109.2
Muscle	17	7.5
Pancreas	9.8	21.8
Sexual_Organs	9.8	8.2
Skin	2.5	7.0
Spleen	9.8	2.9
Stomach	9.8	11.3
Stomach_Lumen	1	1
Thyroid	9.8	20.7
Urinary_Tract	9.8	7.3

Daily food intakes. The most site-specific information on fish and seafood daily intakes for the municipality of Venice is available in the report “Fish production and fish consumption preferences of families in Venice municipality” (Pedenzini, 1996), based on the results of a survey performed in the different areas of Venice municipality (Venice historical centre; islands and coastal villages; mainland/Mestre city). The estimated average daily intake of fish and seafood in the Municipality was equal to 2168 g/month, equivalent to 72.3 g/day. For individuals living in Venice lagoon islands and coastal villages, the average daily intake increased to 94.7 g/day. Typology and quantity of food intake vary depending on the age, it is therefore important to consider age dependent food intakes when simulating life time exposure for the same individual. Age dependent intake rates for Italian population were obtained from the INN-CA national survey (Turrini et al., 2001) performed in 1994–96 by the Italian National Food and Nutrition Research Institute (INRAN) based on the investigation of diet habits through individual questionnaires (7-day based survey technique), involving 1978 individuals stratified into four main geographical areas. Data were aggregated into four age groups: children (1 to 9 years), adolescents (10 to 17 years), adults (18 to 63 years) and elderly people (N 63 years). Data on daily intake of “fish and seafood (fresh and frozen)” for the different age groups were selected. The ratio between age group average daily intakes and overall average daily intake in INNCA survey was used to scale Venice daily intake to different age group intakes to get site-specific age dependent intake values.

Ideally, to reconstruct historical exposure, changing diet patterns across different decades should be considered. However, due to the lack of historical data on diet habits in the area in the past (and generally in Italy), mean daily intakes for different age groups have been assumed as constant for all the simulation period.

The survey by Pedenzini (1996) reported also information on diet preferences of local population for specific typologies of fish/seafood, considering the categories “molluscs”, “crustaceans” and “fish” and including some indications on most consumed species of fish or shellfish. This information has been used to “subdivide” the age group intake values into several aquatic species contributing to the overall intake, in order to link the outputs of the aquatic food web models (namely, concentrations in aquatic organisms from Fish and Invertebrate models) to the Human Intake model in MERLIN-Expo. The age dependent daily intakes of different types of fish and seafood used as input data to MERLIN-Expo Human Intake model are reported in Table 8 for persons

classified as high fish consumers.

Table 8 Age dependent daily intakes of different types of fish and shellfish.

	DAILY INTAKE (kg $_{fw}$/day)			
Food items	**Children (1-9)**	**Adolescents (10-17)**	**Adults (18-63)**	**Elderly (>63)**
Macrobenthos filter feeders (mussel)	0.005	0.007	0.007	0.006
Tapes phillipinarum (Manila clam) and similar sediment dwelling molluscs	0.022	0.032	0.036	0.031
Carcinus mediterraneus (Green crab)	0.008	0.011	0.012	0.010
Atherina boyeri (Sand smelt)	0.003	0.004	0.004	0.004
Chelon labrosus (Grey mullet)	0.006	0.008	0.009	0.008
Dicentrarcus labrax (Sea bass)	0.008	0.011	0.013	0.011
Sparus aurata (Gilt-head bream)	0.008	0.011	0.013	0.011
Zosterisessor ophiocephalus (Grass goby)	0.004	0.006	0.006	0.005

4.4. SIMULATION SETTINGS

Using the chain of models, MERLIN-Expo was applied to simulate PCBs and dioxins exposure of Venice lagoon aquatic organisms and high fish consumers from local population. Simulations were run for a maximum time period of about 27,000 days (74 years), from 1924 (birth year of the oldest individual) to 1998 (collection of human biomonitoring data). Both deterministic and probabilistic simulations were run. Since a long-term exposure scenario was considered, where environmental contamination by persistent pollutants and, consequently, food contamination (i.e., exposure of aquatic organisms in the aquatic food web) change over decades, the year of birth influences the overall internal exposure and it was thus necessary to run separate simulations for different individuals born in different years. The tool in fact does not permit to consider individuals born after the starting date of the simulation. Therefore, individual exposure simulations were run separately with MERLIN-Expo, taking into account the year of birth of study participants (from 1924 to 1972).

4.5. UNCERTAINTY ANALYSIS AND PARAMETER SELECTION

In addition to deterministic assessment, MERLIN-Expo enables an assessor to include uncertainty on input parameters by specifying probabilistic distribution functions (PDFs) for parameter values. For probabilistic analysis, parameters that have been assigned PDFs were selected. 5000 probabilistic simulations of the full model chain were run using Monte Carlo sampling scheme for the period of 24,091 days at 100-

day time step. As a human target, a high-fish consumer individual born in 1932 was selected, so the time period represents 66 years of this individual's lifetime. Environmental and biomonitoring measurement data are available for year 1998, hence it was considered as a final time in all modelling exercises. Considering the five MERLIN-Expo models selected and coupled for this case study, in total 156 parameters were included in the probabilistic assessment.

Two invertebrate species, *Tapes philippinarum* (Manila clam), *Carcinus mediterraneus* (green crab), and two fishes, *Chelon labrosus* (mullet), and *Zosterisessor ophiocephalus* (goby), were included in the probabilistic assessment of time-varying whole body internal concertation of 2,3,7,8-TCDD and PCB126 over the period 1932-1998. The uncertain input parameters used to estimate accumulated contaminant concentration in aquatic biota are represented as probability distributions based on literature review or analysis of available datasets (e.g., phytoplankton lipid content and cell volume) (Hauck et al., 2007, 2011; Hendriks, 1999, 2007; Olenina et al., 2006; Seth et al., 1999). The parameters representing generic trophic levels (fish, invertebrates, and phytoplankton) were probabilistically estimated, providing uncertainty in calibration data, by Hauck et al., (2011), by comparing observed and estimated rate constants for physiological and chemical uptake and elimination. Parameters were originally grouped into three categories reflecting different approaches to their estimation. The first group includes independent parameters whose values can be determined independently from transport coefficients and partial resistances. These parameters are: lipid fraction of organism and its food, fraction of food assimilated, and allometric rate exponent. However, we used organism specific data to parameterize the lagoon food web. The second group was defined as transport coefficients and consists of transport of water through the organism, transport of food through an organism, and the production of biomass – their values were estimated from allometric data. The third group of parameters includes partial resistances, which were derived by comparing the measured and estimated chemical rate constants and minimizing the differences by maximum likelihood estimation (Hauck et al., 2011; Hendriks, 2007).

Contaminant-specific parameter values were derived using QSAR models implemented in EPI Suite software (USEPA, 2012): metabolic half-life of chemicals for organics (Arnot et al., 2008, 2009a), bioconcentration factor for organics (Arnot and Gobas, 2003, 2006), water-organic carbon partition coefficient (Schüürmann et al.,

2007), and octanol/water partition coefficient (Meylan and Howard, 1995). PDFs were estimated based on the primary source where the given QSAR was developed. Assuming identical, independent and normally distributed errors, the uncertainty in a QSAR prediction (X_p) was deduced from reported predicted mean $(\overline{X_p})$, standard error of prediction $\left(SE(\overline{X_p})\right)$, and estimated student t-distribution based on reported number of data in a training set *n* and a number of descriptors *k* used originally in QSAR development (t_{n-k-1}). Described method is given by (Equation 8):

Equation 8

$$X_p \sim \overline{X_p} + t_{n-k-1} \cdot E(\overline{X_p})$$

The probabilistic parameterization of the models is described in details in MERLIN-Expo documentation available on the dedicated website (http://merlin-expo.eu/learn/documentation/model-documentation/).

The 'Human intake' model was used to calculate the total ingested quantity of contaminants from contaminated food, i.e., 'Ingestion rate for food'. It is a step function of age used to assign different rates for different age groups as 'Age group ingestion rate for food' time series. MERLIN-Expo was not designed to assign uncertainty to time-dependent inputs such as age-dependent quantity of ingested food, therefore the 'Human Intake' model was not included in the uncertainty assessment. As for the 'Man' model, body weight and tissue-blood partition coefficients were included in uncertainty analysis. Individual's bodyweight is calculated as a function of age in order to include inter-individual bodyweight variability among same aged persons. Body weight is normally distributed, described by mean and standard deviation (Beaudouin et al., 2010). A tissue-blood partition coefficient is defined as the equilibrium factor represented by ratio of the concentration in a tissue to the concentration in blood. The partition coefficient is a normally distributed variable with mean and standard deviation. Statistics were obtained from reported values in Plowchalk et al., (1992); Shin et al., (2009); Björkman et al., (1996); Ishizaki et al., (1991); Björkman et al., 1990, 1994; Csanády et al., (2002); Gearhart et al., (1993). Several normal probability distribution functions were parameterised for different tissues, separately for 2,3,7,8-TCDD and PCB126 that can be found in the PBPK model documentation (http://merlin-expo.eu/learn/documentation/model-documentation/).

4.6. SENSITIVITY ANALYSIS EXPERIMENT DESIGN

MERLIN-Expo allows the user to select among several tools to perform sensitivity analysis, including local sensitivity analysis methods, screening methods based on optimized experimental designs, global regression methods, global variance-based methods. Sensitivity analysis is the study of how the variation in the output of a model (numerical or otherwise) can be apportioned, qualitatively or quantitatively, to different sources of variation. Sensitivity analysis highlights the inputs that have the greatest influence on the results of a model, therefore, it provides useful insights for model builders and users. Insights from sensitivity analysis can be used for: (i) identification of key sources of uncertainty, (ii) identification of key controllable sources of variability, and (iii) model refinement. SA methods available in MERLIN-Expo were organised in a stepwise structured approach, by starting from computationally 'inexpensive' Morris method to most costly variance-based methods. Since higher tier methods are targeted on those uncertainties that have most influence on the assessment outcome, one can use the screening step (Morris method) to narrow number of input factors to those that are most influential, so that time needed to run final step (e.g., FAST, EFAST, and Sobol methods) can be shortened. The Morris screening method followed by regression based method and EFAST were applied in order to first reduce the number of parameters and then produce three sensitivity measures standardised regression coefficient β_i, first order sensitivity and total order sensitivity indices (S_i and TS_i).

4.6.1. The Morris screening method

Only a summary of the Morris method is presented in this paragraph, the complete description can be found in Morris (1991). The Morris method is a one-factor-at-a-time (OAT) method where the impact of changing the values of each factor (input parameter) is evaluated one by one in each run. It is a qualitative method providing a ranking of input parameters in order of importance but not a decomposition of the output variance. The Morris method is categorized as a global sensitivity analysis because the method covers the entire ranges over which the factors may vary. In the method based on OAT, each input factor may assume a discrete number of values which are selected within the factor's range of variation, and only one input parameter (x_i) is modified by a fixed factor. Each parameter uncertainty interval is first divided into p equally large layers, due to generating a hyperspace Ω, identified by a n-dimensional

p-level grid, where n is the number of parameters. In the Morris method, uncertain parameters are considered to be uniformly distributed, subsequently transformed to the original distribution, which will be used in the model. Model simulation is performed based on a selection of parameters randomly sampled from the previously defined grid. Next, a single parameter is randomly selected and modified by a fix factor Δ, and a second simulation is performed. The Δ is a value in $\{1/(p-1), \ldots, 1-1/(p-1)\}$, but a more economical design is suggested with $\Delta = p/[2(p-1)]$. The model is evaluated for *r* trajectories within the parameter space. The starting point of a trajectory is selected randomly. For each trajectory, every single parameter is changed separately, whereas the new point of this trajectory is an element of the parameter space (Specka et al., 2015). Morris proposed a measure called elementary effects $EE_y(x_i)$ based on calculating for each input $X = (x_1, \ldots, x_n)$ a number of incremental ratios (Equation 9) from which basic statistics are computed to derive sensitivity information.

Equation 9

$$EE_y(x_i) = \frac{y(x_1,\ldots,x_i+\Delta,\ldots,x_n) - y(x_1,\ldots,x_i,\ldots,x_n)}{\Delta}$$

This procedure is repeated r times, which is equal to the sampling number, providing r elementary effects for each parameter. The cost of running screening test is based on the following relation $r(n+1)$. The method can distinguish between factors with negligible effects, linear and additive effects, and factors with non-linear or interaction effects. For each elementary effect $EE_y(x_i)$ two sensitivity measures are computed: μ_i, which assesses the overall influence of the factor on the output, and σ_i, which estimates the non-linear effect and/or the interaction effect with other factors. To classify parameters sensitivity, μ_i values must always considered together with $\sigma_i$values. Campolongo et al., (2007) suggested the use of mean of the absolute elementary effects μ_i^* as to avoid cancelling of positive and negative effects. Morris proposed a method, which is particularly well-suited when the number of uncertain factors is high and/or the model is expensive to compute.

4.6.2. Regression-based methods

Regression sensitivity analysis is performed on probabilistic simulation outputs, and sensitivity indices are calculated after Monte Carlo simulation. The space of the input

factors is sampled via the Monte Carlo method and a linear regression model is built from the model output values. In the regression method, the Standard Regression Coefficients (β_i) quantifies the effects caused by changing a model parameter from its mean by a fraction of its variance, while all others are kept at their initial values. This measure relates directly the sensitivity of the model outputs to the model parameters and are valid only when a model is linear.

The coefficient of determination (denoted by R^2) is a key output of regression analysis. It is interpreted as the proportion of the variance in the output variable that is predictable from the input parameters.

Model coefficient of determination given by

10, can be used for instance to identify nonmonotonic relationships between input and output.

Equation 10

$$R_Y^2 = \sum_{i=1}^{N} \frac{(Y_i^* - \mu_Y)^2}{(Y_i - \mu_Y)^2}$$

Where:

N number of simulations

Y_i the simulation results

Y_i^* is the Y_i derived from regression model

μ_Y mean of output Y

R^2 can take a positive number between (0, 1). R^2 coefficient higher than 0.7 demonstrates that variation in the model output is explained by linear regression.

The standardised regression coefficient is derived from (Equation 11):

Equation 11

$$\beta_i = \left(\frac{\sigma_{X_i}}{\sigma_Y}\right) / b_i$$

Where:

σ_{x_i} standard deviation of the input

σ_y standard deviation of the output

b_i is the estimate of the regression coefficient

Squared standardised regression coefficient (β_i^2) can be derived to show percentage of influence of the parameter on the output's variation (Hall et al., 2009).

4.6.3. EFAST

Extended Fourier Amplitude Sensitivity Test (EFAST) (A. Saltelli, Tarantola, & Chan, 1999) is a variance-based global SA method, which computes the Total Sensitivity Indices (TS_i) of the model inputs. The TS_i measures the main (first order) effect of each individual or a group of inputs on the model output, as well as all higher order effects (i.e. considering interactions) that can be attributed to that parameter. The EFAST method is based on mono-dimensional decomposition of the model along the search curve in the n-dimensional parameter space. The search curve is defined by a set of parametric equations. The range of variation in EFAST is explored for all parameters simultaneously.

Total sensitivity indices not only consider the main effects of inputs but also take into account interaction effects. EFAST is independent of any assumptions regarding the relationship between input parameters and outputs. It provides the fraction of the output variance due to each input parameter. EFAST can compute both first-order (Equation 12) and total sensitivity indices (Equation 13).

Equation 12

$$S_i = \frac{V_{x_i}(E_{x_{-i}}(y|x_i)}{V(y)}$$

Where:

S_i is the first order sensitivity index

V_{X_i} is variance of output due to parameter x_i

$V(y)$ is the total variance of output y

Equation 13

$$TS_i = \sum_{k\#i} S_k$$

Where:

TS_i is the total sensitivity index

$\#i$ represents all of the sets containing index i

4.7. EVALUATION OF BIOACCUMULATION MODEL PERFORMANCE

The performance of the aquatic food web models was evaluated according to the approach proposed by Arnot and Gobas (2004). Model performance can be expressed quantitatively using model bias (MB_j) calculated for all n chemicals in a single species j (Equation 14):

Equation 14

$$\mathrm{MB_j} = 10^{\left(\sum_{i=1}^{n} \frac{[\log(\mathrm{BAF_{p,i}}/\mathrm{BAFo,i})]}{n}\right)}$$

where BAF_p, BAF_o are predicted and observed bioaccumulation factors, and subscripts *i,* and *j* refer to number of chemicals and species respectively. An overall model performance for all *m* species (MB) can be calculated as follows (Equation 15):

Equation 15

$$\mathrm{MB} = 10^{\left[\sum_{j=1}^{m} \frac{\left(\sum_{i=1}^{n} \frac{[\log(\mathrm{BAF_{p,i,j}}/\mathrm{BAFo,i,j})]}{n}\right)}{m}\right]}$$

4.8. ESTIMATION OF HAZARD QUOTIENT

From a regulatory or risk assessment perspective, ecological and human exposure estimates provided by MERLIN-Expo can be evaluated against existing quality standards or threshold values with the aim of deriving an estimate of risks posed to the selected ecological or human targets by the considered environmental contaminants, under the assessed scenario(s).

As for ecological risk assessment, traditionally environmental regulation relies on

media-based quality guidelines, where chemical concentrations in water or sediment are used to quantify environmental risks considering selected target species or the entire ecosystem. However, water quality criteria for bioaccumulative chemicals should incorporate several specific perspectives in order to be sufficiently protective, such as the consideration of chemical uptake through multiple exposure routes including the diet, long term exposure scenarios and inclusion of all factors affecting chemical bioavailability and bioaccumulation (Sappington et al., 2011). To address all these needs, the tissue residue approach (TRA) has been proposed, based on the use of tissue residue as dose metric when evaluating exposure–response relationships (Meador et al., 2011).

The Oregon Department of Environmental Quality (2007) proposed a Critical Tissue Level (CTL) for fish equal to $6.4 * 10^{-6}$ mg/kg $_{fw}$ for dioxins and PCB in both freshwater and marine environments, expressed as 2,3,7,8-TCDD Toxic Equivalent (TE). CTLs correspond to concentrations in tissue at or below which approximately 95% of aquatic organisms bearing this residue would be highly unlikely (b 5% chance) to experience adverse health effects.

To obtain an estimate of ecological risk for each fish species, a Hazard Quotient for all dioxins and dioxin-like PCBs can be calculated by comparing the exposure concentration (expressed as sum of Toxic Equivalents for the investigated substances) with the toxicity threshold (i.e., CTL value), as follows (Equation 16):

Equation 16

$$\mathrm{HQ_i} = \frac{\mathrm{TEQ_i(PCDDs + DL - PCBs)}}{\mathrm{CTL}}$$

where *i* is the fish species. Toxic Equivalent Factors as defined by WHO for fish species have been used (Van den Berg et al., 1998).

CHAPTER 5 RESULTS AND DISCUSSION

5.1. DETERMINISTIC ANALYSIS

The aquatic food web models included in MERLIN-Expo provided as output of the deterministic simulation the time trend of concentrations from 1924 to 1998 of all target chemicals in aquatic organisms included in the Venice lagoon food web.

5.1.1. Environmental Exposure

Time dependent concentrations of 2,3,7,8-TCDD and PCB126 in selected species are reported in Figure 8 and Figure 9 respectively. The results, expressed on a fresh weight basis, show the highest accumulated concentrations for phytoplankton for 2,3,7,8-TCDD and for *Tapes philippinarum* (Manila clam) for PCB126. The lowest concentrations are obtained for *Sparus aurata* (Sea bream) for 2,3,7,8-TCDD, while the species showing the lowest internal concentrations of PCB126 is *Dicentrarcus labrax* (Sea bass). Time dependent concentrations in biota reflect the shape of time trend of contamination in sediment and water compartment, although with appreciable differences in the magnitude of peaks depending on the specific biological and physiological characteristics of each animal and on physico-chemical characteristics of target chemicals. Despite the trend generally observed in dioxins and PCBs environmental contamination, in the case at hand it is possible to notice that each considered substance follows an individual, specific trend, with peaks in specific decades, which is reflected by simulated concentrations in aquatic biota.

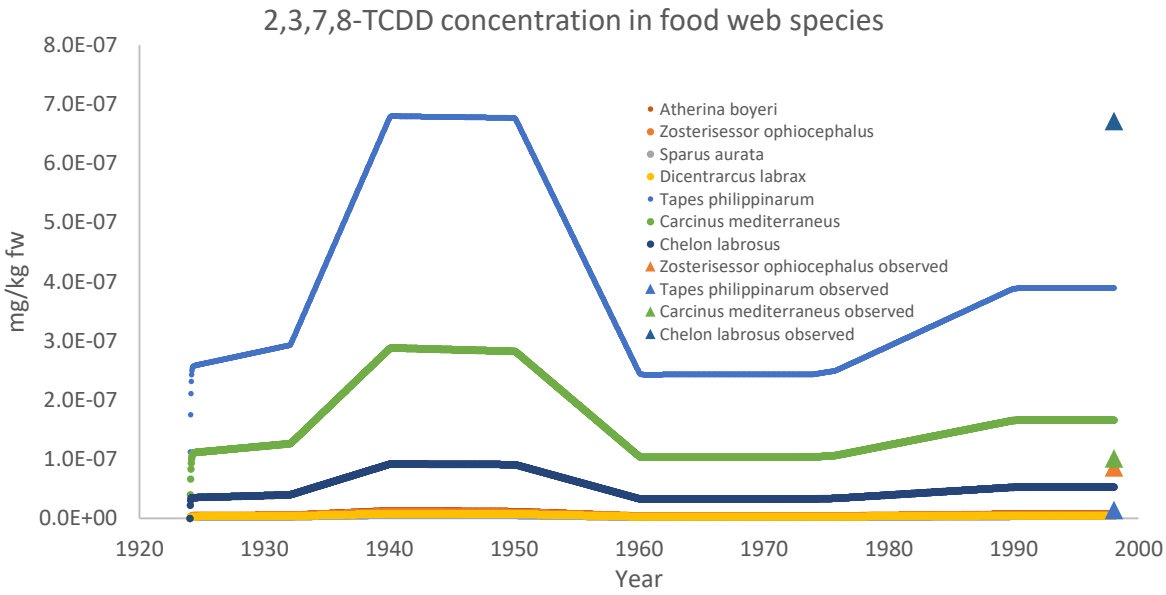


Figure 8 Modelled concentrations of 2,3,7,8-TCDD in selected organisms of the Venice aquatic food web.

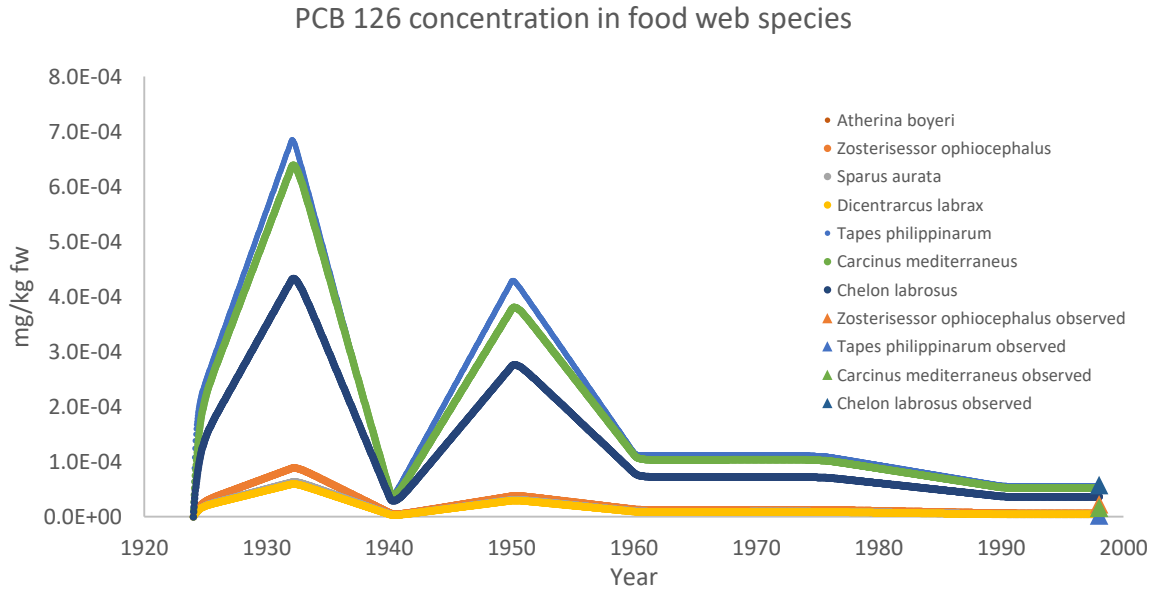


Figure 9 Modelled concentrations of PCB126 in selected organisms of Venice lagoon food web.

With the aim of obtaining a preliminary evaluation of model performance, the estimated concentrations in aquatic organisms were compared to available measurements in organisms sampled in the central lagoon area and in northern lagoon area (in the case of mullet) in 1998 (Venice Water Authority, 1999). Number of sampling locations and number of sampled individuals for each species are reported in Table 9.

Table 9 Number of selected sampling sites in central lagoon and total sampled organisms for chemical concentration measurements in aquatic organisms (Venice Water Authority, 1999).

Species	N° of sampling sites	N° of sampled organisms
Tapes philippinarum (Manila clam)	3	70
Zosterissessor ophiocephalus (Goby)	1	10
Carcinus mediterraneus (Crab)	1	50
Chelon labrosus (Mullet)	4	18

In Table 10, the comparison of measured and simulated concentrations of target chemical compounds in aquatic species is reported. Since biota samples were collected in 1998, simulated concentrations for the same year were selected for the comparison.

Table 10 Comparison of measured and modelled concentrations of chemicals in selected aquatic species (mg/kg $_{fw}$).

	Concentration in aquatic species (mg/kg $_{fw}$)							
	Tapes philippinarum		***Carcinus mediterraneus***		***Chelon labrosus***		***Zosterissessor ophiocephalus***	
Chemical	Measured [a]	Simulated	Measured	Simulated	Measured	Simulated	Measured [b]	Simulated
2,3,7,8-TCDD	1.40E-08*	3.90E-07	1.01E-07	1.66E-07	6.72E-07	5.27E-08	8.58E-08	6.42E-09
1,2,3,7,8-PCDD	2.13E-08*	1.96E-06	1.86E-07	8.33E-07	7.20E-07	2.80E-07	1.54E-07	2.03E-08
1,2,3,4,7,8-HCDD	4.21E-08*	5.35E-06	1.29E-07	2.09E-06	1.54E-07*	7.16E-07	4.76E-08	2.65E-08
PCB 77	1.77E-05	3.57E-04	1.53E-04	2.55E-04	2.64E-04	1.41E-03	1.13E-05	4.88E-04
PCB 126	2.30E-06	5.67E-05	1.62E-05	5.26E-05	5.79E-05	3.65E-05	2.26E-05	6.78E-06
PCB 167	5.37E-05	2.59E-03	5.44E-04	2.12E-03	1.27E-03	1.37E-03	3.31E-04	1.08E-04
PCB 169	2.85E-07	1.68E-05	3.46E-06	1.45E-05	5.28E-06	1.14E-05	6.09E-06	8.90E-07
PCB 170	1.49E-04	8.01E-03	9.31E-04	5.44E-03	5.31E-03	4.24E-03	1.98E-03	1.61E-04
PCB 180	3.91E-04	1.47E-02	2.44E-03	9.82E-03	1.01E-02	7.32E-03	3.85E-03	2.86E-04

Notes:
[a] mean value estimated from data in three sampling sites; [b] mean value estimated from data in four sampling sites.
** at least one of the considered measurement values was below the limit of detection (LOD); value equal to half LOD has been used in the calculation of the mean.*

The observed bioaccumulation factor (BAF_o) was calculated for each species and each chemical as the ratio of the measured concentration in the organism and the corresponding concentration in water, while the predicted bioaccumulation factor (BAF_p) has been calculated as the ratio of the simulated concentration in the organism and the corresponding chemical concentration in water. Figure 10 shows observed versus predicted logBAFs for all target chemicals and for *T. philippinarum*, *C. mediterraneus*, *C. labrosus* and *Z. ophiocephalus*.

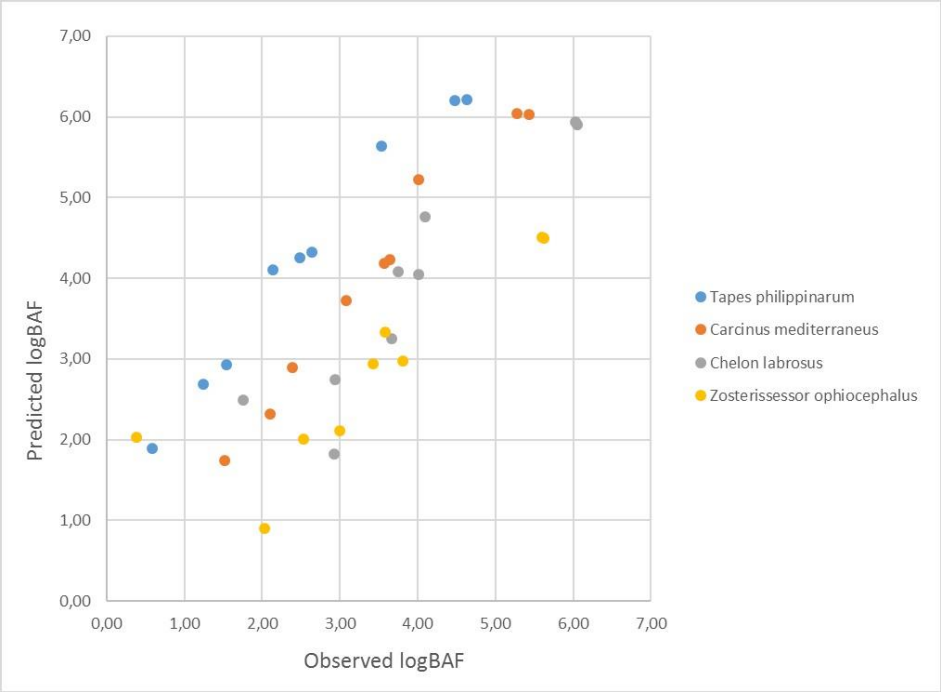


Figure 10 Observed versus predicted log bioaccumulation factors (logBAF) for the target chemicals for the species Tapes philippinarum, Carcinus mediterraneus, Chelon labrosus and Zosterisessor ophiocephalus.

In general terms, a model tends to over-predict when MB>1 and tends to under-predict when MB<1. MB is a geometric mean of the log-normally distributed ratio BAF_p/BAF_o, of all chemicals in all species. Therefore, the 95% confidence interval (CI) of the geometric mean represents the accuracy of the model. MB and its 95% CI include the following sources of error: model parameterisation, model structure, also errors in analytical and empirical data. The analysis of changes in MB values can be used as an indicator of model performance under various scenarios. The calculated model bias indicators for each species and the overall model bias are reported in Table 11.

Table 11 Indicators of model performance for single species and for the overall model.

	Tapes philippinarum	***Carcinus mediterraneus***	***Chelon labrosus***	***Zosterisessor ophicephalus***
Model bias (MB_j) for single species	46.05	3.97	0.95	0.30
Overall model bias (MB)	12.82			

It is possible to observe that the model overestimates of at least one order of magnitude chemical concentrations, and accordingly logBAF, for the species *T.philippinarum* for all considered chemicals. This is confirmed by a high value of the model bias estimated

for this species. It is worth mentioning that the sampling sites where clams were collected was not exactly close to the sampling site of the considered sediment core. Since clams are sessile organisms (not moving across different areas as fish species), the distance between sediment and biota samples might affect significantly the comparability of modelling and monitoring concentrations. It was therefore decided to test the model on an additional set of data, including sediment concentrations (surficial sediment, first 15 cm) and clam concentrations in the same location from the same monitoring campaign, considering only one-time point for 2003 (data available from ICSEL project, 2003). The results are reported in Table 12, from which it can be noticed that the difference between measured and monitored data improved if compared with 1998 data.

Table 12 Chemical concentration in superficial sediment and Tapes philippinarum from ICSEL project (2003) and comparison with simulated concentrations in the same species estimated with MERLIN-Expo

Chemical	Concentration in sediment (mg/kg_{dw})	Concentration in *Tapes philippinarum* (mg/kg_{fw})	
		Measured	Simulated
2,3,7,8 -TCDD	4.00E-04	2.00E-05	3.90E-04
1,2,3,7,8-PCDD	4.00E-04	2.20E-05	6.03E-04
1,2,3,6,7,8-HCDD	6.00E-04	3.00E-05	1.61E-03
PCB 126	1.30E-05	2.00E-06	1.36E-05
PCB 167	1.20E-05	4.10E-05	9.12E-05
PCB 169	5.00E-06	2.00E-06	1.67E-05
PCB 170	9.95E-04	7.80E-05	9.01E-04
PCB 180	2.08E-03	2.26E-04	1.59E-03

Anyway, an overestimation of model predictions in comparison with measurement data can still be observed for *Tapes philippinarum*: further testing of the model on more extended datasets (from both temporal and spatial perspective) can help in understanding better the behaviour of the Invertebrate model for filter feeder organisms under different scenarios and support the identification of possible adjustments to improve its capability of approximate real bioaccumulation measurements. At the same time, it is possible to observe that results obtained for other species are encouraging and turn out to be quite consistent with measured concentrations in biota samples, with differences depending on the species and the individual chemical. This is particularly true in the case of *C. labrosus*, for which the estimated model bias achieves a value of 0.95, therefore quite close to the target value of 1.

The results for all fish species included in the simulated food web are reported in Table 13. It can be observed that the highest HQ is estimated for juveniles of *D. labrax* (equal to 0.27), but in general for all considered species the estimated HQ is below 1, meaning that no potential adverse effects for the considered species are expected as a consequence of the exposure to the two investigated contaminants.

Table 13 Calculation of Hazard Quotient for selected fishes considering dioxins and dioxin-like PCBs included in the ecological exposure estimation

Chemical		*Chelon labrosus*	*Zosterissessor ophiocephalus*	*Atherina boyeri*	*Sparus aurata*	*Dicentrarcus labrax*	*Dicentrarcus labrax juv*	*Sparus aurata juv*	*Chelon labrosus juv*
		Simulated concentrations (mg/kg $_{fw}$)							
2,3,7,8-TCDD		5.27E-08	6.42E-09	6.14E-09	2.38E-09	3.39E-09	1.27E-07	1.15E-08	1.22E-08
1,2,3,7,8-PCDD		2.80E-07	2.03E-08	2.20E-08	1.30E-08	1.88E-08	7.53E-07	3.10E-08	4.91E-08
1,2,3,4,7,8-HCDD		7.16E-07	2.65E-08	2.38E-08	2.21E-08	4.31E-08	1.39E-06	1.96E-08	6.06E-08
PCB 77		1.41E-03	4.88E-04	4.19E-04	3.47E-04	4.12E-04	3.42E-04	1.57E-04	4.73E-04
PCB 126		3.65E-05	6.78E-06	1.11E-05	8.64E-06	8.13E-06	2.63E-05	5.20E-06	1.34E-05
PCB 167		1.37E-03	1.08E-04	7.04E-05	1.09E-04	1.08E-04	5.76E-04	2.77E-05	5.76E-05
PCB 169		1.14E-05	8.90E-07	5.71E-07	9.52E-07	9.42E-07	3.69E-06	1.97E-07	5.56E-07
	WHO TEF for fish	***Concentration (mg TEQ/kg $_{fw}$)***							
2,3,7,8-TCDD	1	5.27E-08	6.42E-09	6.14E-09	2.38E-09	3.39E-09	1.27E-07	1.15E-08	1.22E-08
1,2,3,7,8-PCDD	1	2.80E-07	2.03E-08	2.20E-08	1.30E-08	1.88E-08	7.53E-07	3.10E-08	4.91E-08
1,2,3,4,7,8-HCDD	0.5	3.58E-07	1.33E-08	1.19E-08	1.10E-08	2.16E-08	6.97E-07	9.81E-09	3.03E-08
PCB 77	0.0001	1.41E-07	4.88E-08	4.19E-08	3.47E-08	4.12E-08	3.42E-08	1.57E-08	4.73E-08
PCB 126	0.005	1.83E-07	3.39E-08	5.56E-08	4.32E-08	4.06E-08	1.31E-07	2.60E-08	6.68E-08
PCB 167	0.000005	6.86E-09	5.39E-10	3.52E-10	5.46E-10	5.41E-10	2.88E-09	1.39E-10	2.88E-10
PCB 169	0.000005	5.71E-11	4.45E-12	2.85E-12	4.76E-12	4.71E-12	1.84E-11	9.86E-13	2.78E-12
SUM TEQ		1.02E-06	1.23E-07	1.38E-07	1.05E-07	1.26E-07	1.74E-06	9.42E-08	2.06E-07
HQ		**0.16**	**0.02**	**0.02**	**0.02**	**0.02**	**0.27**	**0.01**	**0.03**

5.1.2. Human Exposure

Taking into account the available human biomonitoring data, MERLIN-Expo has been applied to simulate lifetime internal exposure to 2,3,7,8-TCDD and PCB126 for a group of men classified as “high fish consumers” and born between 1924 and 1972 (Frangipane, 1999). The final outputs of interest provided by the Man model consist of time-dependent chemical concentrations in different human tissues and organs (e.g., blood, adipose tissue, brain, liver), but it is important to remind that MERLIN-Expo can provide additional intermediate outputs (e.g., total quantity of chemical ingested through the dietary pathway at different ages, quantity of chemicals excreted or

metabolised by the organism at different time), which can support the understanding of exposure pathways and toxicokinetic processes.

Figure 11 shows the changing lifetime concentrations of 2,3,7,8-TCDD in human blood for selected individuals born between 1924 and 1972, accompanied (in order to support the interpretation of results) by time trends of chemical concentrations in sediment and water from 1924 to 1998 used as input to the model chain (i.e., inputs to aquatic food web models). Figure 12 illustrates the results for PCB126. In general, the trend in environmental concentrations is in some way reflected into human internal exposure values, but it is “modulated” by absorption, distribution, metabolism and elimination processes regulated by chemical-specific characteristics (such as K_{OW} and metabolic half-life).

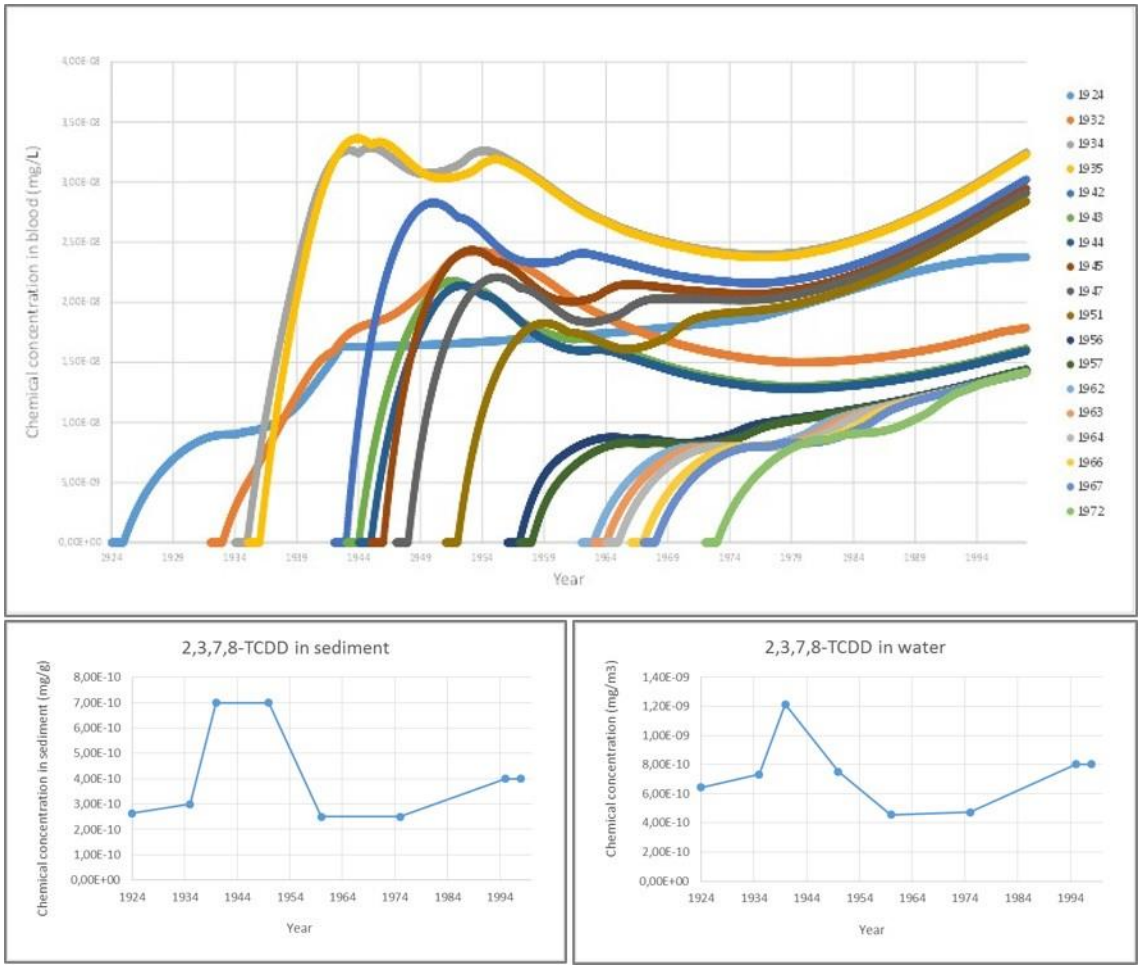


Figure 11 Lifetime concentrations of 2,3,7,8-TCDD in blood of high fish consumers born between 1924 and 1972.

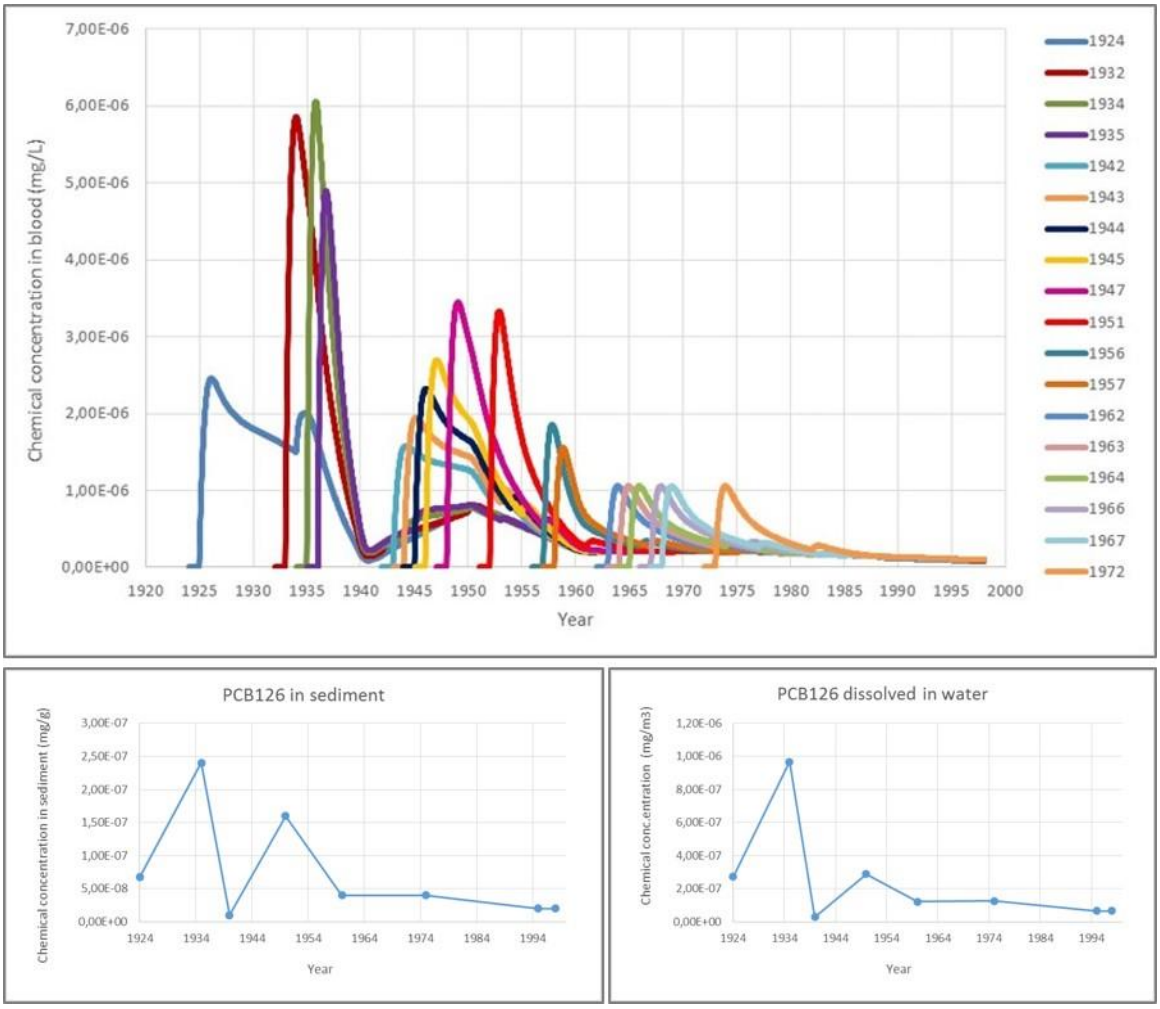


Figure 12 Lifetime concentration of PCB126 in blood of high fish consumers born between 1924 and 1972.

The chart in Figure 11 shows that individuals born after 1956 tend to have lower blood concentrations of 2,3,7,8-TCDD than individuals born before 1951. Body burden of PCBs and dioxins has been shown to increase with age (e.g., Hardell et al., 2010; Sweetman et al., 2000) but this is not the only factor significantly affecting the overall burden. From Figure 11 it is possible to conclude that trends in 2,3,7,8-TCDD concentrations in blood are not only related to the age of individuals but rather reflect a time-dependent chemical input profile, obtained as a combination of changing environmental (and food web) contamination and age-dependent dietary intakes. As for PCB126, lifetime concentrations illustrated in Figure 12 show a similar trend for all individuals, in most cases with a peak of different magnitude (depending on the year of birth) in the first years of life, followed by an overall decrease. These early life peaks can be observed also for 2,3,7,8-TCDD, even if in this latter case they are less evident. These peaks cannot be explained only by a higher level of food contamination,

because they are visible also when simulations with constant environmental concentrations over lifetime are run (a test was performed, without changing the values of other input data). These peaks can be associated to the use of an average daily intake of fish and seafood for children between 1 and 9 years, according to the available food intake statistics. This low resolution in intake rates for toddlers combined with the low weight in early life stages can explain the observed peaks and suggests a refinement of intake input data for young population groups whenever possible.

With the aim of evaluating the performance of applied models in reconstructing human internal exposure, simulated results have been compared to the available human biomonitoring data, i.e., concentrations of PCB126 and 2,3,7,8-TCDD in blood serum of 22 adult males living in Venice municipality and classified as “high fish consumers”. Since MERLIN-Expo provides concentrations of target chemicals in whole blood as output of the Man model, measured concentrations in serum have been properly transformed into equivalent concentrations in blood. Considering that in the case of PCBs and dioxins a significant fraction of chemical tends to distribute in blood serum (Schecter, 2012), the concentration in blood has been assumed half of the concentration in serum, as recommended by Health Canada (Tsuji et al., 2005) for PCBs. In general, the comparison between human biomonitoring data and simulated blood concentrations is not straightforward because cross-sectional data generated through biomonitoring studies are based on group of individuals sampled at the same time, while longitudinal estimates provided by MERLIN-Expo represent single individual over their whole lifetimes. Available biomonitoring data have been compared with the simulated concentrations (22 persons) for year 1998. In Table 9 the comparison of statistics for measured and modelled blood concentrations of 2,3,7,8-TCDD and PCB126 is reported. The available biomonitoring data for PCB126 follow a lognormal distribution, while 2,3,7,8-TCDD concentrations do not follow neither lognormal nor normal distribution (and a significant number of values was below the detection limit), therefore for sake of completeness in Table 9 different statistics are reported.

As an overall outcome, it can be observed that simulated data are in a relatively good agreement with measured data obtained from 1998 survey in Venice municipality from high fish consumers. Measured and simulated data have similar orders of magnitude, the geometric mean (GM) of simulated 2,3,7,8-TCDD values in blood is about three times the GM of measured values, while for PCB126 the geometric mean of simulated

values is one order of magnitude lower than GM of measured data.

It is noteworthy to remind the assumptions related to the assessment framework, which play a relevant role in influencing modelling results and have to be considered in their evaluation. First, a worst-case scenario was adopted in the assessment, where it was assumed that all fish and seafood consumed by the population were caught in a very contaminated area of the lagoon, very close to industrial emission sources. This worst-case assumption helps in exploring the upper bound of human exposure to target chemicals, but it also leads to an overestimation of blood concentrations in comparison with realistic exposure conditions (i.e., fish and seafood from different sources and probably from less contaminated areas, especially after the ban of fishing activities in front of Porto Marghera in the 1990s). At the same time, the contribution to chemical exposure from other food items such as meat or dietary products was not considered in the assessment. Even if fish and seafood can probably be considered among the most relevant sources of TCDD and PCB126 in the diet for high fish consumers, the exclusion of other dietary sources likely leads to an underestimation of internal exposure. Finally, it has to be considered that in the reconstruction of human exposure, only average value of daily intakes of fish and seafood for different age groups were used, since quantitative data on daily consumption of different food types were not available for each participant. This condition hampers the comparison of data at the individual level, because the model provides identical results for all individuals born in the same year if other parameters, such as food intake rates, are not varied.

Blood concentrations (mg/L) of 2,3,7,8-TCDD and PCB126 obtained by MERLIN-Expo simulation for individual high-fish consumers were converted into lipid-adjusted serum concentration by multiplying for a factor of two (blood to serum concentration conversion according to Health Canada, 2003) and by adjusting for the total lipid concentration of individual serum samples as reported by Frangipane (1999). TEQs concentration of PCB126 were obtained by multiplying the concentrations by PCB126 TEF, defined as equal to 0.01 for humans (WHO, 2005).

Table 14 provides a comparison of statistical values for the internal exposure estimates calculated by MERLIN-Expo with the selected BE. It can be noticed that HQ values are all below the value of 1, even in the case of the 95th percentile. However, it is important to remind that the HQ is calculated only taking into account two chemicals, 2,3,7,8-TCDD and PCB126, therefore the inclusion of other dioxins and other dioxin-like PCB congeners in the assessment might lead to higher values.

Table 14 Evaluation of internal exposure estimates of 2,3,7,8-TCDD and PCB126 obtained by MERLIN-Expo against Biomonitoring Equivalent for Dioxin TEQ.

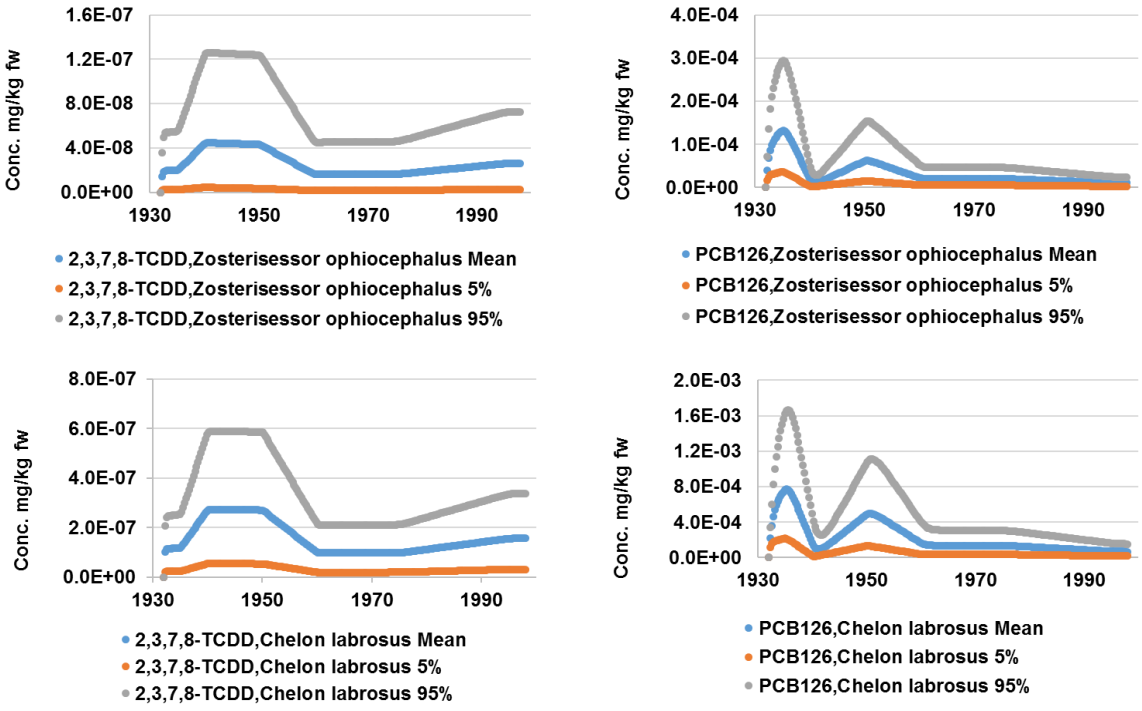
Dioxin TEQs estimated for 22 high fish consumers (sum of 2,3,7,8-TCDD and PCB126) – pg/g serum lipid				
	MEAN	**GEOMETRIC MEAN**	**95th percentile**	**Biomonitoring Equivalent (BE)**
	4,56	4,22	7,69	21
HQ	0,22	0,20	0,37	

5.2. PROBABILISTIC ANALYSIS

The procedure and simulation settings are described in § 4.5.

5.2.1. Environmental Exposure

The accumulation of 2,3,7,8-TCDD and PCB 126 in biota soft tissue was simulated over the time period 1932-1998 with a time step of 100 days. Mean concentrations and 5th and 95th percentile confidence interval are shown in Figure 13.



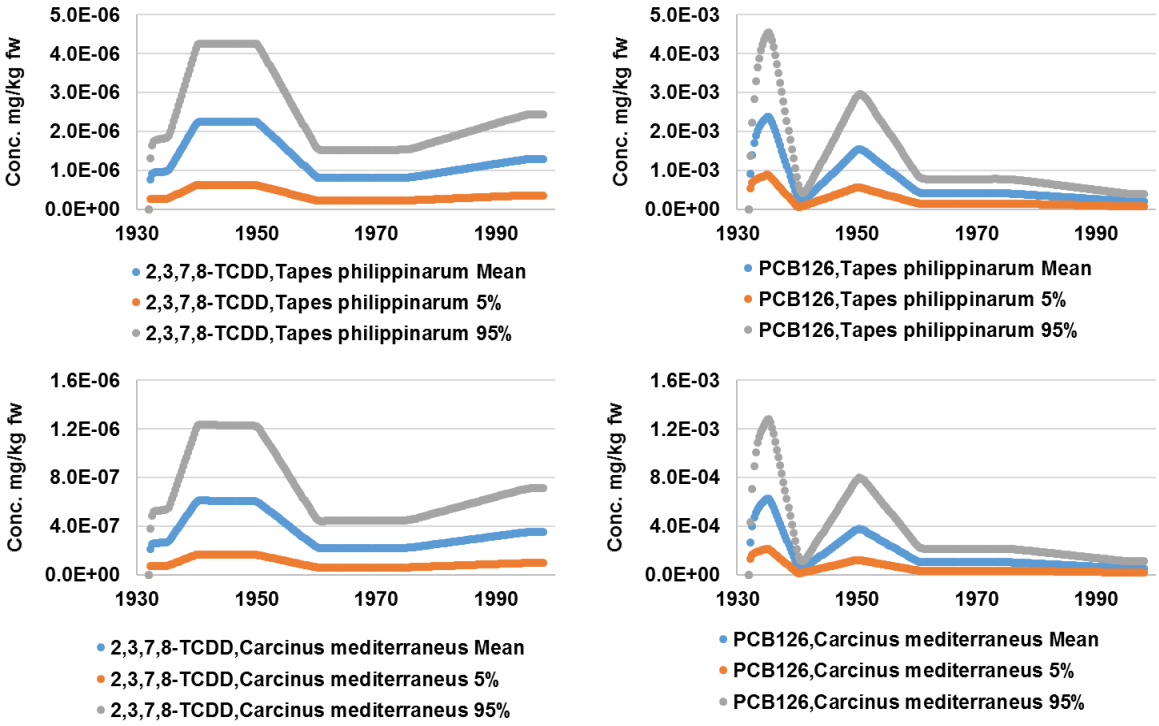


Figure 13 Simulated concentration of PCB126 (mg/kg fw) and 2,3,7,8-TCDD (mg/kg fw) including all routes of exposure and uncertainty ranges of internal concentration (95th-5th %tile interval) in: Tapes philippinarum, Carcinus mediterraneus, Chelon labrosus, and Zosterisessor ophiocephalus, over period 1932-1998.

The curve evolution is specific for the contaminant in question. The accumulated PCB 126 reaches different levels in different species but the concentration trend is similar for all four species. The same observation can be made for 2,3,7,8-TCDD: all species share similar internal concentration trend; however, they differ in the level of accumulated chemical. Two concentration peaks are observed for PCB 126 in all reported species, the first high peak in 1935 and the second, smaller one in 1952. After the second peak, the concentration decreases rapidly until 1960s, and steadily continues to decline for the rest of the simulated period until 1998 (Table 15).

Table 15 Mean concentration and lower and upper confidence intervals for PCB 126 and 2,3,7,8-TCDD at maximum concentration predicted in 1935 and 1940 and concentrations for both chemicals simulated in 1998.

Species	DETERMINISTIC		PROBABILISTIC Mean (5^{th}% ; 95^{th}%)		OBSERVED	
	PCB126 (mg/kg $_{fw}$)	**2,3,7,8-TCDD (mg/kg $_{fw}$)**	**PCB 126 (mg/kg $_{fw}$)**	**2,3,7,8-TCDD (mg/kg $_{fw}$)**	**PCB126 (mg/kg $_{fw}$)**	**2,3,7,8-TCDD (mg/kg $_{fw}$)**
Carcinus mediterraneus	5.26E-05	1.66E-07	5.2E-05 (1.7E-05 ; 1.1E-04)	3.5E-07 (9.6E-08 ; 7.1E-07)	1.62E-05	1.01E-07
Chelon labrosus	3.65E-05	5.27E-08	6.8E-05 (1.5E-04 ; 2.0E-04)	1.6E-07 (3.4E-07 ; 1.3E-06)	5.79E-05	6.72E-07
Tapes philippinarum	5.67E-05	3.90E-07	7.4E-05 (1.0E-05 ; 2.8E-06)	3.6E-07 (2.6E-08 ; 2.8E-09)	2.30E-06	1.40E-08
Zosterisessor ophiocephalus	6.78E-06	6.42E-09	2.4E-05 (1.7E-05 ; 1.1E-04)	7.3E-08 (9.6E-08 ; 7.1E-07)	2.26E-05	8.58E-08

The mean 2,3,7,8-TCDD concentration reaches its maximum in the early 1940′s, reaching a plateau lasting until early 1950s, when a sudden decrease can be observed continuing until early 1960s. Afterwards the concentration is maintained at the same level, and starts building up slowly from mid-1970s until 1998. Overall, PCB 126 accumulates in organisms to higher concentrations than 2,3,7,8-TCDD. Estimated whole body mean concentration of 2,3,7,8-TCDD and PCB 126 is higher for invertebrates in Tapes philippinarum (clam) comparing to Carcinus mediterraneus (green crab), and, among fishes, concentration in Chelon labrosus (mullet) is higher than in Zosterisessor ophiocephalus (goby).

The accumulation of PCB126 is burdened with lower uncertainty than uncertainty on accumulated 2,3,7,8-TCDD. The confidence interval in the case of PCB126, after reaching the second concentration peak, tends to diminish towards the end of the simulation, while uncertainty on accumulated 2,3,7,8-TCDD after mid-1970′s shows growth when approaching 1998. In general, uncertainty varies along the simulated concentration and follows the same behaviour. The difference between uncertainty ranges (95th–5th percentile) is always between 2 and 3x the mean value and invariable between species when all routes of exposure are considered.

5.2.2. Human Exposure

Figure 4 shows the uncertainty on 2,3,7,8-TCDD and PCB 126 concentration in man's blood. PCB126 concentration in blood reaches two distinctive peaks in early 1930's, right after person's birth, and in early 1950's (Figure 14). The former is roughly twice higher than the latter one. PCB126 concentration decreases as simulation approaches the end.

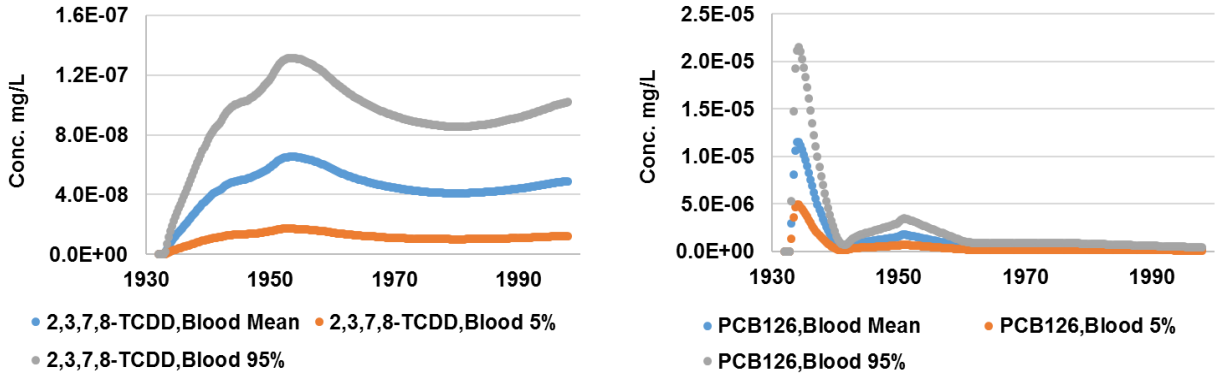


Figure 14 Simulated concentration of PCB126 (mg/L) and 2,3,7,8-TCDD (mg/L) and uncertainty ranges of internal concentration (95th-5th percentile interval) in man's blood over period 1932-1998.

The amount of 2,3,7,8-TCDD in blood slowly increases arriving to a maximum simulated value of $1.3x10^{-7}$ (mg/L) in 1950's. On the contrary, 2,3,7,8-TCDD accumulates in blood in higher concentration than PCB 126 at the final time of the simulation. In fact, Ruiz et al., (2014) noted that the concentrations of 2,3,7,8-TCDD in serum increases with age due to higher environmental dioxin levels in past exposure, the number of years of past exposure, and slower elimination among older persons. Results reported in § 5.1.2. on 2,3,7,8-TCDD levels in blood are in accordance with Ruiz observations. Similarly, to the results from ecological exposure assessment, PCB 126 has the tendency to accumulate to greater level than 2,3,7,8-TCDD (Table 16).

Table 16 Mean concentration and lower and upper confidence intervals for PCB 126 and 2,3,7,8-TCDD in human blood at maximum concetration predicted in 1935 and 1954 respectively, and concentrations for both chemicals simulated in 1998.

	Simulated concentrations of PCB126 in blood (mg/L)		**Simulated concentrations of 2,3,7,8-TCDD in blood (mg/L)**	
Blood	1935	1998	1954	1998
Mean	1.2E-05	2.0E-07	6.5E-08	5.0E-08
5%	4.9E-06	8.0E-08	1.7E-08	1.2E-08
95%	2.2E-05	3.9E-07	1.3E-07	1.0E-07

By comparing concentration trends in biota and environment (Figures A1-A4) one can arrive to the conclusion that the temporal evolution of internal concentration in aquatic organism is shaped mainly by the chemical concentration in exposure media, albeit more by the concentration of contaminants in sediments than in water. A significant drop in the accumulated concentration over simulation period in aquatic species and consequently in human blood can be observed, with regard to the concentration calculated when all food web bioaccumulation routes are active (2,3,7,8-TCDD down by 98% and PCB126 by 94%). Furthermore, exposure concentration in diet (seafood) affects computed temporal variation of concentration levels of the contaminants in blood (Figure A3 bottom pane).

A cross-correlation function (CCF) is used to show potential influence of the environmental concentration time series on the concentration in blood (Figure 15, right pane). The negative line segments correspond to events that are not correlated. In order to apply the cross-correlation function, concentrations in water and sediments were used as input time series and computed concentration in blood as output time series. A positive relationship with positive time lag is characteristic for the dioxin time trend in sediments and blood. Nevertheless, the correlation is weak, largest value at lag -10 reaches 0.49. Concentration of 2,3,7,8-TCDD in water poorly correlates with concentration in blood too. It weakly correlates at lag -10 (0.24), but mostly the lack of relationship is predominant with highest negative values at lag 10 (-0.78). Interestingly, it takes roughly 15 years for 2,3,7,8-TCDD to reach peak concentration in blood after the occurrence of the environmental peak exposure.

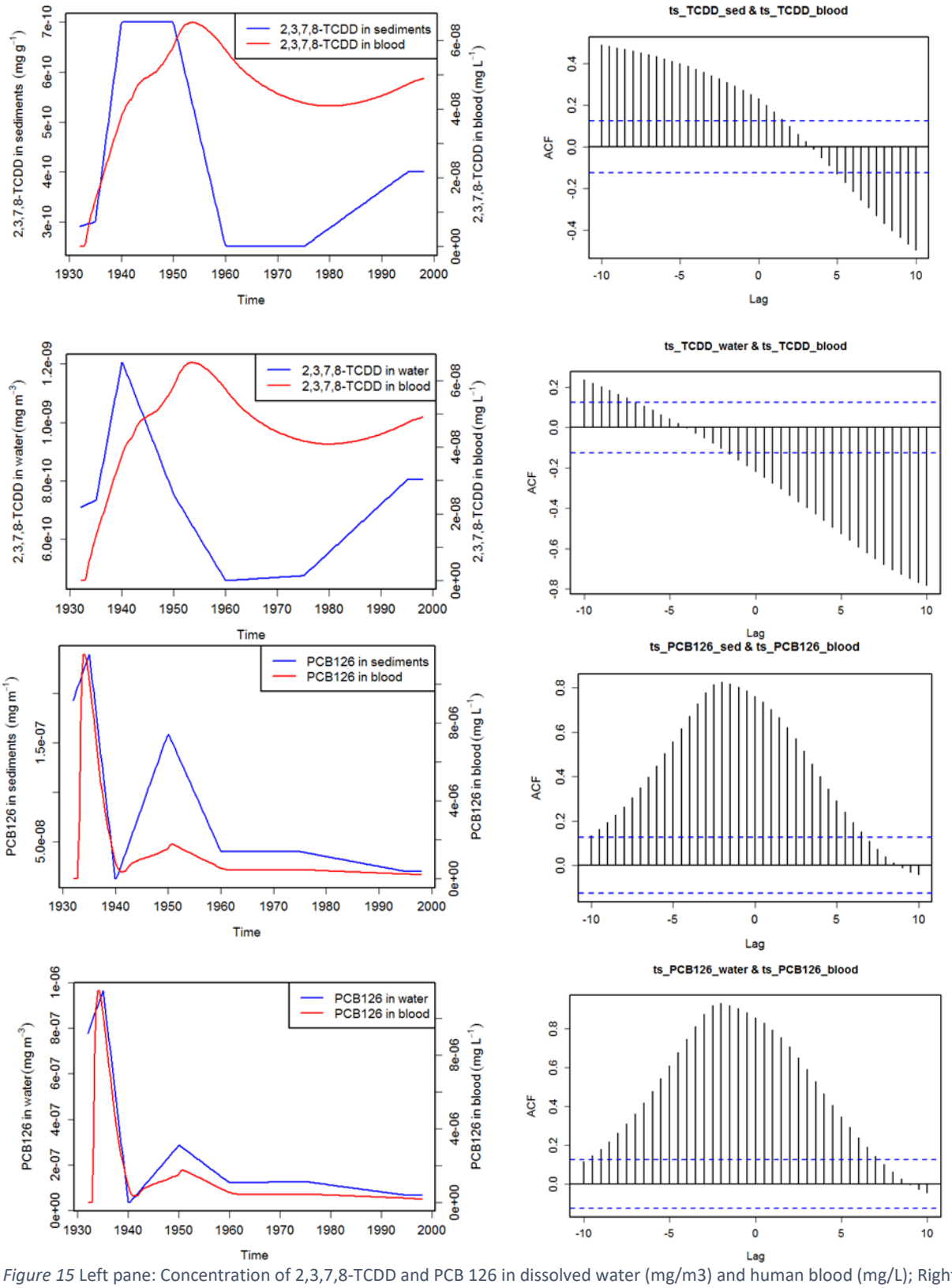


Figure 15 Left pane: Concentration of 2,3,7,8-TCDD and PCB 126 in dissolved water (mg/m3) and human blood (mg/L); Right pane: Cross-correlograms showing influence of chemical concentration in water on concentration in human blood. ACF is defined as autocorrelation function.

The simulated PCB126 concentration response is immediate with respect to the concentration in water and sediments. This is noticeable by pronounced strong positive

correlation over simulation period between both environmental concentration time trends and concertation computed in blood. High peak 0.93 points out strong correlation and it occurrence and lag -2.0 signifies that PCB's concentration in water and sediments slightly leads concentration in blood.

Several studies inform about environmental concentrations as a critical source of uncertainty in modelling bioaccumulation in aquatic food webs (De Laender et al., 2010; Ciavatta et al., 2009; Nfon and Cousins, 2007). It is also well recognised that human dietary exposure concentration together with information on food consumption are one of the most important sources of variability and uncertainty in dietary exposure assessment (Kettler et al., 2015; Kennedy and Hart, 2009). We stress that uncertainties in our reconstructed historical concentration trends are high and remain unquantified. Details on measurements of environmental input concentration and the method applied to calculate historical exposure concentration in water is described in § 5.1.2..

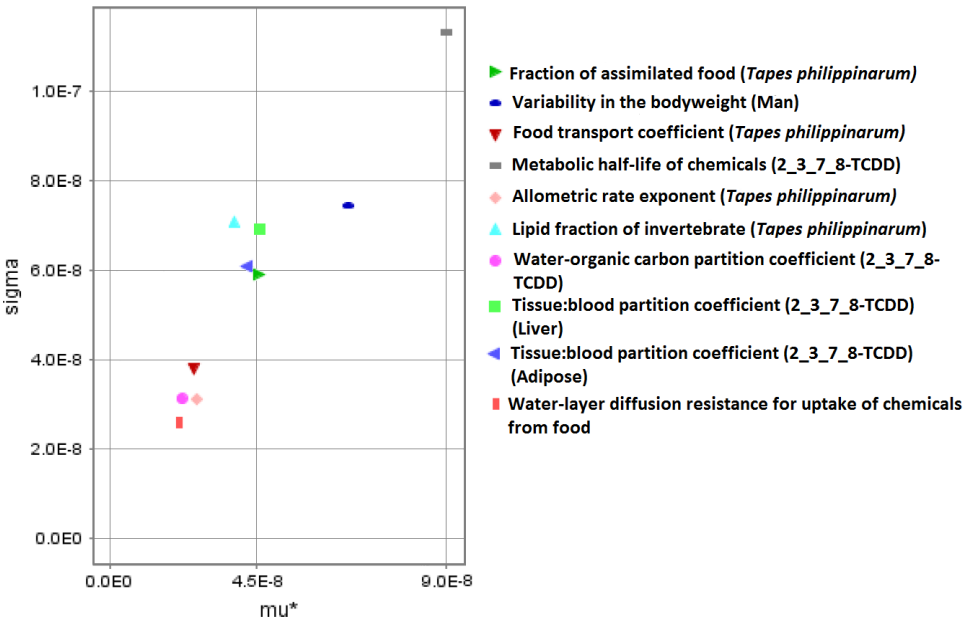
5.3. SENSITIVITY ANALYSIS

In order to account for all important model parameters and their effects, sensitivity analysis was performed as a sequence of methods. The presented results are show for most influential parameters with regard to 2,3,7,8-TCDD and PCB126 concentration in man's blood.

5.3.1. Morris method

The model was run for 24,091 days. 1790 model evaluations were run including 156 parameters in the analysis of PCB 126 and 2,3,7,8-TCDD in blood. Figure 16 presents parameters sorted according to their influence on simulated concentration of the substance in blood in 1998 (that is 24,091st day of the simulation). The results were used to reduce number of parameters to be included in further sensitivity analysis steps. Parameters with μ_i^* (mu*) higher than 2.0×10^{-8} and σ_i (sigma) higher than 1.0×10^{-8} were considered important for calculating concentration of 2,3,7,8-TCDD in blood. Factors deemed as significant for modelling PCB 126 in blood were restricted to those characterised by μ_i^* higher 5.0×10^{-8} and with σ_i higher than 7.0×10^{-8}. Regardless of the considered compound, chemical metabolic half-life and man's body weight were found to be the most important parameters. There are differences in the

two sets of influential parameters, for instance tissue-blood partition coefficient for adipose for 2,3,7,8-TCDD was noted as important but not for PCB 126. On the other hand, lipid content in zooplankton and phytoplankton seems to be more important in case of PCB 126 than for 2,3,7,8-TCDD. Overall, the results of the Morris screening method imply that parameters used in 'Invertebrates' bioaccumulation model for *Tapes philippinarum* (lipid fraction, food assimilation efficiency, water-layer diffusion resistance for uptake of chemicals from food, metabolic half-life of chemicals, allometric rate exponent, food transport coefficient) are predominant among influential parameters and matter most in calculating concentration in blood for both contaminants in question. Those parameters identified as important in the Morris methods (Figure 16) were used in further steps of the sensitivity analysis.



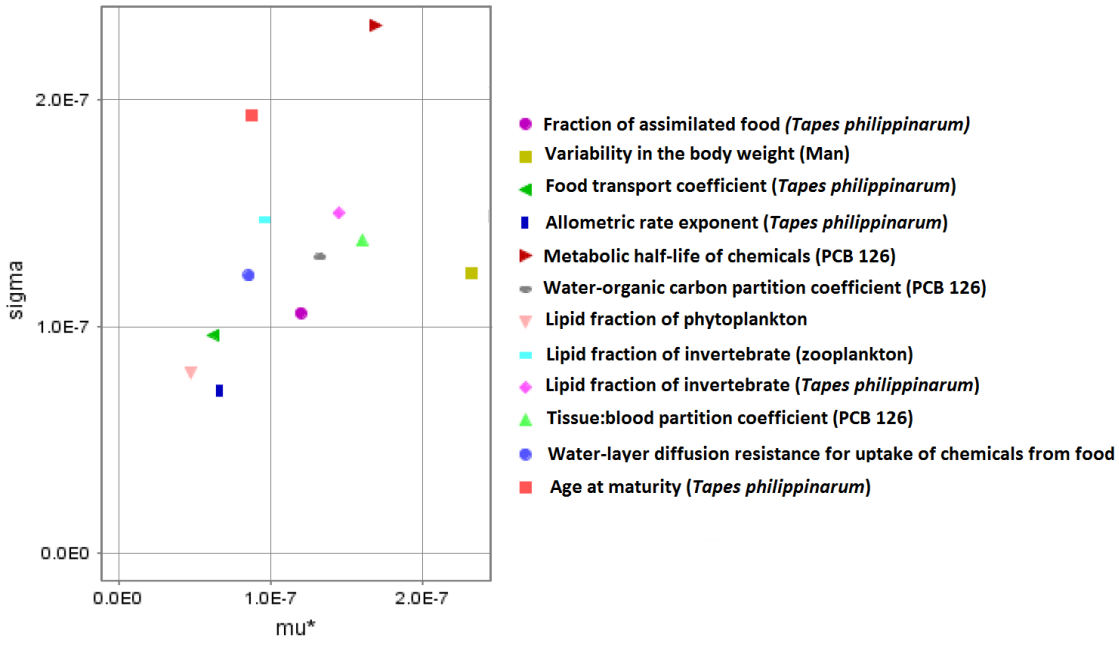


Figure 16 Most influential parameters for calculating 2,3,7,8-TCDD concentration and PCB126 concentration in human blood PCB 126 based on Morris method.

5.3.2. Results of regression-based analysis

A regression-based analysis to assess the influence of uncertain input factors on model output variance was performed using Monte Carlo sampling scheme by drawing 2000 samples. Correlations between 4 uncertain input parameters and probabilistically simulated 2,3,7,8-TCDD and PCB 126 concentration in man's blood are visualised on scatterplots (Figure 17). The parameters included in scatterplots were selected for each contaminant based on the highest mu* and sigma scores indicated in the Morris method. The examination of the scatter plots reveals various patterns between selected input parameters and model output, hence informing about various relationship. The scatter plots reveal metabolic half-lives and lipid content in Manila clam to be positively correlated with computed output, and negative correlation in case of variability in bodyweight and liver-blood partition coefficient with concentration of contaminants in blood. The standardised regression coefficient β_i^2, decomposed according to 10 input factors, captures 73% of the model output variance in case of computed 2,3,7,8-TCDD concentration in blood (Table A5). Table A6 shows individual β_i^2 values for 12 input parameters accounting for 71% of variation in computed PCB 126 concentration in blood. The quality of regression model is assessed by the R2 which for both computed chemicals in blood is above 0.7, indicating that the linear regression fits well model output and that an appreciable fraction of output variance

can be apportioned to linear component of the model (Manache and Melching, 2008). Nevertheless, 25% and 29% of the variation in computed concentration of 2,3,7,8-TCDD and PCB 126, respectively, remains unexplained by the β_i^2. Therefore, further analysis was applied to understand the contribution of uncertain parameters to model output variance.

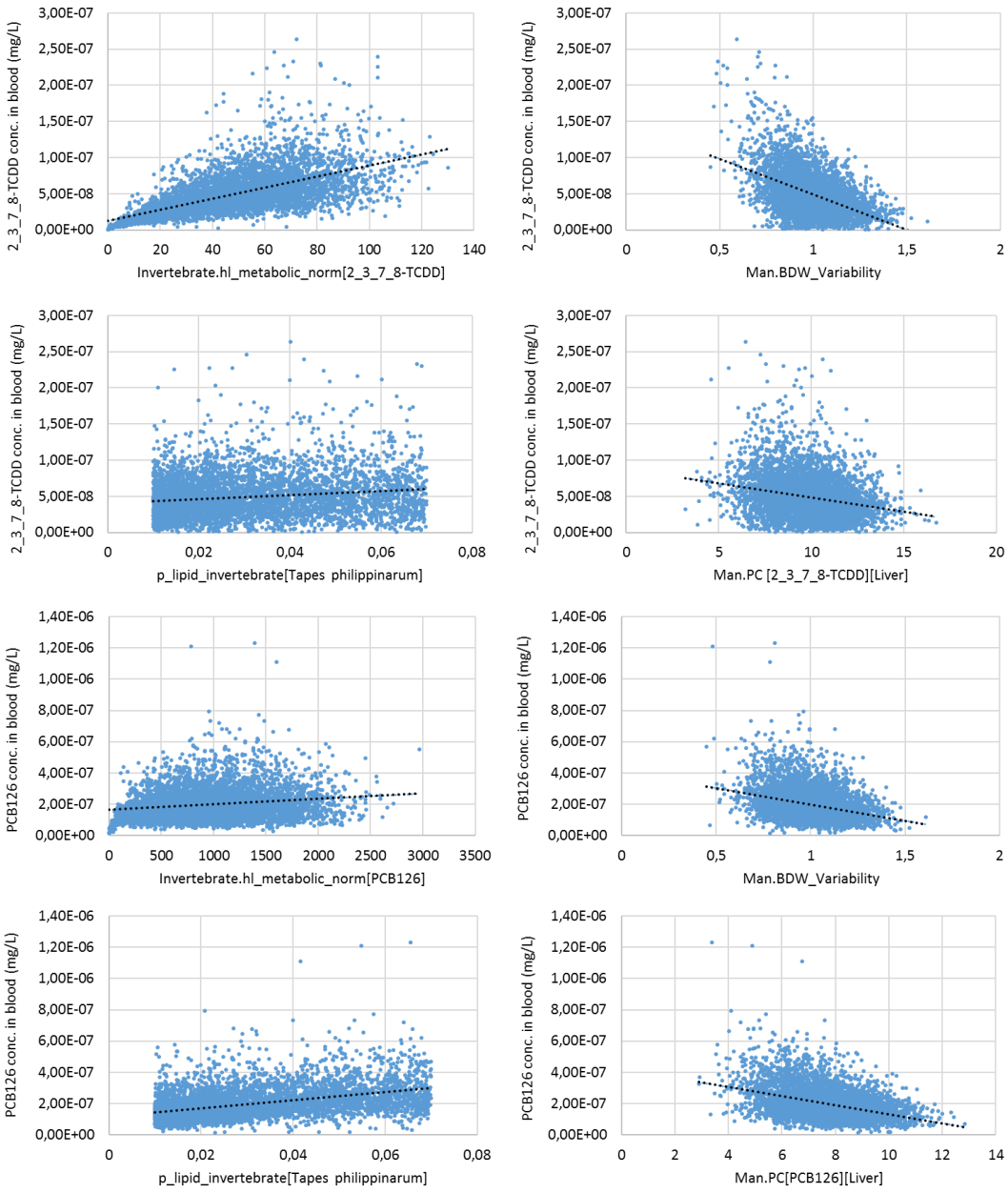


Figure 17 Scatter plots of concentration distribution of 2,3,7,8-TCDD (two upper rows) and PCB 126 (two bottom rows) in blood versus uncertain input parameters at simulation time 24091 (1998).

5.3.3. EFAST

The final step of the sensitivity analysis was performed on parameters selected during the screening step. First and total sensitivity indices (S_i, TS_i) representing the main effect and interactions between parameters were calculated using EFAST method with number of Fourier coefficients set to 4, and sampling size 1000.

5.3.3.1. 2,3,7,8-TCDD in blood

The sum of first order sensitivity indices (S_i) explains 76% of the model output variance and implies that the remaining 24% is due to higher order interactions taking place among the uncertain factors. Two parameters with the highest Si, metabolic half-life of 2,3,7,8-TCDD and variability in the bodyweight, account for 50% of the variation in the computed 2,3,7,8-TCDD concentration in blood.

The total sensitivity indices (TS_i) inform that the most important parameter for computing 2,3,7,8-TCDD concentration in blood is its metabolic half-life, which is used as an input parameter to 'Invertebrate' bioaccumulation model, and turned out to be responsible for 47% of the output variance. The second most important parameter is the inter-individual variability of the body weight, accounting for 24% of output's variation (Table A5). TS_i values computed for metabolic half-life and variability in body weight are respectively 15% and 6% higher than S_i indices, indicating small interaction among the parameters. All global SA methods consistently show metabolic half-life of 2,3,7,8-TCDD and variability in the bodyweight as the most influential parameters for computing 2,3,7,8-TCDD concentration in blood.

The time evolution of the total sensitivity index for a set of parameters is plotted in Figure 18. The key relations are the decreasing index for adipose tissue-blood partitioning coefficient and the increasing index for body weight. Also interesting is the increase in liver tissue-blood partition coefficient total sensitivity index: its importance begins to grow only starting from 1950's. Among parameters specific to aquatic biota, the allometric scaling parameter (kappa), used to model bioaccumulation in Manila clam, shows a significant drop from the beginning of the simulation.

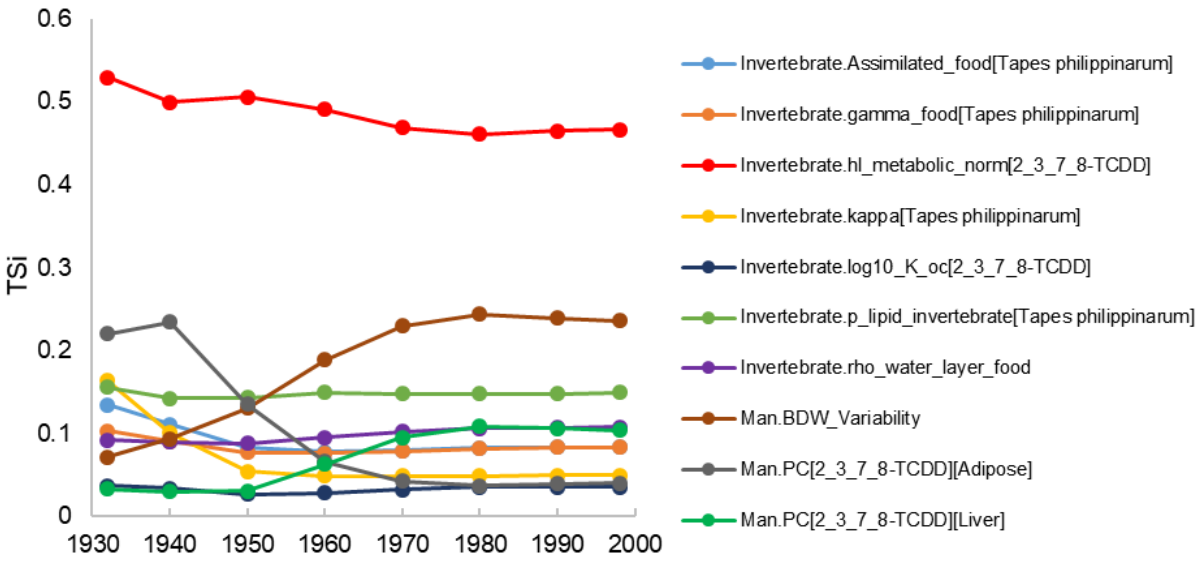


Figure 18 Change of total sensitivity index of 10 parameters over simulation time 1932 – 1998, considering as output the 2,3,7,8-TCDD concentration in blood.

Biotransformation half-lives of organic chemicals are known to affect exposure estimates in aquatic food webs (Arnot et al., 2010). Metabolic half-lives of 2,3,7,8-TCDD is burden with uncertainty attributable to QSAR modelling, which was originally intended to provide screening level predictions of the fish whole body biotransformation half-lives of chemicals restricted to model's applicability domain (Arnot et al., 2009b). In the applied PBPK model the bodyweight is expressed as a function of age in order to account for inter-individual variability of the bodyweight for persons of the same age (Bois et al., 2010). Variations of the bodyweight in adulthood are assumed to be variations of the volumes of the adipose tissues, possibly for that reason variability in the body weight is responsible for more variance in computing 2,3,7,8-TCDD concentration in blood than adipose tissue.

5.3.3.2. PCB126 in blood

The most important parameters detected by the three computed global sensitivity indices are lipid content and fraction of assimilated food specific to Manila clam (Table A6). S_i calculated for 12 input parameters arrives at 81% of variation leaving 19% to be explained by interaction between parameters. First order effects computed for lipid content and fraction of assimilated food capture together 35% of output variance. Total effects show that 28% percent of variation in the simulated internal concentration of PCB 126 is explained by uncertainty in lipid content of Manila clam (*Tapes*

philippinarum) and 22% is due to the fraction of assimilated food. It is interesting to note that contribution of these factors to the output variance through interaction is weak as the difference between TS_i and Si is small, respectively 8% and 7% for each of the parameter (Table A6). The estimated TS_i for the top three parameters reported in Table A6 captures 86 % of the variation in concentration of 2,3,7,8-TCDD, while 66% of variation in concentration of PCB 126 is explained by the three most influential parameters (Table A6). Overall, the fractions of variation in PCB 126 concentration in blood given parameters are responsible for, are less discernible than in the case of 2,3,7,8-TCDD.

The time evolution of total sensitivity index for the most influential parameters for accumulation of PCB 126 in blood does not show any significant changes over time, as depicted in Figure 19, possibly due to the weak interactions between parameters. Two bumps, one in 1940s and the other one smaller in 1960s, for several parameters (Figure 19) seem to be related to two distinctive spikes in environmental concentration of PCB126 in sediments and water.

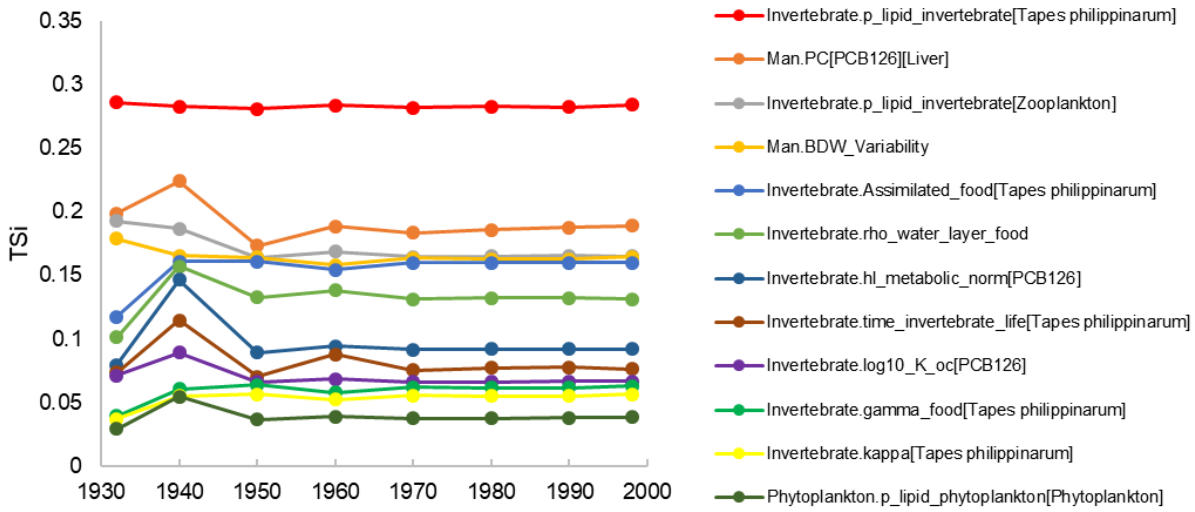


Figure 19 Change of total sensitivity index of 12 parameters over simulation time 1932 – 1998 considering as output PCB 126 concentration in blood.

The calculation of respiratory and dietary uptake and elimination kinetics of organic chemicals in aquatic species is based on parameters related to animal physiology, such as food assimilation efficiency, and partition of hydrophobic organic chemicals to lipid content, hence these factors are expected to have an effect on bioaccumulation and human exposure estimates. This is confirmed in our study of influential parameters where, indeed, parameters representing lipid content and food assimilation efficiency

are the most important ones. The estimation of PDF assigned to assimilation efficiency for Manila clam should attract more attention then, given that it was originally estimated as a generic factor describing efficiency of assimilation in aquatic invertebrates (and not specifically for clam).

Overall, sensitivity analysis yielded a R^2 value close to 0.7, that helps to classify model as quasilinear (Cariboni et al., 2007). S_i estimated around 80% for both chemicals implies that only a small part of output variation can be attributed to interaction between parameters. The fact that no particular differences between total and first order indices exist would confirm this observation (Saltelli, 2004).

One significant factor having the potential to strongly influence obtained results is the food intake rate for man, which for Manila clam is the highest among the considered seafood items (Figure 20) This may be the reason why five parameters directly related to the clam are relevant for estimating PCB 126 in man's blood and four for estimating 2,3,7,8-TCDD. Both chemicals are highly hydrophobic ($\log K_{OW\_2,3,7,8\text{-}TCDD}$ = 6.9, $\log K_{OW\_PCB126}$ = 6.8). 2,3,7,8-TCDD tends to concentrate in lipid-rich tissues, as discussed by Diliberto et al., (2001) and TCDD lipid solubility is particularly important at low doses. However, despite high hydrophobicity of PCB126, higher concentration was found in liver than in fat due to most likely protein binding (Lohitnavy et al., 2008). This difference between the two chemicals could be addressed in the PBPK by additional data collection and model parameterisation.

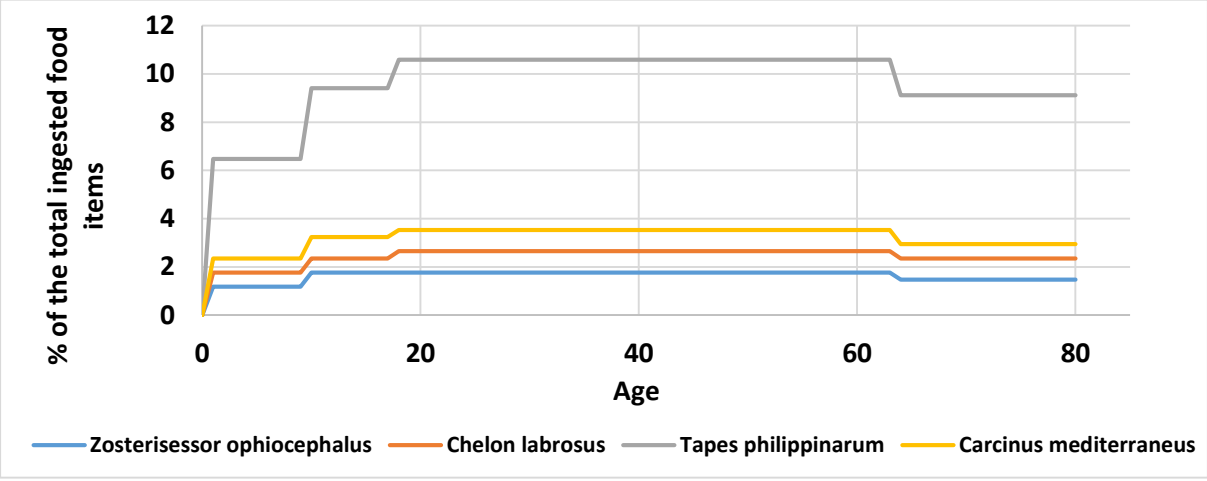


Figure 20 % contribution of the four selected species to the total ingested seafood by human as a function of age (years).

CHAPTER 6 CONCLUSIONS

Overall conclusion of the study is that it is feasible to incorporate human receptor and expanding the typical concepts of assessing environmental quality adopted for instance in WFD (2000) where quality of waters is assessed through integrity of ecological, physical, chemical and biological parameters.

Integrated exposure modelling was applied to assess the bioaccumulation of a set of dioxins and PCBs in the aquatic food web of the Venice lagoon and the exposure of local population (high fish consumers sub-group) through the intake of contaminated seafood from the same lagoon. The source of chemical concentrations input data are sediment cores which are known to be a good record of past contamination trends of organic contaminants. In order to address exposure problem in the Lagoon of Venice, first three bioaccumulation models were developed and later implemented in EU-backed MERLIN-Expo tool. Overall, the five models from MERLIN-Expo library were combined (Phytoplankton, Invertebrate, Fish, Human Intake and Man models) and both deterministic and probabilistic simulations were run for a time period of several decades, from 1930s to 2000s. For the considered classes of chemicals, the project demonstrated the feasibility of reconstructing detailed long-term exposure scenarios addressing both ecological and human exposure issues and considering different targets. The flexibility of the modular structure of MERLIN-Expo made possible to reconstruct a rather complex aquatic food web, representative of the Venice lagoon ecosystem and including 17 different aquatic species. Moreover, simulated concentrations in edible species were used, together with age dependent food intake rates, to reconstruct human internal exposure for local population subgroup (adult males, high fish consumers). The ecological exposure assessment targeted different congeners of PCBs and dioxins, demonstrating the possibility to run simulations for several contaminants at the same time (not mixtures). This feature facilitates easy comparison of the behaviour of the chemicals with different physico-chemical characteristics and helps to explore their potential for bioaccumulation and/or bio-magnification in a straightforward way. MERLIN-Expo proved to be flexible and suitable to support integrated exposure assessment of dioxins and PCBs where both ecological and human targets are considered, even for long term scenarios, and the model performance, evaluated against real monitoring data, are satisfying if all the assumptions included in the assessment framework are considered.

The scarcity of historical contamination data represented a challenge to the PhD project, nevertheless the available data give the idea about the general contamination trend over several decades, which in fact agrees well with pollution emissions in the Lagoon of Venice (contamination peaks in 1940s and 1950s). Despite the uncertainties associated with the assessment framework and data availability and treatment (e.g., reconstruction of water concentrations, interpolation, blood to serum transformation), the results of the described application can already show that the proposed integrated exposure modelling framework for PCBs and dioxins is valid to reconstruct real biomonitoring data with a good approximation (comparable orders of magnitude between simulated and measured concentrations in blood).

The results obtained from the application were used to perform a preliminary ecological and human health risk assessment for the considered chemicals, by comparing the internal exposure estimates against existing benchmark values available in literature. No conditions of significant ecological or health risk have been detected for the considered worst-case scenario, however it is important to remind that the case study application included only a very restricted set of target chemicals and only dietary pathway regarding human exposure and it would be relevant to extend the application to other substances with similar modes of action in order to perform a full risk assessment for local ecosystem and human population. Moreover, the consideration of different and more complete exposure scenarios would permit to better explore the implications of temporal and spatial changes in environmental contamination and exposure variable (such as diet habits) on the overall exposure. The uncertainties associated with the presented exposure assessment in the Venice lagoon should be properly identified and assessed, with the aim of quantifying the margin of variability of the model outputs attributable to uncertainty and variability in input parameters.

The scope of the probabilistic exposure assessment was to provide transparency and credibility to the historical lifetime exposure assessment of a human individual and of a food web, through the application of different uncertainty and sensitivity analysis tools offered by MERLIN-Expo software. The main driver for ecological exposure to POPs resulted to be environmental concentration, especially in sediments. After disabling the consumption of sediments by the considered aquatic species, it was found that this exposure route is the most important one (sediment is a part of diet items but its ingestion is modelled separately due to water-organic carbon partitioning and organic carbon content used specifically in the sediment uptake model). Even though 2,3,7,8-

TCDD concentration in water and sediments is not well correlated with blood concentration, a huge decrease was observed in biota and human blood concentration after shutting down the sediment ingestion exposure route. The general conclusion with regard to the obtained results after first two steps of SA (i.e., application of Morris method and regression-based analysis) is that model is quasilinear. With regard to the uncertain model parameters related to simulated concentration of PCB126 and 2,3,7,8-TCDD in man blood, EFAST yielded body weight and liver tissue-blood partition coefficient and, additionally, adipose tissue-blood partition coefficient in the case of exposure to 2,3,7,8-TCDD was distinguished. However, the indices values are low, suggesting that other more dominant sources of uncertainty exist. Overall, human exposure to POPs depends on a significant number of parameters, processes and behaviours. Results from SA are spread across many model parameters and do not clearly identify a reduced number of influential factors. However, in case of exposure to 2,3,7,8-TCDD there is still some contribution from metabolic half-life used in invertebrate model (when human body weight variability and clam lipid content are considered, this contribution goes up to almost 90%). These factors and the high ingested quantity of seafood with major presence of clam in the daily intake would add up to factors strongly affecting concentration of 2,3,7,8-TCDD in human blood. The environmental concentration of the dioxin shows, however, very weak correlation with concentration in human blood. PCB126 in blood, on the other hand, is noticeably more correlated to environmental concentration both in sediments and water. Also PCB's contribution from seafood intake is larger than that of 2,3,7,8-TCDD. SA does not show any major driver of PCB126 concentration in blood among model parameters. The obtained results suggest that environmental concentrations and eating behaviours should be scrutinized better in order to elucidate contribution of uncertainty to model outputs and also encourage to include functionalities in MERLIN-Expo for considering uncertainty in time series inputs in UA/SA. While ecological parameters affect the level of accumulated concentration in biota, and should be better considered in order to obtain more accurate bioaccumulation estimates, for human exposure to POPs they do not play such an important role, what is confirmed by rather low values of the SA measures.

As a further development of this work, a more refined characterization of exposure scenarios could be carried out in order to make the predicted results and the biomonitoring data fully comparable and provide a quantitative evaluation of modelling

performance. To complement or support the interpretation of existing monitoring data or to explore future exposure scenarios it is advisable to test these potentialities for other classes of chemicals (e.g., PAHs, phthalates, pesticides, metals).

The potential for practical application of integrated ecological and human exposure to both organic and inorganic substances is substantial. One instance where application of integrated exposure modelling may be further explored regards direct and indirect effects of human activities (e.g. maintenance of waterways, navigation or fishing) affecting quality of the Venetian lagoon. An example of such impact is resuspension of the sediments and subsequent remobilisation of buried contaminants which re-enter into the environment resulting in lower status, and creating situation of increased chance of exposure of ecological and human receptors to chemicals stressors. Similarly, the workflow adopted in the presented PhD project could be used to conceptualise such an event by coupling of ecological and human exposure models, including the uncertainty and sensitivity analysis, enabling the environmental decision-makers to explore series of various actions and corresponding uncertainties.

Further testing of the applied models on new environmental and human biomonitoring datasets and on an expanded set of bioaccumulative chemicals, as well as the refinement of the selected input data for the most influential parameters (through additional literature data or experimental activities) can support an improvement of the model capability to reconstruct real bioaccumulation data.

It would be of interest to explore integration of other components in order to expand the presented exposure assessment by for instance integrating exposure and effects modelling. Such opportunity is offered by the GUTS model (Jager et al., 2011). It would make possible to expand the current modelling framework of additional module aimed at stimulating stochastic death and individual tolerance. At the same time, this should stimulate experimental activity towards collection of ecotoxicological data in order to support the validation of the effect model. Apart from addressing bioaccumulation in individual organisms the bioaccumulation models could be expanded to address accumulation/magnification/effects phenomena in populations and communities. Following the currently broadly addressed topic of global climate change the presented integrated modelling framework could include temporal changes in exposure parameters, such as temperature, for the analysis of bioaccumulation metrics and effects on human internal exposure in the context of climate change.

Overall, the study demonstrates that integrated human and ecological exposure

modelling used in complex exposure scenario can be useful for supporting experimental analysis (§ 5.1 & 5.2) and pointing out gaps in knowledge such as variables that should be studied in more details and which strongly affect the assessment of external/internal exposures to environmental organic contaminants (§ 5.3).

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APPENDIX A

Equations

The concentration in fishes caught for human food at time corresponding to mature age, taking into account continuous renewal of fish population (i.e. death of old potentially contaminated individuals replaces by newborn non contaminated individuals), is given by:

Equation A 1

$$C_{fish\_human\_food}[t] = \max(0; \frac{(Q_{fish\_respiratory\_system}[t] + Q_{fish\_GIT\_system}[t])}{W_{fish}} - \frac{Q_{fish\_respiratory\_system}[t - time_{fish\_life}] + Q_{fish\_GIT\_system}[t - time_{fish\_life}]}{W_{fish}} \cdot \exp(-(k_{excretion} + k_{egestion} + k_{growth} + \lambda_{metabolism}) \cdot time_{fish\_life})$$

Where:

$Q_{fish\_respiratory\_system}$ is a quantity of a chemical in fish respiratory system (mg)

$Q_{fish\_GIT\_system}$ is a quantity of a chemical in fish gastro intestinal system (mg)

W_{fish} is weight of a fish (kg_{fw})

$time_{fishlife}$ is fish age at maturity (d)

$k_{excretion}$ is respiratory elimination rate constant (d^{-1})

$k_{egestion}$ is dietary egestion rate constant (d^{-1})

k_{growth} is growth rate constant (d^{-1})

$\lambda_{metabolism}$ is metabolism rate constant (d^{-1})

The concentration in invertebrate caught for human food at time corresponding to mature age, taking into account continuous renewal of invertebrate population (i.e. death of old potentially contaminated individuals replaces by newborn non contaminated individuals), is given by:

Equation A 2

$$C_{Invertebrate\_human\_food}[t] = \max(0; \frac{(Q_{Invertebrate\_respiratory\_system}[t] + Q_{Invertebrate\_GIT\_system}[t])}{W_{Invertebrate}} - \frac{Q_{Invertebrate\_respiratory\_system}[t - time_{Invertebrate\_life}] + Q_{Invertebrate\_GIT\_system}[t - time_{Invertebrate\_life}]}{W_{Invertebrate}} \cdot \exp(-(k_{excretion} + k_{egestion} + k_{growth} + \lambda_{metabolism}) \cdot (time_{Invertebrate\_life})$$

Where:

$Q_{Invertebrate\_respiratory\_system}$ is a quantity of a chemical in invertebrate respiratory system (mg)

$Q_{Invertebrate\_GIT\_system}$ is a quantity of a chemical in invertebrate gastro intestinal system (mg)

$W_{Invertebrate}$ is weight of a invertebrate (kg_{fw})

$time_{Invertebrate}$ is invertebrate age at maturity (d)

$k_{excretion}$ is respiratory elimination rate constant (d^{-1})

$k_{egestion}$ is dietary egestion rate constant (d^{-1})

k_{growth} is growth rate constant (d^{-1})

$\lambda_{metabolism}$ is metabolism rate constant (d^{-1})

Differential equation for calculating amount of chemicals in phytoplankton cell

Equation A 3

$$\frac{dQ_{Phytoplankton}}{dt} = W_{Phytoplankton} \cdot \left(k_{uptake} \cdot C_{diss\_water}\right) - \left(k_{ecretion} + k_{growth}\right) \cdot Q_{Phytoplankton}$$

Where:

$Q_{Phytoplankton}$ is a quantity of a chemical in phytoplankton (mg)

$W_{Phytoplankton}$ is a phytoplankton weight (kg_{fw})

k_{uptake} is a direct uptake from overlaying water ($L*kg_{fw}^{-1}*d^{-1}$)

C_{diss_water} concentration of the chemical dissolved in water ($mg*m^{-3}$)

$k_{excretion}$ is esxrection rate constant (d^{-1})

k_{growth} is growth rate constant (d^{-1})

Differential equations for blood (ArterialBlood, VenousBlood)

Equation A 4

$$\frac{dArterialBlood(t)}{dt} = Q_C \times \left(\frac{C_{Lungs}(t)}{PC_{Lungs}} - C_{BloodArterial}(t)\right) - ArterialBlood\_to\_MetabolitesArterialBlood - ArterialBlood\_to\_Excretion$$

Where:

ArterialBlood is the amount in the arterial blood (mg),

$C_{Blood\_Arterial}$ is the concentration in the arterial blood (mg/L),

C_{Lungs} is the concentration in the lungs (mg/L),

Q_C is the cardiac output (total blood flow) (L/min),

PC_{Lungs} is the lungs:blood partition coefficient (unitless)

ArterialBlood_to_MetabolitesArterialBlood is a transfer describing the metabolism in the arterial blood (mg/min),

ArterialBlood_to_Excretion is a transfer describing the excretion in the arterial blood (mg/min).

The equation for venous blood is:

Equation A 5

$$\frac{dVenousBlood(t)}{dt} = \sum_{Cpt} Q_{Cpt} \times \frac{C_{Cpt}(t)}{PC_{Cpt}} + (Q_{Liver} + Q_{Gut} + Q_{Pancreas} + Q_{Spleen} + Q_{Stomach}) \times \frac{C_{Liver}(t)}{PC_{Liver}} - Q_C \times C_{BloodVenous}(t) - VenousBlood\_to\_MetabolitesVenousBlood - VenousBlood\_to\_Excretion$$

Where:

C_{pt} designates the following compartments: adipose, adrenal, bone, brain, breast, heart, kidney, marrow, muscle, sexual organs, skin, thyroid, and urinary tract,

VenousBlood is the amount in the venous blood (mg),

C_{Blood_Venous} is the concentration in the venous blood (mg/L),

C_{Cpt} is the concentration in the compartment Cpt (mg/L),

Q_C is the cardiac output (total blood flow) (L/min),

Q_{Cpt} is the blood flow entering in the compartment Cpt (L/min),

PC_{Cpt} is the C_{pt}:blood partition coefficient (unitless)

VenousBlood_to_MetabolitesVenousBlood is a transfer describing the metabolism in the venous blood (mg/min),

VenousBlood_to_Excretion is a transfer describing the excretion in the venous blood (mg/min).

Description of input parameters to the used models

Table A 1 Input parameters to FISH/INVERTEBRATE models

Name	Unit	Description	Full name
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.	Intercept of weight-length relationship
Assimilated_food	unitless	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.	Fraction of assimilated food
b_W_fish	unitless	The relationship between weight and length is expressed by an allometric formula including slope b_W_fish. Used in calculating weight of fish at a given age, based on a given fish length.	Slope of weight-length relationship
gamma_food	kg.fw kg.fw-1 d^-1	Food transport coefficient represents delay in advective transport of chemical substances through organism due to limited supply of new food.	Food transport coefficient
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. Half lives are normalised values to 0.01 kg fish and 15°C.	Metabolic half-life of chemicals
kappa	unitless	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	Allometric rate exponent
L_fish	cm	Used in calculating weight of fish at maturity according to an allometric relationship	Fish length at maturity
log10_BCF_organic	L kg.fw^-1	Represents the partitioning at equilibrium of chemicals between fish organism and water in absence of diet contribution	Bioconcentration Factor for organics
log10_K_oc	unitless	Organic carbon is assumed to be the main particulate media interacting with hydrophobic chemicals potentially present in water bodies. The Water-Organic Carbon partition coefficient represents the ratio at equilibrium of the chemical associated to particulate organic matter and present in water respectively.	Water-organic carbon partition coefficient

log10_K_ow	kg kg-1	Kow partition cooefficient is used as a measure of hydrophobicity of the organic substance at equilibrium concentrations between octanol and water phase. Experimental Kow values are provided for number of chemicals.	Octanol/water partition coefficient
p_carbon_sediment	unitless	Organic carbon is considered to be the main sorbing phase in water and sediments for neutral organic compounds. It is used to express affinity of organic contaminants to organic matter in sediments.	Fraction of organic carbon in sediment
p_lipid_fish	unitless	Lipid fraction of fish. Used to calculate the partition of chemical between water and lipids in fish	Lipid fraction of fish
p_lipid_invertebrate	unitless	Lipid fraction of invertebrates. Used to calculate the partition of chemical between water and lipids in invertebrates	Lipid fraction of invertebrate
p_lipid_phytoplankton	unitless	Lipid fraction of phytoplankton. Used to calculate the partition of chemical between water and lipids in phytoplankton	Organinc carbon fraction of phytoplankton
pref_diet	unitless	Fish diet preference for food item 1 used to calculate the concentration of the chemical substance that is absorbed with ingested diet 1. Ten potential diets are arbitrary defined in the MERLIN-Expo model. All diet preferences for food items, inlcuding sediments, should sum up to 1.	Fish diet preference for food items
pref_diet_sed	unitless	Fish diet preference for sediment used to calculate the concentration of the chemical substance that is absorbed with ingested sediments. It should be inlcuded in the overall diet preferences of a given animal.	Fish diet preference for sediments
rho_lipid_layer	kg d kg-1	Represents time of passive diffusion of organic contaminant through lipid membranes.	Lipid-layer permeation resistance
rho_water_layer	kg d kg-1	Represents time of diffusion of organic contaminant contained in water through aqueous layer.	Water-layer diffusion resistance for uptake of chemicals from water
rho_water_layer_food	kg d kg-1	Represents time of diffusion of organic contaminant from ingested food through aqueous layer	Water-layer diffusion resistance for uptake of chemicals from food
time_fishlife	d	It is used for correcting concentration of contaminant in fish body for a given age of fish.It is also used for calculating growth rate.	Fish age at maturity

Table A 2 Input parameters to PHYTOPLANKTON model

Name	Unit	Description	Full name
a_growth	unitless	Coefficient a_growth describe relationship between cell size and growth rate in the size scaling model of phytoplankton growth applied in the MERLIN-Expo.	Intercept of phytoplankton growth rate
b_growth	unitless	Coefficient b_growth describe relationship between cell size and growth rate in the size scaling model of phytoplankton growth applied in the MERLIN-Expo.	Slope of phytoplankton growth rate
b_lipid_layer_resistance	unitless	Exponent is a measure of nonlinearity of the relation between concentration of sorbate and concentration of chemical in the solution. b_lipid_layer_resistance coefficient is influenced by exposure concentration of metals.	Lipid layer resistance exponent
kappa	unitless	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	Allometric rate exponent
log10_K_ow	kg kg-1	Kow partition cooefficient is used as a measure of hydrophobicity of the organic substance at equilibrium concentrations between octanol and water phase. Experimental Kow values are provided for number of chemicals.	Octanol/water partition coefficient
p_carbon_phytoplankton	unitless	Carbon fraction represents phytoplankton cell carbon pool, where accumulation of metals may take place. Carbon fraction is used in expressing excretion of the metals (k_ excretion_metals) from the planktonic cell.	Organic carbon fraction of phytoplankton
p_lipid_phytoplankton	unitless	Lipid fraction of phytoplankton. Used to calculate the partition of chemical between water and lipids in phytoplankton	Lipid fraction of phytoplankton
rho_lipid_layer	kg d kg-1	Represents time of passive diffusion of organic contaminant through lipid membranes.	Lipid-layer permeation resistance
rho_water_layer	kg d kg-1	Represents time of diffusion of organic contaminant contained in water through aqueous layer.	Water-layer diffusion resistance for uptake of chemicals from water
V_cell	µm^3	Cell biovolume is used in estimating phytoplankton cells weight.	Phytoplankton cell volume
volume_to_weight	µg µm-3	volume_to_weight conversion factor is used in transforming cell biovolume to weight of phytoplankton cell.	Volume to weight conversion factor

Table A 3 Input parameters to PBPK model

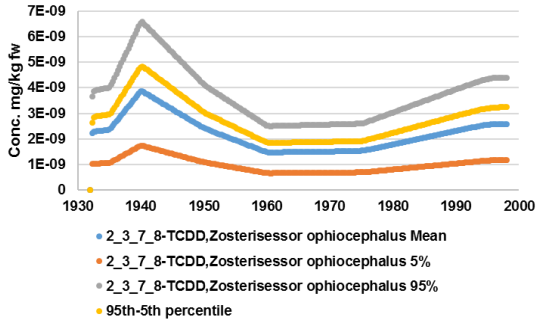
Name	Unit	Description	Full name
Abs_ingestion	unitless	The fraction of contaminant absorbed via ingestion was defined to control the quantity of contaminant that is absorbed by ingestion (via the gastro-intestinal tract or modelled as a direct input in the liver). This fraction was introduced to take into account mechanisms that may not be well described for some contaminants between an intake and the subsequent concentrations in the body. $Abs_{ingestion}$ is used to compute the quantity of contaminant ingested.	Fraction of contaminant absorbed via ingestion
Abs_inhalation	unitless	The fraction of contaminant absorbed via inhalation ($Abs_{inhalation}$) was defined to control the quantity of contaminant that is absorbed by inhalation. This fraction was introduced to take into account mechanisms that may not be well described for some contaminants between an intake and the subsequent concentrations in the body. $Abs_{inhalation}$ is used to compute the quantity of contaminant inhaled.	Fraction of contaminant absorbed via inhalation
BDW_Variability	unitless	The parameter $BDW_{Variability}$ was created to allow inter-individual variability of the bodyweight for persons of the same age. The user can use this parameter to constraint the bodyweight to a specific value or to simulate a random population.	Variability in the bodyweight
BIND	mg/L	Parameter to take into account the partitioning in blood for chemicals such as lead. For most of the chemicals, we assume no partitioning in blood. The parameter BIND is the maximum capacity of erythrocytes to bind the contaminant.	Capacity of erythrocytes to bind contaminants

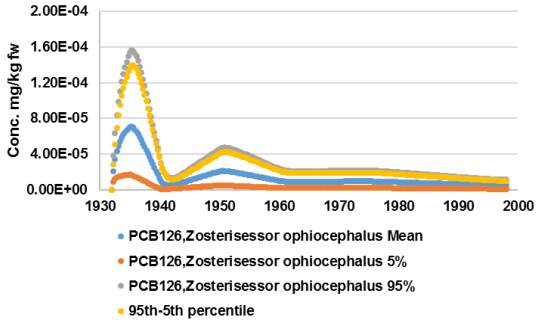
CL_perBDW	L/min*kg	The clearance is the rate at which a substance is removed or cleared from an organ or the body by metabolism (here). In the PBPK, the user has to inform the clearance per kg of bodyweight (CL_{perBDW}) that will be used together with the calculated bodyweight to compute the clearance. The clearance then increases with age. The parameter CL_{perBDW} is used to compute the quantity of contaminant metabolized.	Clearance per kg of bodyweight
DensityOrgan	kg/L	This parameter gives the organ density for each compartment. The density of an organ or a tissue is its mass per unit of volume.	Density of organs
InitialAge	year	The age of the individual when the simulation starts (in years).	Age at the beginning of the simulation
Ka_gut	1/min	Ka_{gut} is a rate constant of the absorption of the contaminant in the intestines. The absorption is modelled by a diffusion process.	Absorption from the gut lumen to the gut wall
Ka_stomach	1/min	$Ka_{stomach}$ is a rate constant of the absorption of the contaminant in the stomach. The absorption is modelled by a diffusion process.	Absorption from the stomach lumen to the stomach wall
KBIND	mg/L	Parameter to take into account the partitioning in blood for chemicals such as lead. For most of the chemicals, we assume no partitioning in blood. The parameter KBIND is the half-saturation constant used in describing the partition of contaminants between blood and plasma.	Saturation constant for partitioning between blood and plasma
Ke_bile	1/min	This parameter reflects the elimination of the contaminant from liver through bile. A compound excreted in bile may be reabsorbed from the gastro-intestinal tract and returned to the general circulation. If the user wants to model the excretion of the compound from the body without the reabsorption, he/she should use the parameter Kex_perBDW.	Biliary excretion rate

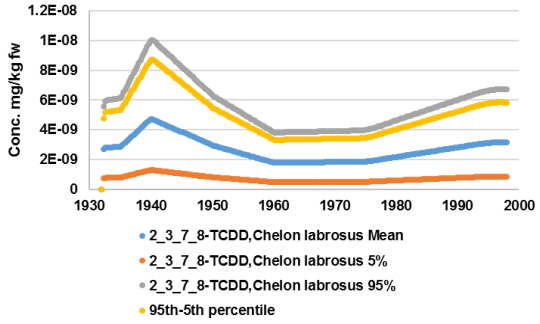
Kex_perBDW	1/min*kg	The excretion rate reflects the process of excretion of the contaminant by the different organs (for instance, the urinary excretion in the kidneys or blood). In the PBPK, the user has to inform the excretion rate per kg of bodyweight (Kex_{perBDW}) that will be used together with the calculated bodyweight to compute the excretion rate as a function of age. The excretion rate increases with age. This parameter is used to compute the quantity of contaminant excreted.	Excretion rate per kg of bodyweight
Km	mg/L	The Michaelis constant (Km) is a parameter of the Michaelis-Menten equation used to describe the metabolism of the contaminant. Km is defined as the substrate concentration at which the reaction rate is half of Vmax and is an inverse measure of the affinity of the contaminant for the enzymes (a small Km indicates a high affinity). The parameter Km is used to compute the quantity of contaminant metabolized.	Michaelis constant
MainCYP	unitless	The parameter MainCYP allows the user to indicate which cytochrome P450 (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP2E1, CYP3A, CYP3A4, CYP3A5, CYPC18, CYPC19, and others) is mainly involved in the metabolism of the contaminant. If none of the cytochromes proposed metabolizes the contaminant, the user has to choose the option "Others". The parameter MainCYP is used to select a function that apportions the clearance or Vmax values to the enzyme content as a function of the age of the individual in order to compute the quantity of contaminant metabolized.	Main cytochrome P450 involved in metabolism
Option_BindingRBC	unitless	The parameter is chosen by the user when the partioning of the contaminants between blood and plasma is modeled. This parameter informed the general variable BindingRBC. The value is 1.	Binding to erythrocytes - option
Option_IngestionGIT	unitless	The parameter is chosen by the user to inform the general variable Ingestion, when he/she wants to model the absorption via the gastro-intestinal tract of ingested contaminants. The only possible value is 1.	Ingestion via the GI tract

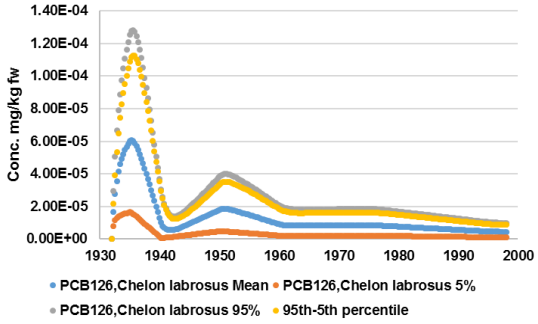
Option_IngestionSkipGIT	unitless	The parameter is chosen by the user to inform the general variable Ingestion, when he/she wants to model a direct input in the liver of ingested contaminants. The only possible value is 2.	Ingestion via the liver
Option_NoBindingRBC	unitless	The parameter is chosen by the user when the partioning of the contaminants between blood and plasma is not modeled. This parameter informed the general variable BindingRBC. The value is 0.	No binding to eryhtrocytes - option
PC	unitless	This parameter is the partition coefficients tissue over blood in the different tissues. A tissue:blood partition coefficient is defined as the ratio of the concentration in a tissue to the concentration in blood.	Tissue:blood partition coefficients
PC_BloodAir	unitless	The blood:air partition coefficient is the ratio of the concentration in blood over the concentration in air at equilibrium. The blood:air partition coefficient is used to describe the uptake and the elimination via lungs.	blood:air partition coefficient
scQ_adult	unitless	The relative blood flows in adults are scaling factors used to compute the blood flow entering in an organ/tissue according to the cardiac output.	Relative blood flows in adults
scW_adult	unitless	The relative organ weights in adults (scW_{adult}) are the proportions of the weight of an organ/tissue compared to the bodyweight for an adult person.	Relative organ weight in adults
Vmax_perBDW	mg/min*kg	The maximum velocity of the metabolic reaction per kg of bodyweight (Vmax_perBDW) is used to compute the maximum velocity (Vmax), a parameter of the Michaelis-Menten equation used to describe the metabolism of the contaminant. The parameter Vmax_perBDW is used to compute the quantity of contaminant metabolized.	Maximum velocity per kg of bodyweight

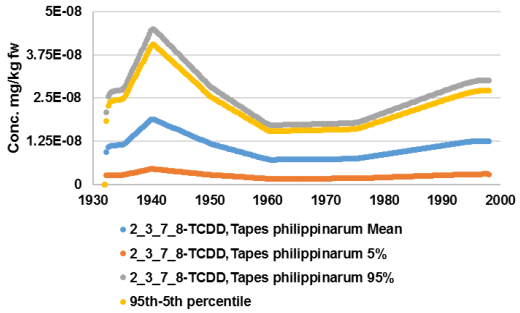
Exposure pathways contribution to the level of internal exposure concentration of PCB126 and 2,3,7,8-TCDD

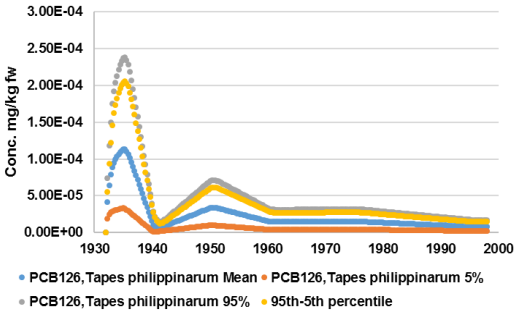


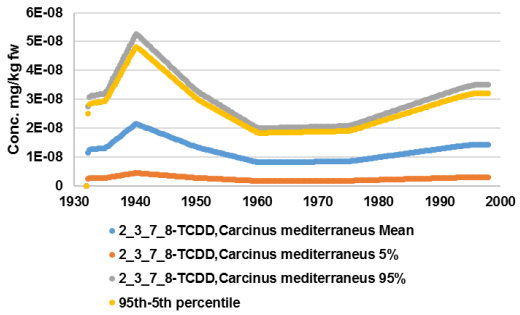


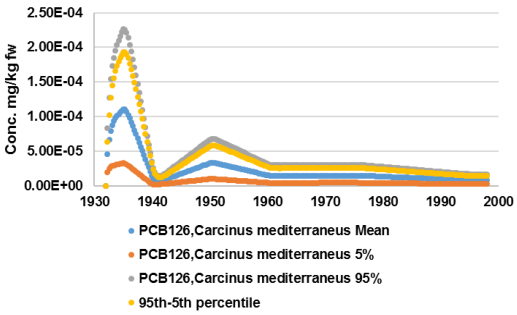












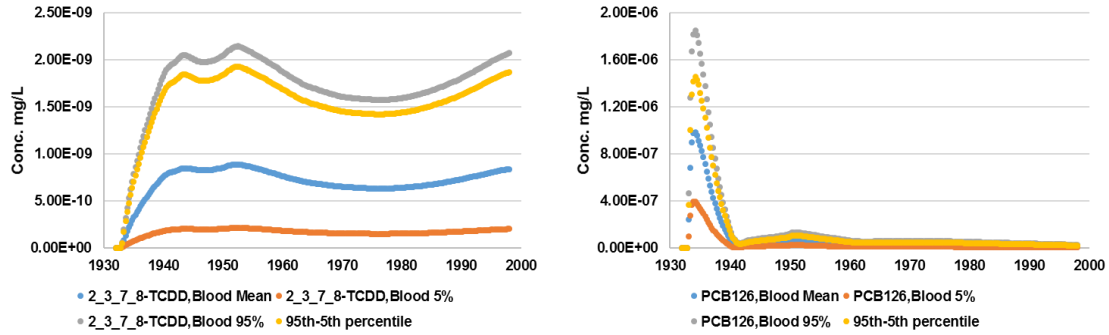
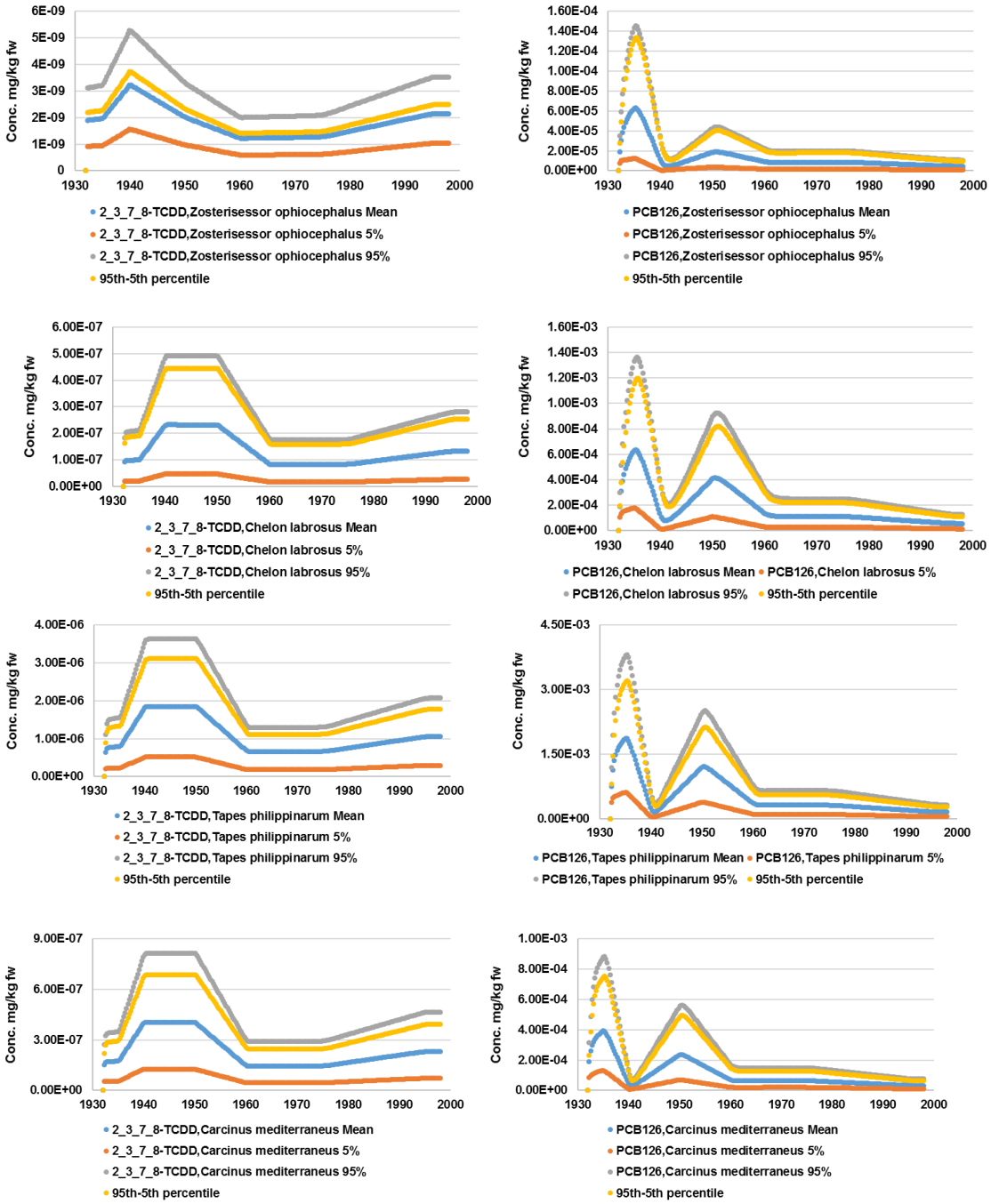


Figure A 1 Effect on computed concentration of 2,3,7,8-TCDD (mg/kg fw) and PCB 126 (mg/kg fw) and on uncertainty range in selected aquatic species and man's blood when uptake from sediment is disabled



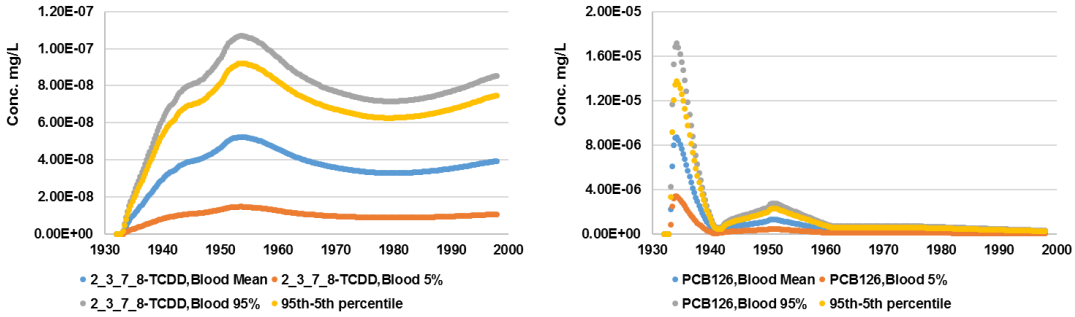
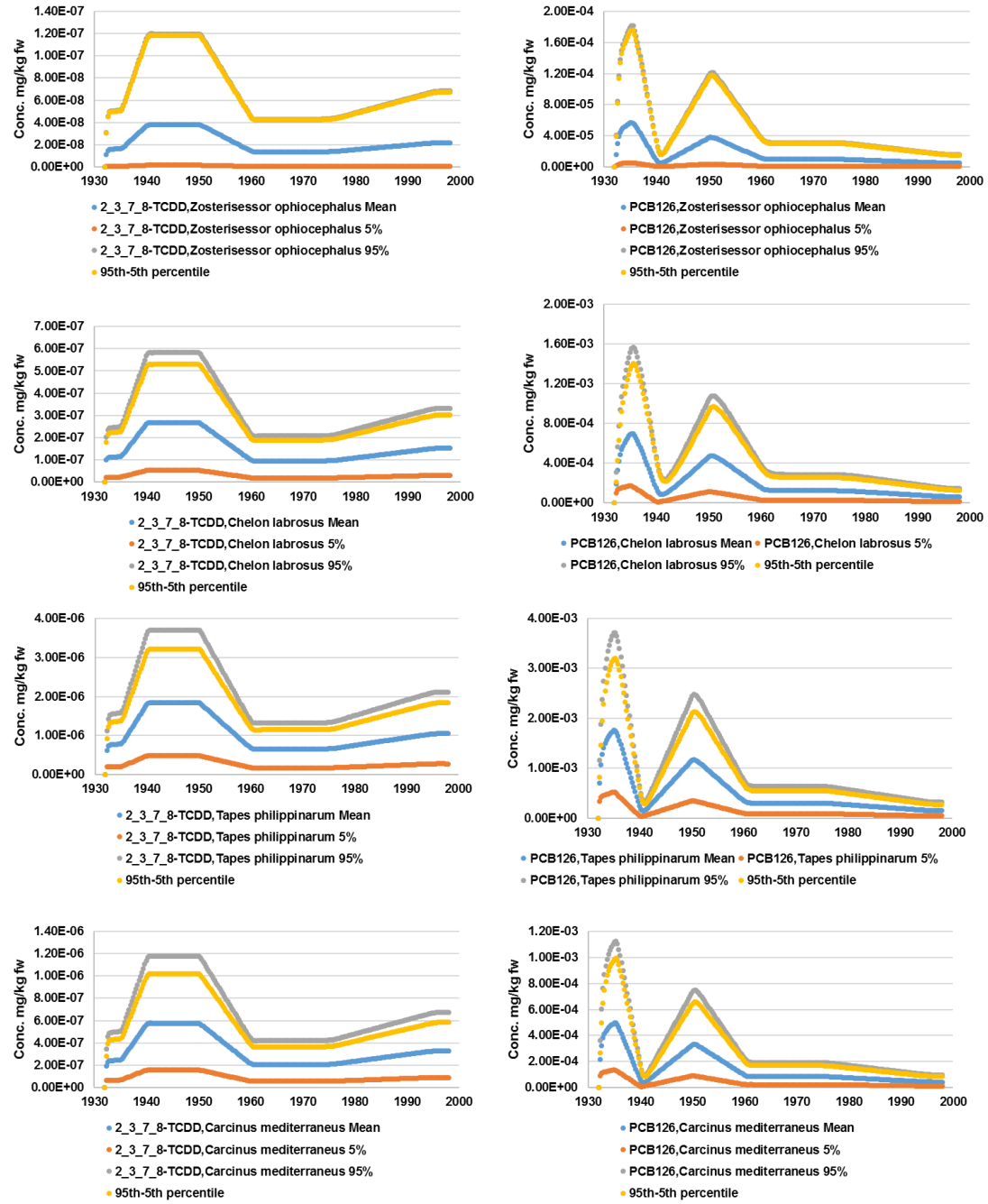


Figure A 2 Effect on computed concentration of 2,3,7,8-TCDD (mg/kg fw) and PCB 126 (mg/kg fw) and on uncertainty range in selected aquatic species and man's blood when uptake from diet is disabled



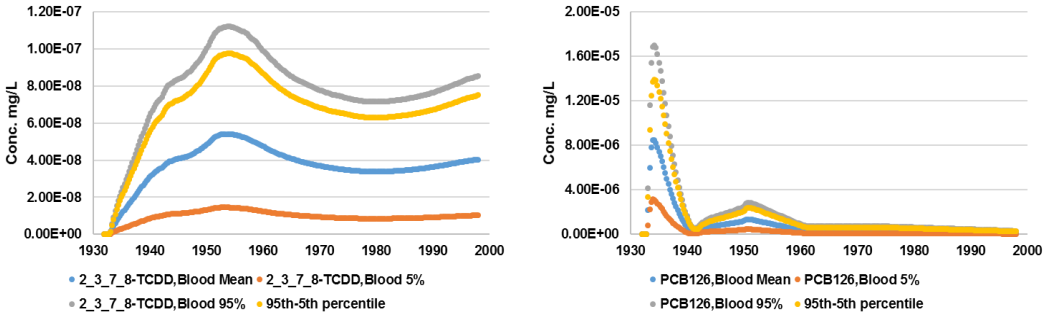


Figure A 3 Effect on computed concentration of 2,3,7,8-TCDD (mg/kg fw) and PCB 126 (mg/kg fw) and on uncertainty range in selected aquatic species and man's blood when uptake from water is disabled

Summary of the calculated sensitivity indices

Table A 4 Squared standardised regression coefficient (β_i^2) and coefficient of determination (R^2), first order sensitivity index (S_i), total order sensitivity index (TS_i) for 10 input parameters accounting for variance in computed 2,3,7,8-TCDD concentration in blood, in year 1998. Scores are ordered according to decreasing TS_i.

Input	**Squared standardised regression coefficient (β_i^2)**	**First order sensitivity index (*Si*)**	**Total Order Sensitivity Index (*TSi*)**
	$R^2 = 0.75$		
Metabolic half-life of chemicals (2,3,7,8-TCDD)	0.27	0.32	0.47
Variability in the bodyweight (Man)	0.21	0.18	0.24
Lipid fraction of invertebrate (Tapes philippinarum)	0.06	0.07	0.15
Water-layer diffusion resistance for uptake of chemicals from food	0.00	0.02	0.11
Tissue:blood partition coefficients (2,3,7,8-TCDD) (Liver)	0.06	0.06	0.10
Fraction of assimilated food (Tapes philippinarum)	0.08	0.04	0.08
Food transport coefficient (Tapes philippinarum)	0.03	0.04	0.08
Allometric rate exponent (Tapes philippinarum)	0.01	0.02	0.05
Tissue:blood partition coefficients (2,3,7,8-TCDD) (Adipose)	0.01	0.01	0.04
Water-organic carbon partition coefficient (2,3,7,8-TCDD)	0.00	0.00	0.04

Table A 5 Squared standardised regression coefficient (β_i^2) and coefficient of determination (R^2), first order sensitivity index (S_i) total order sensitivity index (TS_i) for 12 input parameters accounting for variance in computed PCB126 in blood, in year 1998. Scores are ordered according to decreasing TS_i.

Input	**Squared standardised regression coefficient (β_i^2)**	**First order sensitivity index (*Si*)**	**Total Order Sensitivity Index (*TSi*)**
	$R^2 = 0.71$		
Lipid fraction of invertebrate (Tapes philippinarum)	0.18	0.20	0.28
Fraction of assimilated food (Tapes philippinarum)	0.14	0.15	0.22
Variability in the bodyweight (Man)	0.09	0.10	0.16
Tissue:blood partition coefficients (PCB 126) (Liver)	0.14	0.10	0.16
Lipid fraction of invertebrate (Zooplankton)	0.04	0.08	0.15
Metabolic half-life of chemicals (PCB126)	0.03	0.05	0.12
Water-organic carbon partition coefficient (PCB126)	0.03	0.03	0.11
Water-layer diffusion resistance for uptake of chemicals from food	0.00	0.04	0.11
Invertebrate age at maturity (Tapes philippinarum)	0.02	0.02	0.07
Food transport coefficient (Tapes philippinarum)	0.02	0.02	0.07
Lipid fraction of invertebrate (Phytoplankton)	0.01	0.01	0.05
Allometric rate exponent (Tapes philippinarum)	0.01	0.01	0.04