4FUN

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MERLIN-Expo: Lessons learned from the case studies

One of the objectives of the 4FUN project was to increase the confidence in the applicability of the MERLIN-Expo tool through targeted demonstration activities based on complex realistic case studies. In particular, we aimed at demonstrating: (i) the reliability of the modelling predictions through a comparison with actual measurements; (ii) the feasibility of building complex realistic exposure scenarios satisfying the needs of stakeholders; and (iii) how uncertainty margins can improve risk governance. The case studies can be seen as reference cases that provide guidance to future users on how to apply the tool in different situations and how to interpret the results from the assessments with the tool taking into account relevant regulatory frameworks. The three case studies are presented thoroughly in separate deliverables (D5.1, D5.2, and D5.3). Here the main features of the MERLIN-Expo tool that were explored using these case studies are summarised.

Reliability of the MERLIN-Expo predictions

One of the major achievements of the case studies was to assess the reliability of the predictions obtained by MERLIN-Expo. In most cases, a factor less than 3 was observed between the model predictions and the actual experimental data (see case studies 1 and 3, for example). Such an agreement between predictions and measurement is generally judged acceptable in a purely predictive framework, i.e., the models are sufficiently generic to be applied to a large number of substances and situations, even when the measurement data were not used to calibrate the models. Although the number of case studies is relatively low to generalize these results, our testing approach gives a quite reasonable confidence in MERLIN-Expo predictions. It is important to notice that confidence increases because some modules of the modelling chain had already been studied on their own (for example, the PBPK model has already been developed and evaluated on a separate dataset).

Unsurprisingly, MERLIN-Expo performed best when model parameters were set to values specific to the sites and the populations (see case study 1), allowing to tailor the assessment to local conditions. Most of the modules implemented in the MERLIN-Expo library are mechanistic models, so their parameters refer to physico-chemical, physical or biological processes that have already been measured or estimated. MERLIN-Expo integrates and organizes the available knowledge in order to improve exposure assessment and, subsequently, risk assessment. In the case there is no prior information, default values are provided in MERLIN-Expo and guidance on how to obtain additional, more specific data is given in the documentation of each module.

Flexibility in building complex exposure scenarios

One of the main features of MERLIN-Expo is its ability to build realistic site-specific scenarios in an intuitive fashion, making use of a library of models that covers a wide spectrum of exposure assessment contexts. MERLIN-Expo was tested on three case studies exhibiting very different characteristics in order to cover a wide range of: (i) substances (e.g. metals, persistent organic pollutants, emerging pollutants); (ii) contamination sources (water, wastes, soil, dust, air, food); (iii) environmental policy endpoints (e.g. waste, land management, water quality); (iv) spatial/temporal scales (e.g. close vicinity of industry, lagoon). The case studies offered the opportunity to explore the applicability of the tool at several levels of complexity, ranging from very simple to rather complex scenarios. The complexity depends on the description of the environment and exposure pathways (number of modules selected and their interconnections, default values or site-specific values for parameterization), but also on the statistical analyses performed (deterministic or probabilistic). All these different levels of complexity were effectively handled with MERLIN-Expo. Using the same tool also allows a direct comparison of the results obtained from different hypotheses. Moreover, MERLIN-Expo can be used to combine ecological and human exposure assessment using a single tool (see case study 2), supporting the integrated evaluation of chemical fate and effects also for long-term scenarios.

Incorporating uncertainty in risk assessment

All the case studies performed probabilistic analyses to study the impact of uncertainty and variability in parameter values of the different modules on the final model outputs, such as a biological measure in humans. The probabilistic simulation tools implemented in MERLIN-Expo were used together with the default probability density functions (pre-)defined for model parameters. These analyses produced a mean prediction associated to an interval of confidence for the model outcomes of interest. In some cases (e.g., in case study 3), we showed that the experimental data were encompassed in the predicted interval of confidence at 95%, a result that further supports the accuracy of the tool. Sensitivity analyses were also run to identify and rank the key input parameters of the exposure, and also to assess the relative contribution of the different sources, pathways, and routes of exposure on the overall modelled exposure (e.g., in case study 1).

The availability of different options for uncertainty and sensitivity analysis in MERLIN-Expo, from simple local methods to more computational expensive non-local methods, is targeted to a wide range of end-users and should facilitate the incorporation of such issues in future decision making. Such analyses then provide valuable information for both risk assessors and decision-makers by supporting decisions to conduct additional analyses or prioritise resource allocations for additional research and/or data collection efforts. This is also in line with the recommendations of international agencies (EFSA, 2015; BFR 2015; WHO 2008) and makes MERLIN-Expo an appealing tool for advanced exposure assessment.

An evolving tool

Modelling tools are usually in constant evolution. At the beginning of the 4FUN project, the MERLIN-Expo tool was not suitable to implement all the case study specificities. All along the project, there were discussions with the model and software developers to make some adjustments in order to improve the tool. Few examples of functionalities and features included in MERLIN-Expo and used in the case studies are: capability of modelling larger populations, performing simulation for several individuals at the same time; including individual time-activity patterns (e.g. individual moving between areas with varying levels of contamination); developing a food web model to describe the transfer of contamination between species and across trophic levels (prey and predator model, implemented for the aquatic environment); adding a module (“human intake”) to combine the human intakes from several sources; allowing time-varying intake (e.g., food consumption evolves with the age of the individual), including and parametrizing new substances originally not included in the database.

MERLIN-Expo is now ready to be used for various exposure scenarios but will need to be maintained and updated to include new models and/or features that could further facilitate scenario building and/or the interpretation of the results. For instance, the tool could be linked to databases or *in silico* models (QSARs) to ease the parameterization of the models. End-users with not all the required information at hand find guidance in the model documentation supplemented to the tool. Extending this guidance and documentation may be particularly relevant for physico-chemical parameters specific to the contaminants (e.g., the partition coefficients between two media, or between blood and tissue in humans), or for the integration of default values for food consumption of predefined products (e.g., referencing the database developed by the European Food Safety Authority).

1 Introduction

1.1 Context and objectives

The case study 2 of 4FUN project focuses on ecological and human exposure to Persistent Organic Pollutants (POPs) in Venice lagoon and its surrounding areas.

The lagoon of Venice (Figure 1) is a superficial basin, located along the northwestern coast of the Adriatic Sea, with an area of 549 km^2 (Guerzoni and Tagliapietra, 2006). The lagoon of Venice can be defined as a transitional environment, characterized by shallow waters (i.e., the mean water depth is 1.2 meters) (Guerzoni and Tagliapietra, 2006), and influenced by several anthropogenic activities such as industry, tourism, and fishery. These activities have caused in the past and still cause the release in environmental media of a wide range of chemical substances, including Persistent Organic Pollutants (POPs). The most significant sources of POPs can be identified in the industrial settlement of Porto Marghera (where many chemical industries, oil refining plants, and waste incineration plants were present, today partially dismissed), the treated and untreated municipal effluents from the city of Venice and surrounding urban centres, the freshwater loads from the catchment area and atmospheric depositions (Collavini et al., 2005; Guerzoni et al., 2005). The lagoon sediments, which keep trace of historical time trends of emissions (Frignani et al., 2005; Dalla Valle et al., 2005), represent the most important secondary source of POPs.

Figure 1. Satellite image of Venice lagoon and its position in Italy.

Despite the implementation of environmental protection regulations and the use of technologies for emissions control in the last two decades, the presence of polychlorinated dibenzo-p-dioxins and –furans (PCDD/Fs) and polychlorinated biphenyls (PCBs) in lagoon sediments, due to their affinity to lipids and organic matters and their significant persistence (Ritter et al., 2007), may still represent a hazard to ecosystems and population health. These compounds might indeed be directly available to benthic species and can then indirectly be transferred to other organisms, accumulating along the aquatic food chain and reaching high concentrations in top predators.

As a further consequence, local population is potentially exposed through the consumption of local fish and shellfish, which are caught or cultivated in the lagoon. Biological resources of

the lagoon have been exploited since centuries by traditional fishing and farming activities. Moreover, 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 they are most abundant (Pranovi et al., 2003) but illegal harvesting in highly contaminated areas close to Porto Marghera has been often detected as well.

Since the annual consumption rate of fish and seafood turned out to be relatively high if compared to other Italian regions, especially in some areas of Venice municipality (Pedenzini, 1996), the diet might represent a significant exposure route to POPs for local population. Only very few human biomonitoring studies have been conducted in the area (Raccanelli et al., 2007) and the available data will be compared with the results provided by MERLIN-Expo tool. However, the reduced availability of population exposure data, especially in the last decade, highlights the potential utility of predictive modelling tools to better investigate the relationships between environmental contamination distribution, diet patterns and associated health risks.

In conclusion, the main objective of the case study is to estimate ecological exposure and human life time exposure to POPs through the diet, in order to support the characterization of risks to lagoon ecosystems and the assessment of human health risks associated with local fish and seafood consumption. In order to achieve this objective, three main steps can be identified, namely:

- the evolution of environmental contamination in recent decades should be reconstructed, modelling the transfer and distribution of contaminants between the different environmental media;
- the bioaccumulation processes in the aquatic food chain will be investigated, considering also edible organisms which are constituents of the diet for local population;
- the life-long dietary intake of the chemicals of interest will be estimated, considering the contributions both from local fish/seafood (modelled concentrations) and from other food items such as dairy products, meat, vegetables/fruits (monitoring concentrations from literature), and taking into account population habits.

The following chemicals have been included in the assessment:

a) Polychlorinated biphenyls (PCBs): PCB77, PCB126, PCB167, PCB169, PCB170, PCB180.
b) Dioxins: 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 1,2,3,4,7,8-HCDD

The selection has been driven by the availability of environmental, biota and human biomonitoring data, needed to run the models and to check the reliability of results of intermediate simulation steps by comparison with real data. For the full chain assessment (i.e., ecological and human exposure assessment), PCB126 and 2,3,4,8-TCDD were selected. The remaining chemicals have been included only in the ecological assessment phase.

1.2 The conceptual model of CS2 and its implementation in MERLIN-Expo

For the assessment of ecological and human exposure to POPs, a conceptual model for the Venice case study has been developed. It is represented in Figure 2.

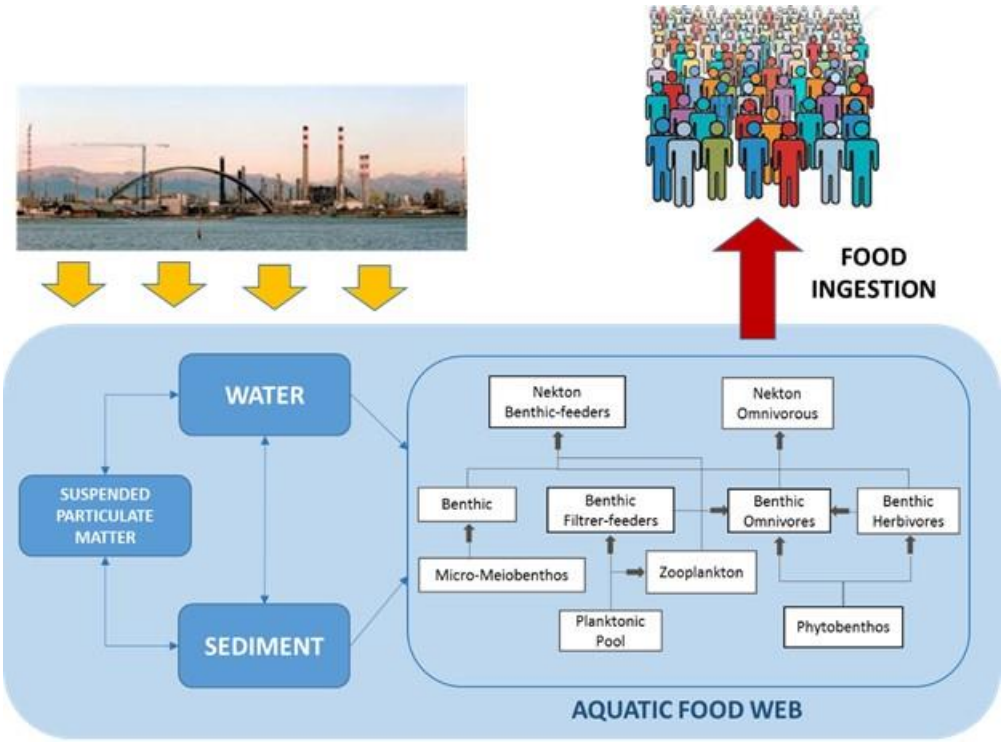


Figure 2. Conceptual framework of 4FUN case study n.2.

Three new models have been specifically developed and implemented in MERLIN-Expo with the purpose of simulating the bioaccumulation of contaminants in aquatic food web, namely **Phytoplankton**, **Invertebrate** and **Fish** models, which are now part of MERLIN-Expo library.

These models, based on the approach proposed by Hendriks and colleagues (2001a, 2001b), feature characteristics of biodynamic approach: they describe accumulation rates of contaminants, are mechanistically based, and reflect variability of organisms and contaminants by considering both biological and physicochemical specific parameters (Luoma et al., 2005). A kinetic approach is used rather than an equilibrium-based approach to simulate the accumulation of both organic and inorganic contaminants by aquatic animals. Using rates to express accumulation processes allows models to be flexible and predictive under changing conditions. The models provide mechanistic understanding of bioaccumulation phenomena of organics and metals, and accounts for main transport processes between water and organism compartments, i.e. uptake via respiratory route, uptake via dietary route, elimination via respiratory route (excretion), elimination via gastro intestinal track (egestion), metabolism, and growth. While processes involved in bioaccumulation were assumed to be similar for fish and invertebrates species (Figure 3), for phytoplankton only uptake from water is considered (Figure 4), since phytoplankton consist of autotrophic species, and dietary uptake can be disregarded (Frouin et al., 2013). A detailed description of these models is provided in the corresponding “model documentation” reports, which will be freely available on 4FUN project website.

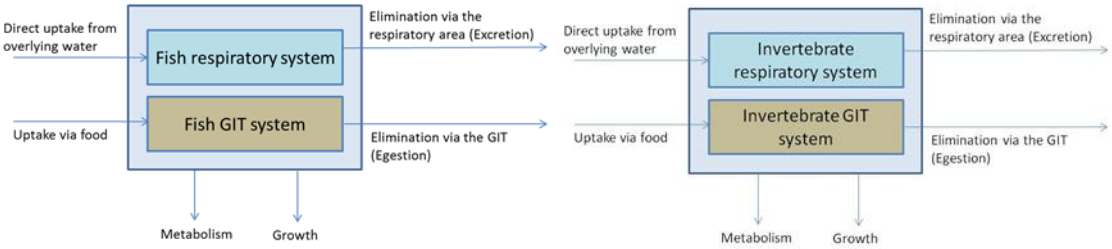


Figure 3. Bioaccumulation processes included in Fish and Invertebrate models.

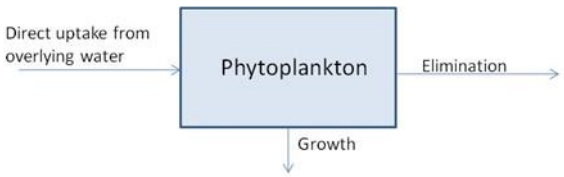


Figure 4. Bioaccumulation processes included in Phytoplankton model.

Each of the three models can be used as a stand-alone model (to simulate accumulation in one or more species), or it can be coupled to other models of the "aquatic food web system" to recreate aquatic food web of different dimensions and complexity, supporting the simulation of bio-magnification processes across different trophic levels.

According to the goals of case study 2, Phytoplankton, Invertebrate and Fish models have been combined to recreate a food web representative of Venice lagoon ecosystem (detail description will be provided in Paragraph 2.1). Furthermore, in order to simulate the consumption of seafood by local population and estimate the internal human exposure to POPs associated to the diet, the aquatic food web models have been coupled to the Human Intake and to the Man models available in MERLIN-Expo library. Some of the organisms included in the Venice lagoon food web are indeed edible organisms, which are commonly caught or harvested in the lagoon (e.g., Manila clam, mussel, mullet, seabass).

Figure 5. The models applied for the Venice case study visualized in the MERLIN-Expo matrix interface.

For sake of simplicity, the ecological exposure assessment and the human exposure assessment will be illustrated in two separate chapters, i.e. in Paragraph 2 respectively.

1.3 Study area

The lagoon of Venice can be divided into three main basins: southern lagoon, central lagoon and northern lagoon. For the ecological exposure assessment of case study 2, the central lagoon was selected as target area.

The central lagoon is closed to Porto Marghera industrial area and has been strongly influenced by input of contaminants associated to industrial activities. Many studies on surficial sediments showed that the concentrations of persistent organic pollutants such as dioxins and PCBs in the central basin are higher than other areas (Frignani et al., 2001; Marcomini et al., 1997; MAV, 2000a, 200b), as reported in Figure 6.

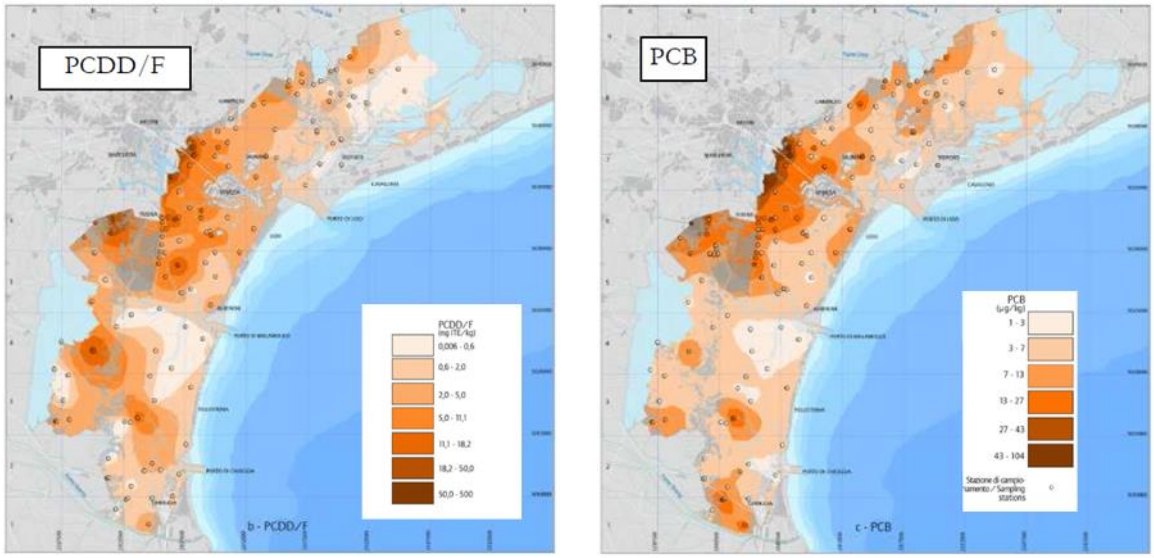


Figure 6. Spatial distribution of PCDD/Fs and PCBs sediment contamination in Venice lagoon (Source: Atlante della Laguna, 2006).

The central basin has been largely exploited by mechanical clam harvesting, especially during the 90's (Pranovi et al., 2003). 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 extensively in the most contaminated areas. Therefore, according to a conservative approach, the selection of central lagoon as target areas for exposure modelling allows to consider the worst-case scenario for both ecological and human exposure assessment.

2 Ecological exposure assessment

2.1 Aquatic food web of Venice lagoon

In order to simulate the accumulation of target chemicals in aquatic organisms of Venice lagoon, it is first necessary to define a site-specific food web structure. 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 bio-magnification phenomena as the consequence of dietary uptake (Mackay and Fraser, 2000; Kelly et al., 2007). The characterization of predator-prey interaction 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 and colleagues (2008), based on extended literature on Venice lagoon ecosystems assessment and modelling (e.g., Carrer and Opitz, 1999; Pranovi et al., 2003; Libralato et al., 2003). This food web has been slightly adapted for the application of MERLIN-Expo and it finally includes 17 species plus the sediment compartment (which constitutes part of the diet for some benthic organisms). For some species (*Manila clam, 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, i.e. they are caught by local fisherman and enter the human diet (thus, they are also relevant for the 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).

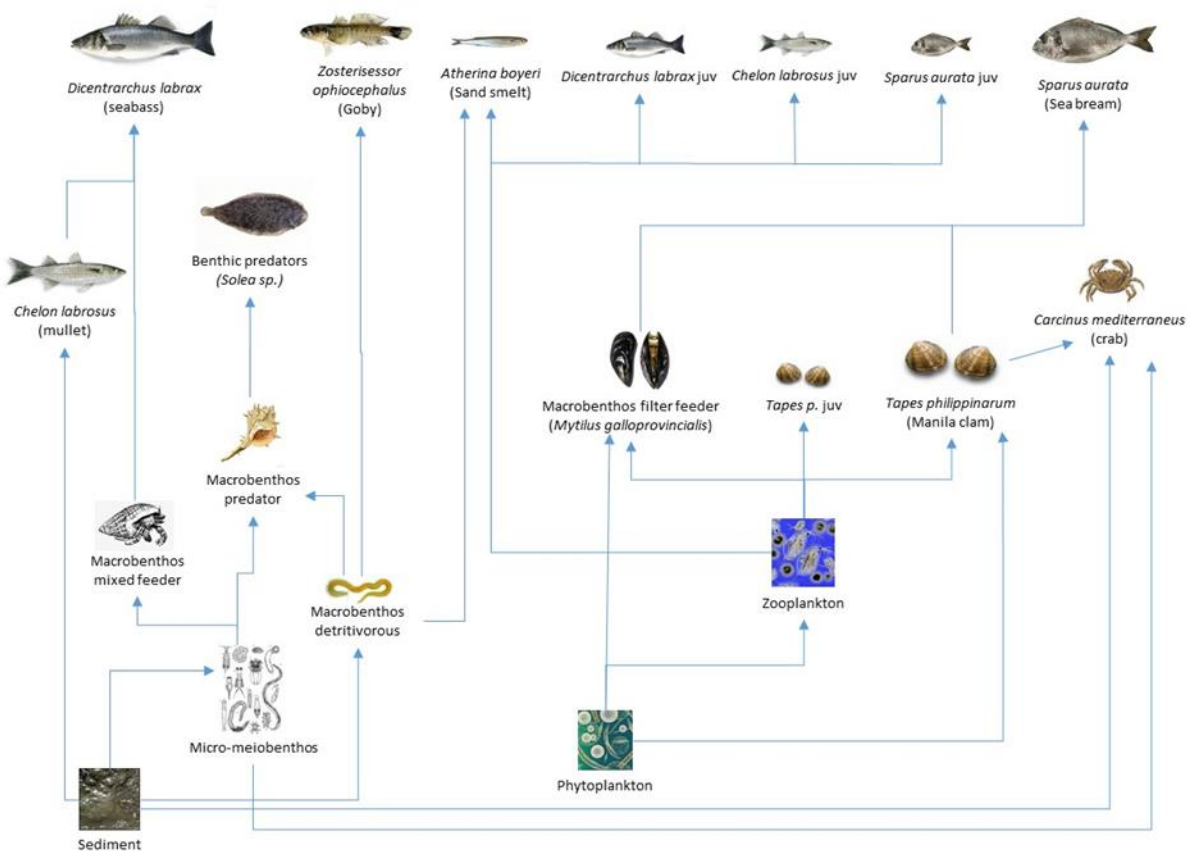


Figure 7. Food web structure adopted for Venice lagoon (modified from Libralato et al., 2002; Micheletti et al. 2008).

2.2 Input data

In this paragraph, all input data required to run MERLIN-Expo models for ecological exposure assessment for the Venice case study are reported.

Input data are classified as:

a) “parameters”, which are constant over each simulations 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).

Since the final goal of case-study 2 is to assess the lifetime human exposure to PCBs and dioxins, and available human biomonitoring data date back to 1997-1998, the objective is to dynamically simulate concentrations in aquatic organisms since about 1940 (this period correspond also to the beginning of PCB production and use in Italy). However, historical data of PCB emissions suitable to reconstruct the historical development of PCBs contamination in the lagoon of Venice are not available. As an alternative, sediment cores proved to be useful in supporting the reconstruction of temporal trends of environmental contamination, as demonstrated by many authors (Marcomini et al., 1999; Frignani et al., 2005), also in combination with environmental modelling approaches (Dalla Valle et al., 2005).

Studies focused on the reconstruction of temporal trends of POPs contamination in radio-dated sediment cores from the Venice lagoon have been reviewed (e.g., Marcomini et al.,

1999; Frignani et al., 2001, 2004, 2005; Piazza et al., 2003; Pavoni et al., 1987). In general, a significant increase of organic pollutants in lagoon sediments has been observed since the 40's; the maximum inputs of contaminants is associated to the period 60's-70's, after this period a relevant decrease has been observed. The appearance of PCB in lagoon sediment has been generally measured in sediment layers corresponding to the 60's, while dioxins and furans has been observed since about 1920, with a peak around 1980. The analysis of dated sediment cores by Marcomini and colleagues (1999) and Frignani and colleagues (2005) have been selected because they provide individual concentrations for the chemicals of interest (instead of total concentrations) at specific cores depths, corresponding to specific time period. The time series used as input data to MERLIN-Expo will be illustrated in detail in Paragraph 0.

2.2.1 Chemical-related parameters

Phytoplankton, Invertebrate and Fish models have been parameterized for the selected target chemicals. The required chemical-related parameters (octanol-water partition coefficient, bio-concentration factor, metabolic half-life of chemicals) are common to the three models, moreover the Phytoplankton model requires also the water-organic carbon partition coefficient. The selected values are reported in Table 1, while all references for the selected parameters values are reported in Appendix A.

Parameter	Octanol-water partition coefficient		Water-organic carbon partition coefficient		Bioconcentration factor		Metabolic half-life of chemical	
Abbreviation	log Kow		log Koc		BCF		λ_metabolism	
Unit	unitless		unitless		unitless		d^{-1}	
Chemicals	value	PDF	value	PDF	value	PDF	value	PDF
PCB77	6.34	norm(p1=5.0,x1=5.98,p2=95.0,x2=6.7)	474	norm(p1=5.0,x1=3.97,p2=95.0,x2=5.51)	5.03	norm(p1=5.0,x1=4.19,p2=95.0,x2=5.87)	2254	norm(p1=5.0,x1=3.51,p2=95.0,x2=144.88)
PCB126	6.8	norm(p1=5.0,x1=6.44,p2=95.0,x2=7.16)	4.93	norm(p1=5.0,x1=4.16,p2=95.0,x2=5.7)	5.08	norm(p1=5.0,x1=4.24,p2=95.0,x2=5.92)	288.4	norm(p1=5.0,x1=44.87,p2=95.0,x2=1853.57,trmin=0.0)
PCB167	7.5	norm(p1=5.0,x1=7.14,p2=95.0,x2=7.86)	5.22	norm(p1=5.0,x1=4.45,p2=95.0,x2=5.99)	4.75	norm(p1=5.0,x1=3.91,p2=95.0,x2=5.59)	271.64	norm(p1=5.0,x1=42.27,p2=95.0,x2=1745.86,trmin=0.0)
PCB169	7.46	norm(p1=5.0,x1=7.1,p2=95.0,x2=7.82)	5.17	norm(p1=5.0,x1=4.4,p2=95.0,x2=5.94)	5.64	norm(p1=5.0,x1=4.8,p2=95.0,x2=6.48)	413	norm(p1=5.0,x1=64.27,p2=95.0,x2=2654.67,trmin=0.0)
PCB170	8.27	norm(p1=5.0,x1=7.91,p2=95.0,x2=8.63)	5.64	norm(p1=5.0,x1=4.87,p2=95.0,x2=6.41)	3.69	norm(p1=5.0,x1=2.85,p2=95.0,x2=4.53)	799.83	norm(p1=5.0,x1=124.45,p2=95.0,x2=5140.55)
PCB180	8.27	norm(p1=5.0,x1=7.91,p2=95.0,x2=8.63)	5.29	norm(p1=5.0,x1=4.85,p2=95.0,x2=5.73)	6.20	norm(p1=5.0,x1=5.7,p2=95.0,x2=6.7)	716.2	norm(p1=5.0,x1=129.6,p2=95.0,x2=3958.6,trmin=0.0)
2,3,7,8-TCDD	6.92	norm(p1=5.0,x1=6.56,p2=95.0,x2=7.28)	4.83	norm(p1=5.0,x1=4.06,p2=95.0,x2=5.6)	3.54	norm(p1=5.0,x1=3.29,p2=95.0,x2=3.79)	16	norm(p1=5.0,x1=3.0,p2=95.0,x2=88.0)
1,2,3,7,8-PeCDD	7.56	norm(p1=5.0,x1=7.2,p2=95.0,x2=7.92)	4.74	norm(p1=5.0,x1=3.97,p2=95.0,x2=5.51)	3.74	norm(p1=5.0,x1=3.49,p2=95.0,x2=3.99)	27.3	norm(p1=5.0,x1=4.9,p2=95.0,x2=150.8,trmin=0.0)
1,2,3,7,8-HxCDD	8.21	norm(p1=5.0,x1=7.85,p2=95.0,x2=8.57)	5.38	norm(p1=5.0,x1=3.7,p2=95.0,x2=5.54)	3.64	norm(p1=5.0,x1=3.39,p2=95.0,x2=3.89)	46.5	norm(p1=5.0,x1=8.4,p2=95.0,x2=257.1,trmin=0.0)

Table 1. Values of chemical parameters for the selected substances.

2.2.2 Biota-related parameters

Input values for parameters related to biological parameters for the species included in the Venice food web have been derived from available literature and databases.

Single values and probability density functions (PDFs) selected for Phytoplankton, Invertebrate and Fish models are reported in Table 2, Table 3, and Table 4, respectively.

Parameter	**Unit**		
Allometric rate exponent	unitless	value	0.25
		PDF	norm(mean=0.25,sd=0.11,trmin=0.0)
Intercept of phytoplankton growth rate	unitless	value	0.22
		PDF	logn(p1=5.0,x1=0.13,p2=95.0,x2=0.34)
Slope of phytoplankton growth rate	unitless	value	0.15
		PDF	logn(p1=5.0,x1=0.12,p2=95.0,x2=0.19)
Lipid fraction of phytoplankton	unitless	value	0.02
		PDF	unif(min=0.01,max=0.03)
Lipid layer permeation resistance	kg d /kg	value	97
		PDF	logn(p1=5.0,x1=32.0,p2=95.0,x2=298.0)
Lipid layer resistance exponent	unitless	value	0.41
		PDF	norm(mean=0.28,sd=0.53)
Organic carbon fraction of phytoplankton	unitless	value	0.29
		PDF	unif(min=0.11,max=0.46)
Phytoplankton cell volume	μm^3	value	7.68
		PDF	logn(p1=5.0,x1=2.9,p2=95.0,x2=12.04)
Water layer diffusion resistance for uptake of chemicals from water	kg d / kg	value	0.0068
		PDF	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)

Table 2. Input values for parameters of Phytoplankton model.

Parameter	Invertebrate age at maturity		Weight at maturity	Allometric rate exponent		Lipid fraction of invertebrate		Food transport coefficient	
Unit	d		Kg_{fw}	unitless		unitless		$kg_{fw} / kg_{fw} d$	
SPECIES	value	PDF	value	value	PDF	value	PDF	value	PDF
Zooplankton	20	unif(min=1.0,max=365.0)	3.42E-05	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)	0.05	logu(min=0.01,max=0.07)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)
Micro-meiobenthos	20	unif(min=1.0,max=365.0)	1.00E-04	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)	0.014	logu(min=0.01,max=0.07)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)
Macrobenthos Detritivorous	90	unif(min=1.0,max=180.0)	3.20E-04	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)	0.014	logu(min=0.01,max=0.07)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)
Macrobenthos Omnivorous Filter Feeder	548	unif(min=420.0,max=730.0)	6.71E-03	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)	0.012	logu(min=0.01,max=0.07)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)
Tapes philippinarum juv	90	unif(min=10.0,max=180.0)	1.00E-03	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)	0.0125	logu(min=0.01,max=0.07)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)
Tapes philippinarum	910	unif(min=365.0,max=1095.0)	7.00E-03	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)	0.0125	logu(min=0.01,max=0.07)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)
Macrobenthos Omnivorous Mixed Feeder	90	unif(min=1.0,max=180.0)	1.41E-03	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)	0.0262	logu(min=0.01,max=0.07)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)
Carcinus mediterraneus	730	unif(min=365.0,max=1095.0)	1.02E-02	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)	0.05	logu(min=0.01,max=0.07)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)
Macrobenthos Omnivorous Predator	545	unif(min=365.0,max=1095.0)	1.57E-03	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)	0.05	logu(min=0.01,max=0.07)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)

Parameter	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	
Unit	unitless		kg d / kg		kg d / kg		kg d / kg	
SPECIES		PDF		PDF		PDF		PDF
Zooplankton	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0)	97	logn(p1=5.0,x1=32.0,p2=95.0,x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0, x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Micro-meiobenthos	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0)	97	logn(p1=5.0,x1=32.0,p2=95.0,x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0, x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Macrobenthos Detritivorous	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0)	97	logn(p1=5.0,x1=32.0,p2=95.0,x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0, x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Macrobenthos Omnivorous Filter Feeder	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0)	97	logn(p1=5.0,x1=32.0,p2=95.0,x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0, x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Tapes philippinarum juv	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0)	97	logn(p1=5.0,x1=32.0,p2=95.0,x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0, x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Tapes philippinarum	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0)	97	logn(p1=5.0,x1=32.0,p2=95.0,x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0, x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Macrobenthos Omnivorous Mixed Feeder	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0)	97	logn(p1=5.0,x1=32.0,p2=95.0,x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0, x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Carcinus mediterraneus	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0)	97	logn(p1=5.0,x1=32.0,p2=95.0,x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0, x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Macrobenthos Omnivorous Predator	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0)	97	logn(p1=5.0,x1=32.0,p2=95.0,x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0 ,x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)

Table 3. Input values of biota-related parameters for Invertebrate model

Parameter	Fish age at maturity		Fish lenght at maturity		Intercept of lenght-weight relationship		Slope of lenght-weigth relationship		Allometric rate exponent	
Unit	d		cm			unitless	unitless		unitless	
SPECIES	value	PDF	value	PDF	value	PDF	value	PDF	value	PDF
Atherina boyeri	730	unif(min=365.0,max=1460.0)	10.5	unif(min=7.8,max=14.0)	0.00603	logn(p1=5.0,x1=0.00487,p2=95.0,x2=0.00745)	3.07	norm(p1=5.0,x1=3.01,p2=95.0,x2=3.13)	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)
Chelon labrosus	1095	unif(min=730.0,max=4575.0)	30.3	unif(min=22.6,max=40,6)	0.00794	logn(p1=5.0,x1=0.00577,p2=95.0,x2=0.01093)	3.12	norm(p1=5.0,x1=3.03,p2=95.0,x2=3.21)	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)
Chelon labrosus juv	730	unif(min=365.0,max=760.0)	3	unif(min=1.7,max=7.5)	0.0091	logn(p1=5.0,x1=0.007,p2=95.0,x2=0.011)	3.02	norm(p1=5.0,x1=2.846,p2=95.0,x2=3.195)	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)
Dicentrarcus labrax	1460	unif(min=730.0,max=1460.0)	35.9	unif(min=26.8,max=48.1)	0.00891	logn(p1=5.0,x1=0.00724,p2=95.0,x2=0.01097)	3.05	norm(p1=5.0,x1=2.99,p2=95.0,x2=3.11)	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)
Dicentrarcus labrax juv	75	unif(min=730.0,max=1460.0)	3	unif(min=2.0,max=10.2)	0.0076	logn(p1=5.0,x1=1.0,p2=95.0,x2=2.0)	3.2	norm(p1=5.0,x1=2.9,p2=95.0,x2=3.3)	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)
Nekton carnivorous benthic feeder	1058.5	unif(min=730.0,max=2500.0)	32.5	unif(min=24.3,max=43.6)	0.0123	logn(p1=5.0,x1=0.01043,p2=95.0,x2=0.01451)	2.96	norm(p1=5.0,x1=2.92,p2=95.0,x2=3.0)	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)
Sparus aurata	949	unif(min=730.0,max=1460.0)	30	unif(min=22.4,max=40.2)	0.01259	logn(p1=5.0,x1=0.01114,p2=95.0,x2=0.01423)	3.03	norm(p1=5.0,x1=-3.0,p2=95.0,x2=3.06)	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)
Sparus aurata juv	50	unif(min=45.0,max=480.0)	3	unif(min=1.6,max=6.1)	0.00923	logn(p1=5.0,x1=1.0,p2=95.0,x2=2.0)	3.28	norm(p1=5.0,x1=2.9,p2=95.0,x2=3.3)	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)
Zosterisessor ophiocephalus	1659.5	unif(min=730.0,max=1825.0)	16.3	unif(min=12.2,max=21.9)	0.00813	logn(p1=5.0,x1=0.00497,p2=95.0,x2=0.01329)	3.07	norm(p1=5.0,x1=2.93,p2=95.0,x2=3.21)	0.25	norm(mean=0.25,sd=0.11,trmin=0.0)

Parameter	**Lipid fraction of fish**		**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**	
Unit	unitless		$kg_{fw} / kg_{fw} d$		unitless		kg d / kg		kg d / kg		kg d / kg	
SPECIES	value	PDF	value	PDF	value	PDF	**value**	PDF	**value**	PDF	value	PDF
Atherina boyeri	0.096	logu(min=0.01, max=0.2)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0	97	logn(p1=5.0,x1=32.0,p2=95.0, x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0,x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Chelon labrosus	0.068	logu(min=0.01, max=0.2)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0	97	logn(p1=5.0,x1=32.0,p2=95.0, x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0,x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Chelon labrosus juv	0.068	logu(min=0.01, max=0.2)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0	97	logn(p1=5.0,x1=32.0,p2=95.0, x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0,x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Dicentrarcus labrax	0.1338	logu(min=0.01, max=0.2)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0	97	logn(p1=5.0,x1=32.0,p2=95.0, x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0,x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Dicentrarcus labrax juv	0.1338	logu(min=0.01, max=0.2)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0	97	logn(p1=5.0,x1=32.0,p2=95.0, x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0,x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Nekton carnivorous benthic feeder	0.08	logu(min=0.01, max=0.2)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0	97	logn(p1=5.0,x1=32.0,p2=95.0, x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0,x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Sparus aurata	0.0973	logu(min=0.01, max=0.2)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0	97	logn(p1=5.0,x1=32.0,p2=95.0, x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0,x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Sparus aurata juv	0.0973	logu(min=0.01, max=0.2)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0	97	logn(p1=5.0,x1=32.0,p2=95.0, x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0,x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)
Zosterisessor ophiocephalus	0.1	logu(min=0.01, max=0.2)	0.03	logn(p1=5.0,x1=0.022,p2=95.0,x2=0.041)	0.73	beta(alpha=50.0,beta=18.5,trmin=0.0,trmax=1.0	97	logn(p1=5.0,x1=32.0,p2=95.0, x2=298.0)	0.0002	logn(p1=5.0,x1=3.6E-6,p2=95.0,x2=0.011)	0.0068	logn(p1=5.0,x1=0.0037,p2=95.0,x2=0.013)

Table 4. Input values of biota-related parameters for the Fish model.

The Fish and Invertebrate models require also defining the diet preferences of each species to be model. Components of the organisms' diet can be either other organisms, such as invertebrate, fish or phytoplankton (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 the fraction of each prey/food item (over the total dietary intake) for each considered species. Diet preferences for the Venice lagoon organisms have been defined according to available literature data and adapting the diet matrix proposed by Micheletti and colleagues (2008). The diet matrix is reported in

Table 5.

	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			

Table 5. Diet matrix for the Venice lagoon food web (adapted from Micheletti et al., 2008).

2.2.3 Time series

The food web models (Phytoplankton, Invertebrate and Fish) are all linked to the model "River measurements", which is used to define the environmental conditions the organisms are living in (despite the name "River", this module represents a generic aquatic environment).

The "River measurement" block requires input values to the following forcing variables:

- Water temperature (input to Phytoplankton, Invertebrate and Fish models);
- Chemical concentrations in dissolved water (input to Phytoplankton, Invertebrate and Fish model);
- Chemical concentrations in sediment (input to Invertebrate and Fish models).

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.

Besides ecological exposure, this case study aims at assessing the lifetime human exposure to POPs, therefore it is necessary to simulate concentrations in edible species for several decades in the past in order to reconstruct dietary uptake for local population. However, historical data of PCBs and dioxins emissions suitable to reconstruct the historical development of contamination in the lagoon of Venice are not available. As an alternative, sediment cores proved to be useful in supporting the reconstruction of temporal trends of environmental contamination, as demonstrated by many authors (Marcomini et al., 1999; Frignani et al.,2004; 2005), also in combination with environmental modelling approaches (Dalla Valle et al., 2005a).

Concentration of chemicals in different layers of dated sediment cores have been used to reconstruct historical trends of sediment and water contamination.

Taking into account the partitioning process of chemicals between the aqueous and the solid phase, the total concentration of chemical in water (C_{wt} in g/L) can be calculated according to Eq. 1.

$$Cwt = \frac{Cs}{Ksw} \qquad \text{(Eq. 1)}$$

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 (Kow) proposed by Seth and colleague (1999):

$$Ksw = \mathrm{foc} \cdot 0.35 \cdot \mathrm{Kow} \cdot d_{bs} \qquad \text{(Eq. 2)}$$

where f_{oc} is the fraction of organic carbon in the sediment and d_{bs} (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) (Eq.3).

$$Cwd = \frac{Cwt}{1+\left(\frac{Kow \cdot MOM}{d_{OM}}\right)} \qquad \text{(Eq. 3)}$$

where MOM is the organic matter concentration in water (Kg/L), and d_{OM} is the organic matter density (Kg/L). MOM 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.

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	

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

Studies focused on the reconstruction of temporal trends of POPs contamination in radio-dated sediment cores from the Venice lagoon have been reviewed (e.g., Marcomini et al., 1999; Frignani et al., 2001, 2004, 2005; Piazza et al., 2003; Pavoni et al., 1987). The study by Frignani and colleagues (2005) has been selected because it provides individual concentrations for the individual congeners of interest (while other studies often reported only total concentrations) at specific cores depths (corresponding to specific time period).

Specifically, from this study the data related to sediment core “E”, collected in the central lagoon area close to Porto Marghera industrial zone, have been used (see map in Figure 8).

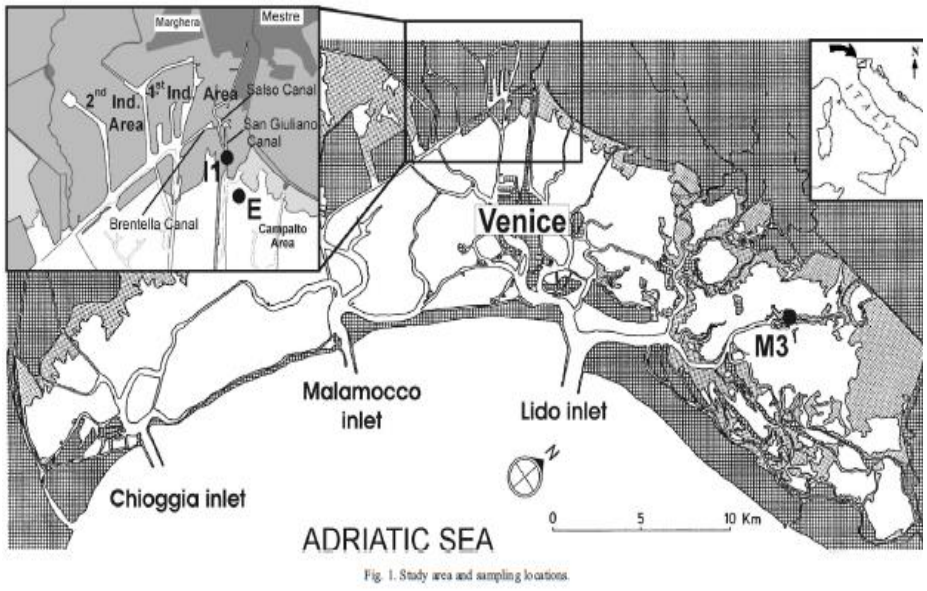


Figure 8 Sediment core sampling site (map from Frignani et al., 2001)

Concentrations of target chemicals in sediment corresponding to different ages, and related concentrations dissolved in water calculated for the same years are reported in Table 7 and

Table 8. To reconstruct temporal trend, values between the measured points have been interpolated.

Year	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
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

Table 7. Concentration of chemicals (mg/Kg) for each layer of the dated sediment core and corresponding year.

Year	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
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

Table 8. Concentration of chemicals dissolved in water (mg/kg^3) estimated for different years.

2.3 Results and discussion

In this paragraph, the results of the application of MERLIN-Expo models to estimate concentrations in aquatic organisms of the Venice lagoon are presented, with the description of outcomes of both deterministic and probabilistic simulations. Moreover, an appraisal of models' performance will be proposed, as well as the results of sensitivity analysis.

2.3.1 Deterministic simulations

MERLIN-Expo provides as output of the deterministic simulation the time trend of concentrations of all target chemicals in aquatic organisms included in the Venice lagoon food web, for the selected period of 21900 days (from 1940 to 1998). Based on sediment radio dating, 1940 is the earliest record on content of contaminants in sediments. The latest available data on chemical concentrations in sediments, used as simulation input, are available for year 1998 (21170 day).

An example of the temporal trend of PCB126 in selected organisms is presented in Figure 9.

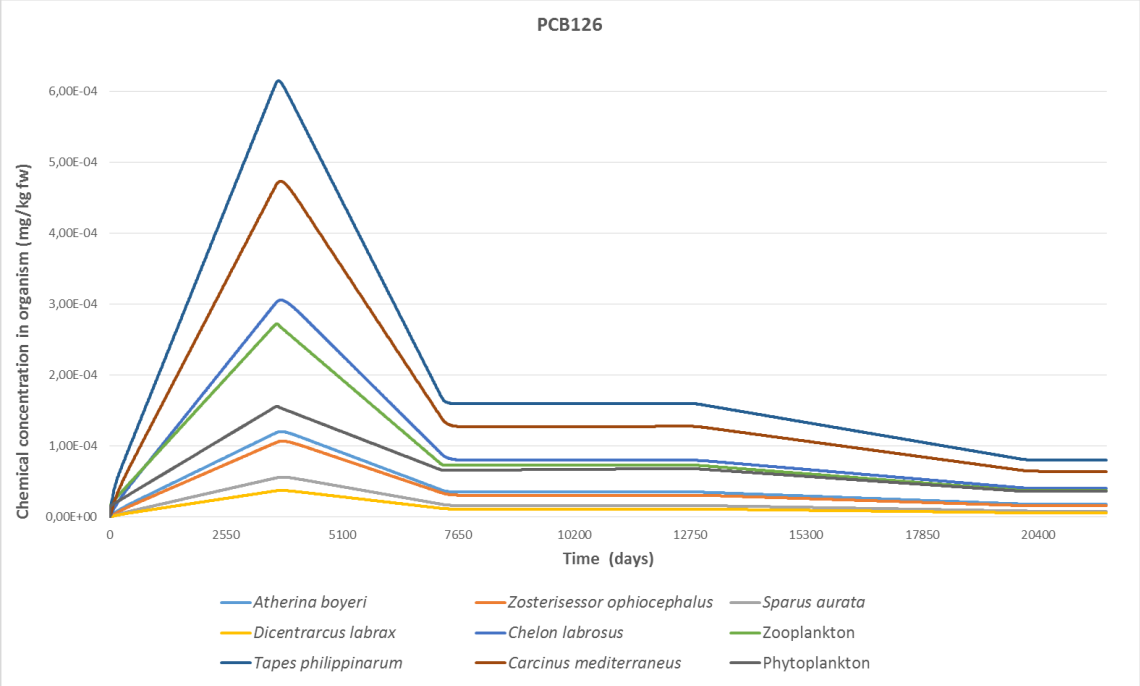


Figure 9. Time trend of PCB126 concentration (mg/Kgfw) in selected aquatic organisms considered in the Venice lagoon food web modelling.

Simulation results depicted in Figure 9 represent the comparison of accumulated chemical in different species. Aquatic organisms were assumed to be exposed to dissolved concentration in water through respiration, and ingested chemicals bound to sediments and accumulated in other species. Results, expressed on a fresh weight basis, show the highest accumulated concentration in *Tapes philippinarum* (Manila clam), while the lowest simulated concentration was obtained for *Dicentrarcus labrax* (European seabass). If simulated concentrations in aquatic species are compared to historical trend of reconstructed chemical concentrations in water, it can be noticed that they follow a similar trend.

The estimated concentrations in aquatic organisms have been compared to available measurements in organisms sampled in the central lagoon (namely, goby, Manila clam, and crab) shown in Figure 10, and northern lagoon area (in the case of mullet) in 1998 (Mappatura project; MAV, 2000a).

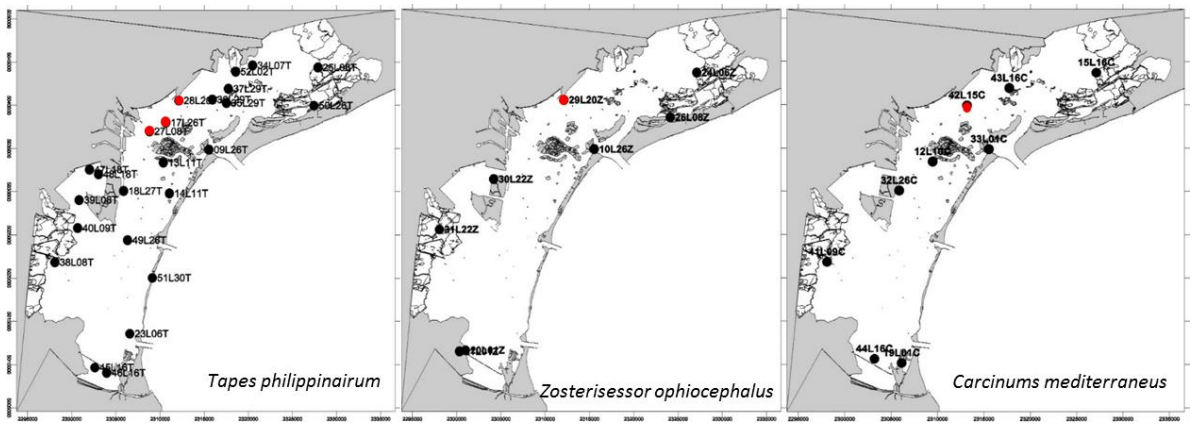


Figure 10. *Tapes philippinarum*, *Zosterisessor ophiocephalus* and *Carcinus mediterraneus* sampling sites in the Central Lagoon (Mappatura project, 1999)

The list of sampled species, the number of selected sampling sites and the overall number of sampled individuals are reported in Table 9.

Species		N° Sampling site	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

Table 9. List of aquatic species and selection of data for the central area of Venice lagoon.

In Table 10, a comparison of measured and simulated concentrations of chemical compounds in aquatic species is reported. Since biota samples have been collected in 1998, simulated concentrations for the same year have been selected from the MERLIN-Expo time trend results.

	Tapes philippinarum **(mg/kg$_{fw}$)**		***Carcinus mediterraneus*** **(mg/kg$_{fw}$)**		***Chelon labrosus*** **(mg/kg$_{fw}$)**		***Zosterissessor ophiocephalus*** **(mg/kg$_{fw}$)**	
Chemical	Measured	Simulated	Measured	Simulated	Measured	Simulated	Measured	Simulated
2,3,7,8-TCDD	1.40E-08*	4.84E-07	1.01E-07	1.96E-07	6.72E-07	5.70E-08	8.58E-08	1.92E-08
1,2,3,7,8-PCDD	2.13E-08*	2.41E-06	1.86E-07	9.53E-07	7.20E-07	3.00E-07	1.54E-07	6.48E-08
1,2,3,4,7,8-HCDD	4.21E-08*	6.34E-06	1.29E-07	2.23E-06	1.54E-07*	7.43E-07	4.76E-08	7.09E-08
PCB 77	1.77E-05	4.44E-04	1.53E-04	2.83E-04	2.64E-04	6.29E-05	1.13E-05	5.67E-05
PCB 126	2.30E-06	8.03E-05	1.62E-05	6.43E-05	5.79E-05	4.01E-05	2.26E-05	1.56E-05
PCB 167	5.37E-05	3.46E-03	5.44E-04	2.41E-03	1.27E-03	1.43E-03	3.31E-04	3.33E-04
PCB 169	2.85E-07	2.32E-05	3.46E-06	1.73E-05	5.28E-06	1.24E-05	6.09E-06	2.83E-06
PCB 170	1.49E-04	9.58E-03	9.31E-04	5.56E-03	5.31E-03	4.13E-03	1.98E-03	3.77E-04
PCB 180	3.91E-04	1.75E-02	2.44E-03	1.00E-02	1.01E-02	7.14E-03	3.85E-03	6.61E-04

Notes:

[a] mean value estimated across three sampling stations

[b] mean value estimated from data in three sampling stations

* = 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.

Table 10. Comparison of simulated and measured concentrations of chemicals in selected aquatic species.

The observed bioaccumulation factor (BAF_o) has been calculated for each species and each chemical as the ratio of the measured concentration in the organism and the corresponding concentration dissolved in water (at the same time). The predicted bioaccumulation factor (BAF_p) has been calculated as the ratio of the simulated concentration in the organism and

the corresponding concentration of chemical in water. The comparison of values of BAF_o and BAF_p for each species and each target chemical is presented in Table 11.

	Tapes philippinarum		*Zosterisessor ophiocephalus*		*Carcinus mediterraneus*		*Chelon labrosus*	
Chemical	**logBAFo**	**logBAFp**	**logBAFo**	**logBAFp**	**logBAFo**	**logBAFp**	**logBAFo**	**logBAFp**
2,3,7,8-TCDD	1.24	2.78	2.03	1.38	2.10	2.39	2.92	1.85
1,2,3,7,8-PCDD	2.13	4.19	2.99	2.62	3.08	3.79	3.66	3.28
1,2,3,4,7,8-HCDD	3.53	5.71	3.58	3.76	4.02	5.25	4.09	4.78
PCB 77	0.58	1.98	0.39	1.09	1.52	1.79	1.76	1.13
PCB 126	1.54	3.08	2.53	2.37	2.39	2.99	2.94	2.78
PCB 167	2.64	4.45	3.43	3.43	3.65	4.29	4.02	4.07
PCB 169	2.48	4.39	3.81	3.48	3.56	4.27	3.75	4.12
PCB 170	4.48	6.29	5.60	4.88	5.27	6.05	6.03	5.92
PCB 180	4.63	6.29	5.63	4.86	5.43	6.04	6.05	5.90

Table 11. Comparison of observed ($logBAF_o$) and simulated Bioaccumulation Factors ($logBAF_p$) for the selected aquatic species.

2.3.2 Model performance (deterministic simulations)

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

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

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:

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

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 12. Indicators of model performance for single species and for the overall model.

	Tapes philippinarum	*Zosterisessor ophicephalus*	*Carcinus mediterraneus*	*Chelon labrosus*
Model bias (MB_j) for single species	58.30	0.61	3.84	0.73
Overall model bias (MB)	15.87			

Table 12. Indicators of model performance for single species and for the overall model.

The high MB for *Tapes philippinarum* (associated to the significant difference between simulated and measured concentrations) can be explained if we consider that, the place where clams were collected does not correspond with the place where the sediment core was sampled. 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, considering only 1 time point for 2003 (data provided by ICSEL project, 2003).

With this new dataset, the distance between model outcomes and monitoring data seems to reduce, as results from the comparison on measured and simulated data for *Tapes* presented in Table 13.

Compound	Sediment ICSEL 2003 (superficial) mg/kg dw	*Tapes philippinarum*	
		Measured ICSEL	Simulated starting from ICSEL data
2,3,7,8 -TCDD	4.00E-04	2.00E-05	4.85E-04
1,2,3,7,8-PCDD	4.00E-04	2.20E-05	7.42E-04
1,2,3,6,7,8-HCDD	6.00E-04	3.00E-05	1.90E-03
OCDD	2.36E-02	2.31E-04	3.31E-02
PCB 126	1.30E-05	2.00E-06	1.94E-05
PCB 167	1.20E-05	4.10E-05	1.24E-04
PCB 169	5.00E-06	2.00E-06	2.31E-05
PCB 170	9.95E-04	7.80E-05	1.11E-03
PCB 180	2.08E-03	2.26E-04	1.95E-03

Table 13. Measured and simulated concentration of contaminants in *Tapes philippinarum* using new dataset

The model bias was re-calculated by substituting these new values for *Tapes* (values for other species are the same as before) and it can be noticed that the difference between

measured and monitored data improved as reported in Table 14. Anyway, an overestimation of model predictions in comparison with measurement data can 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 behavior of the model under different scenarios and support the identification of possible adjustments to improve its capability of approximate bioaccumulation measurements.

	Tapes philippinarum	*Zosterisessor ophicephalus*	*Carcinus mediterraneus*	*Chelon labrosus*
Model bias (MB_j) for single species (ICSEL, 2003)	18.92	0.61	3.84	0.73
Overall model bias (MB) (ICSEL, 2003)	6.48 (corrected only of concentration for Tapes)			

Table 14. Model bias calculated using data on concentration in biota (*Tapes philippinarum*) and water from ICSEL project (2003).

2.3.3 Probabilistic simulations

In order to evaluate the uncertainty associated with exposure estimates, the bioaccumulation factors (BAF) were probabilistically estimated with MERLIN-Expo (Monte Carlo approach, 100 simulations) taking into account uncertainty for the following parameters: fraction of assimilated food, lipid content (carbon content in case of phytoplankton), water layer diffusion resistances from food and water, lipid layer resistance. The rationale for taking into account these parameters in probabilistic analysis was reported by Hauck et al. (2011): the variation in rate constant for chemical intake from water and food is driven mainly by variation in partial resistances (water resistances from water and food, and lipid resistances) and lipid content.

An example of results from probabilistic simulations is the time trend of the mean concentration in organisms (e.g., *Zosterisessor ophiocephalus* in the example reported in Figure 11) accompanied by the time trend of 5^{th} and 95^{th} percentile of the estimated concentration.

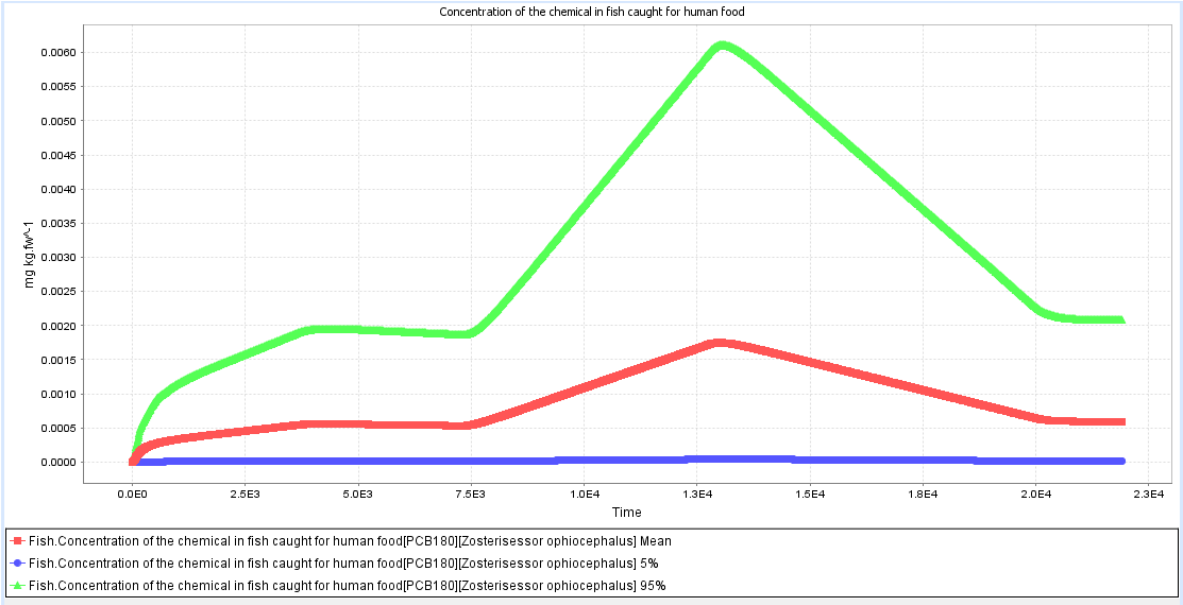


Figure 11. An example of results of probabilistic simulations: concentration of PCB180 in *Z. ophiocephalus* (mean, 5th and 95th percentile).

The mean concentrations in biota and the mean BAF have been calculated for selected points in time, corresponding to years for which sediment and water measurement data were available (i.e., 1940, 1950, 1960, 1975, 1995). Simulations were performed on a daily resolution, therefore internal concentrations in biota (mg/kg fw) and BAF estimates are plotted on time scale in day units (see Table 15 for correspondence between days and years). BAF values were obtained by dividing concentration of contaminant in organism by concentration in water. Statistics, mean and standard deviation values were derived from probabilistic simulation (100 simulations). From Figure 12 to

Figure 15 the temporal change in internal concentration and BAF of 1,2,3,7,8-PeCDD, PCB169, PCB167, PCB126 in four selected species are illustrated.

Days (x)	Years
1	1940
3650	1950
7300	1960
12775	1975
20075	1995
21900	1998

Table 15. Simulation time scale and corresponding years, based on available data on contaminant concentration in sediment.

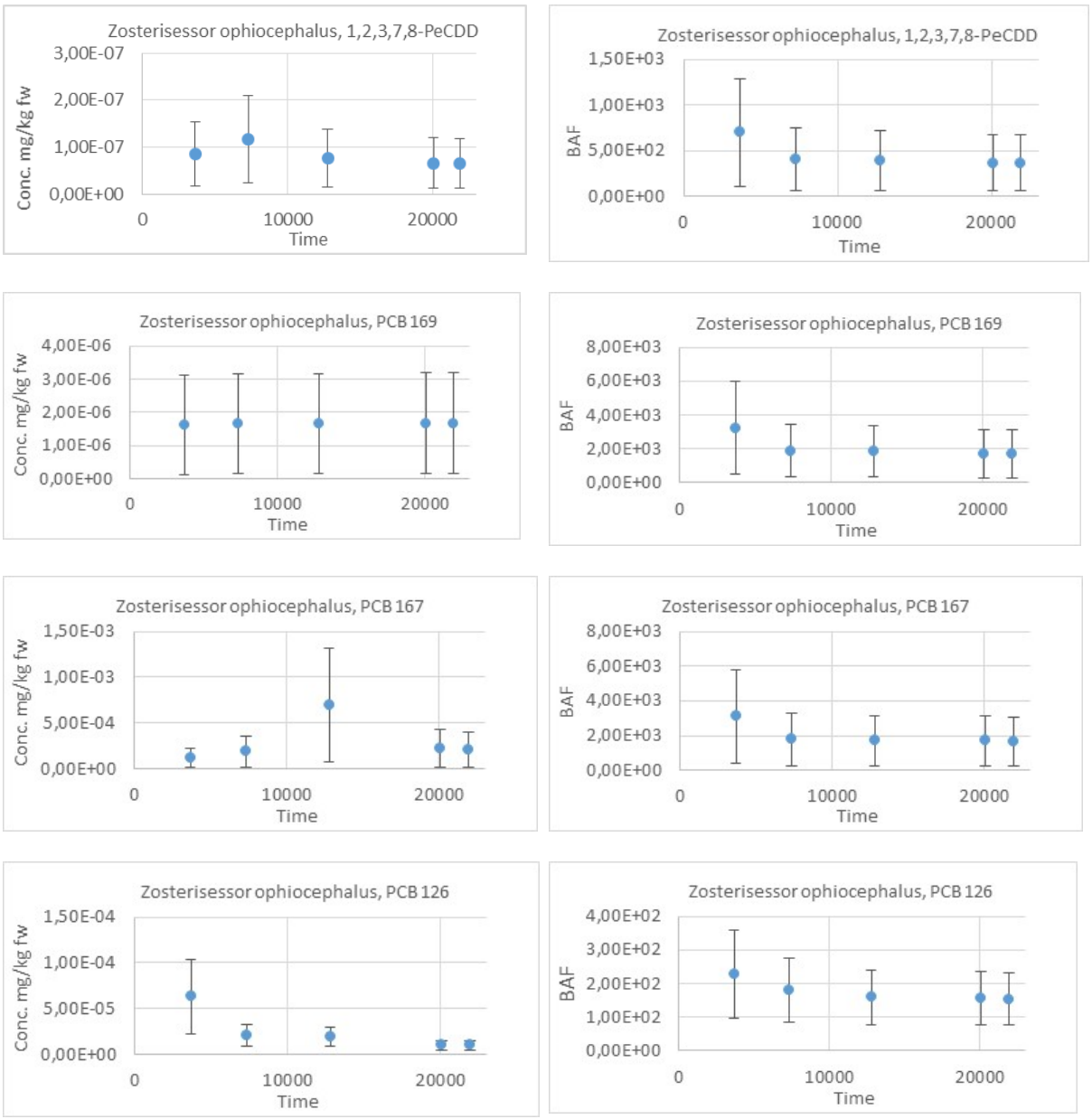


Figure 12. Temporal mean internal concentration + SD and mean BAF + SD for *Zosterisessor ophiocephalus*

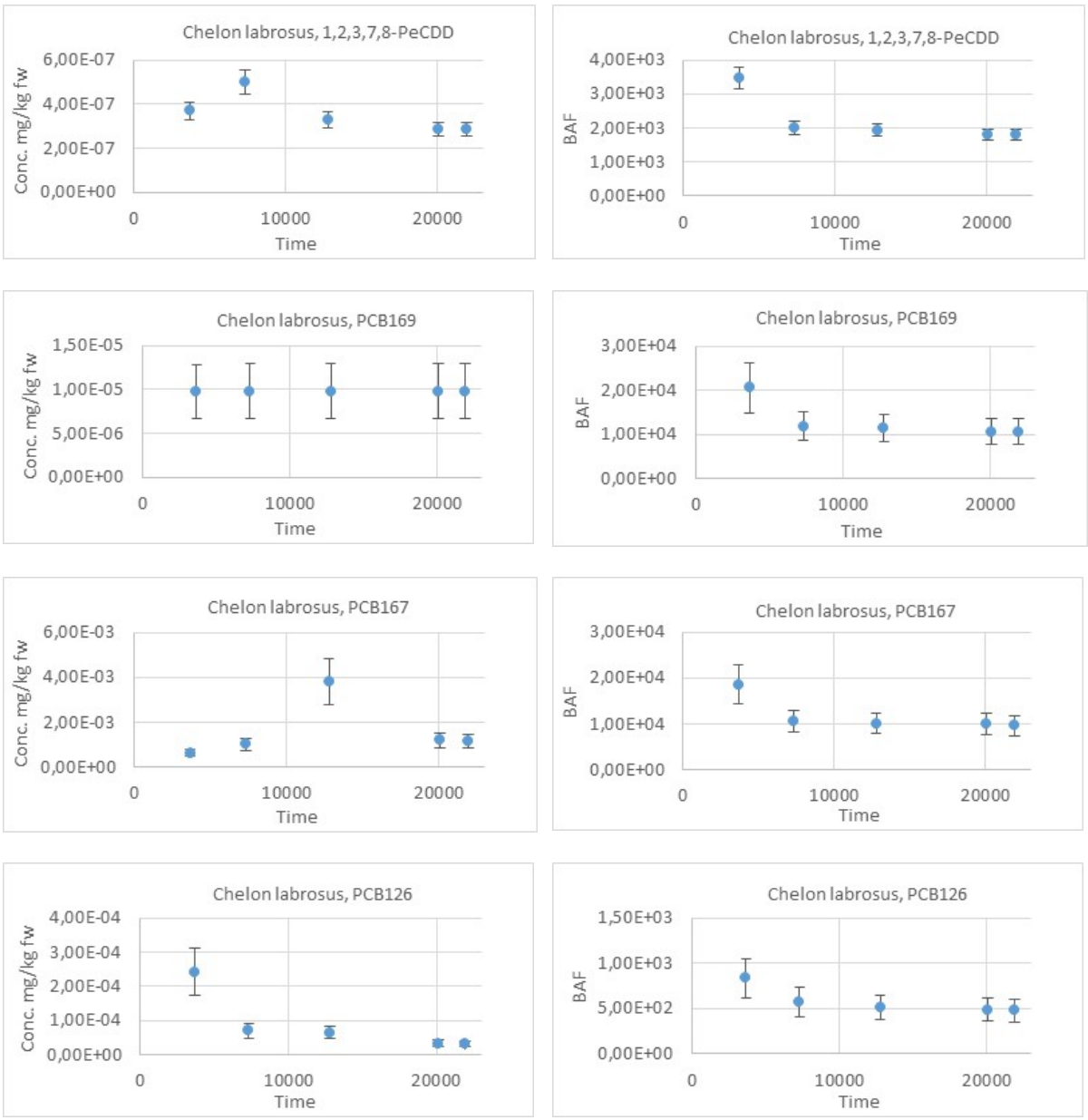


Figure 13. Temporal mean internal concentration + SD and mean BAF + SD for *Chelon labrosus.*

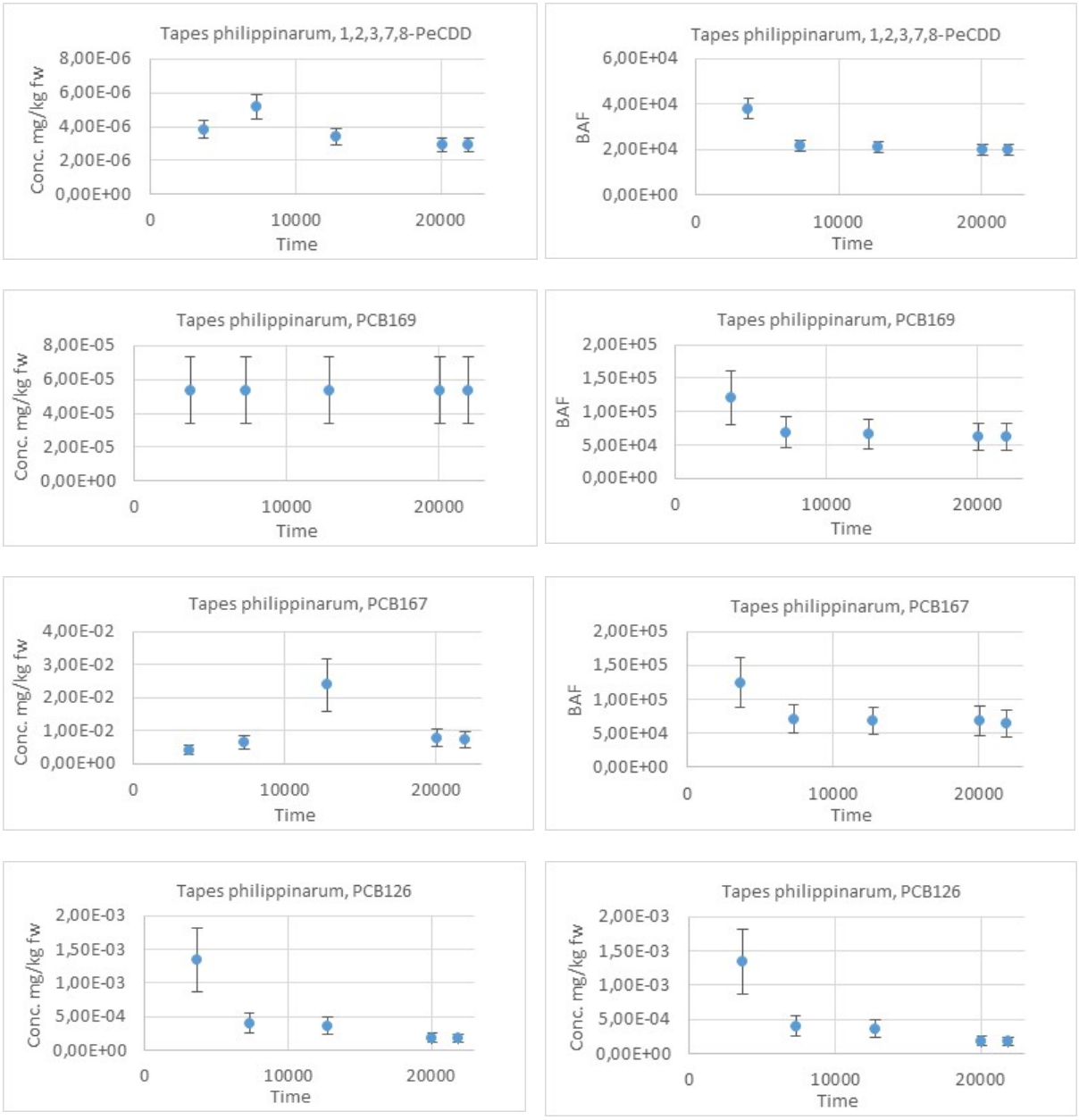


Figure 14. Temporal mean internal concentration + SD and mean BAF + SD for *Tapes philippinarum*

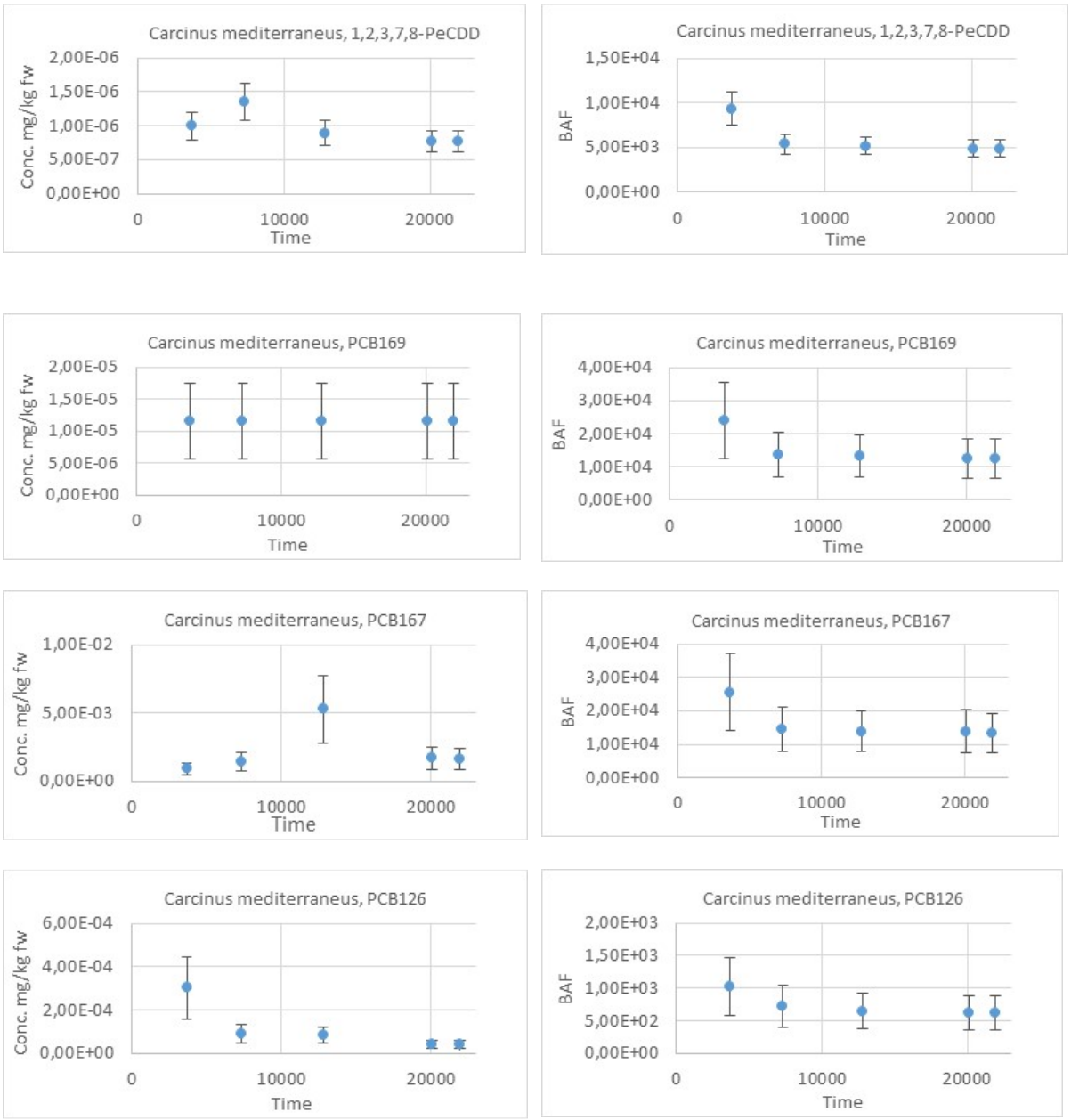


Figure 15. Temporal mean internal concentration + SD and mean BAF + SD for Carcinus mediterraneus

Predicted values of BAF (BAFp) (mean values obtained from probabilistic simulations for year 1998) were compared with observed BAF (BAFo) values calculated from measured concentrations (1998), as reported in Table 16.

Chemical	Organism	Predicted logBAF (logBAFp)	Observed logBAF (logBAFo)
1,2,3,7,8-PeCDD	Zosterisessor ophiocephalus	2.6	3.0
PCB169	Zosterisessor ophiocephalus	3.2	3.8
PCB167	Zosterisessor ophiocephalus	3.2	3.4
PCB126	Zosterisessor ophiocephalus	2.2	2.5
PeCDD	Chelon labrosus	3.3	3.7
PCB169	Chelon labrosus	4.0	3.7
PCB167	Chelon labrosus	4.0	4.0
PCB126	Chelon labrosus	2.7	2.9
PeCDD	Tapes philippinarum	4.3	2.1
PCB169	Tapes philippinarum	4.8	2.5
PCB167	Tapes philippinarum	4.8	2.6
PCB126	Tapes philippinarum	3.5	1.5
PeCDD	Carcinus mediterraneus	3.7	3.1
PCB169	Carcinus mediterraneus	4.1	3.6
PCB167	Carcinus mediterraneus	4.1	3.6
PCB126	Carcinus mediterraneus	2.8	2.4

Table 16. Comparison between predicted (mean values obtained from probabilistic simulations for 1998) and observed BAF values for different aquatic species and chemicals

Uncertainty ranges (5th and 95th percentiles) on internal concentration at time 21900 (1998) are presented in Table 17.

Chemical	Kow	Conc. (5th – 95th) *Zosterisessor ophicephalus (ZO)*	Conc. (5th – 95th) *Chelon labrosus (CL)*	Conc. (5th – 95th) *Tapes philippinarum (TP)*	Conc. (5th – 95th) *Carcinus mediterraneus (CM)*
PCB126	6.8	3.91E-06 – 2.07E-05	1.63E-05 – 4.61E-05	7.75E-05 - 2.73E-04	3.98E-05 - 7.32E-05
PCB169	7.46	1.59E-07 – 4.65E-06	4.17E-06 – 1.42E-05	2.00E-05 - 8.37E-05	1.04E-05 - 2.22E-05
PCB167	7.5	1.23E-05 – 6.15E-04	5.66E-04 – 1.62E-03	2.85E-03 - 1.10E-02	1.50E-03 - 2.88E-03
1,2,3,7,8-PeCDD	7.56	1.78E-09 – 1.75E-07	2.35E-07 – 3.30E-07	2.18E-06 - 3.49E-06	7.57E-07 - 1.03E-06

Table 17. Uncertainty ranges on simulated internal concentration (mg/kg fw) for the last time point (1998) and different values of log K_{OW} in four selected species

In Figure 16, uncertainty ranges calculated as the difference between 95th and 5th percentile are plotted against log Kow (as proposed by de Laender et al., 2010).

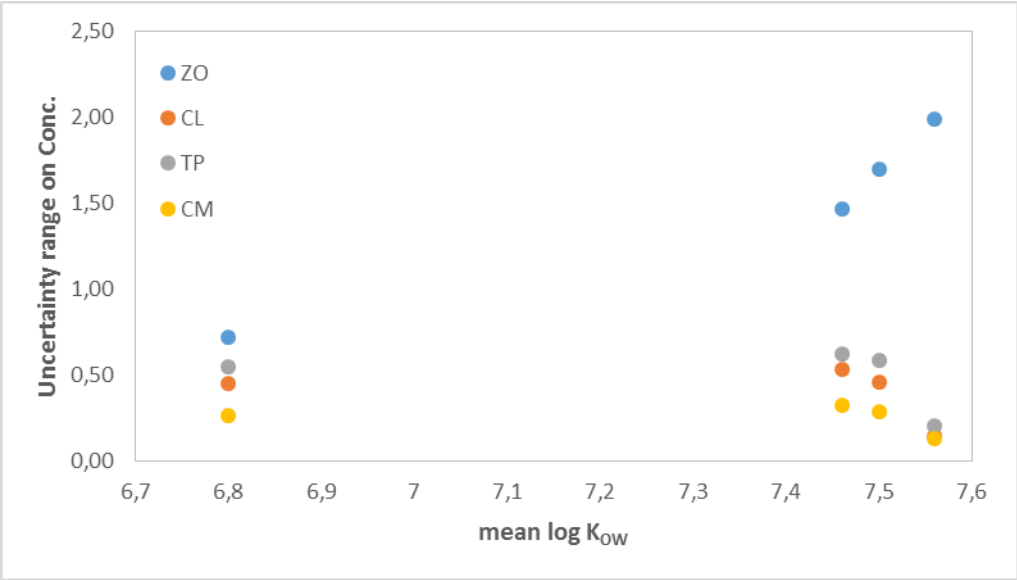


Figure 16. Uncertainty range of the internal concentration (95th–5th percentile of log Concentration) of substances with different log K_{OW} in four species (two fishes: ZO, CL, and two invertebrates: TP, CM)

Uncertainty on internal concentration differ between species and selected chemicals. Several studies indicated that uncertainties on chemicals parameter such as K_{OW} had the largest influence on model output uncertainty (MacLeod et al., 2002, Ciavatta et al., 2009, De Laender et al., 2010). Table 17 and Figure 16 show how uncertainty varies with species and different K_{OW} values, for year 1998. In the case of *Zosterisessor ophiocephalus,* uncertainty on internal concentration increases with log K_{OW}, and this is in agreement with findings by De Laender and colleagues (2010), who applied OMEGA bioaccumulation model to study propagation of uncertainty of chemical and physiological parameter on variation in internal concentration in generic fish. However, results obtained for *Chelon labrosus*, *Tapes philippinrum*, and *Carcinus mediterraneus* suggest that other species- or chemical-specific parameters may play important role in affecting uncertainty of internal concentration. In fact, Ciavatta and colleagues (2009), report that for clam (*Tapes philippinarum*) uncertainty on other parameters such as organic carbon–octanol proportionality constant, used in calculating freely dissolved concentration in pore water, contributes more than uncertainty in K_{OW} to the output variance. Sensitivity analysis application was performed in order to better clarify key parameters in bioaccumulation modelling performed with MERLIN-Expo (see Paragraph 2.3.4).

2.3.4 Sensitivity analysis

Sensitivity analysis using the Morris method was performed, making use of the functionalities offered by MERLIN-Expo. The Morris method (Morris, 1991) 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. This OAT method is categorized as a global sensitivity analysis because the method covers the entire ranges over which the factors may vary, whereas in a local sensitivity analysis, the factors vary only around their nominal values. The method is used to identify which factors are (a) negligible, (b) linear and additive, or (c) non-linear or involved in interaction with other factors (Campolongo et al, 2007). For each factor, the method computes two sensitivity measures: $\boldsymbol{\mu}$, which assesses the overall influence of

the factor on the output, and ***σ***, which estimates the non-linear effect and/or the interaction effect with other factors.

Considering the complexity of the simulated food web, sensitivity analysis was performed focusing only on a sub-set of chemicals and on two species, one invertebrate (*Tapes philippinarum*) and one fish (*Chelon labrosus*). The species have been selected because they are also part of human diet, so they contribute to human exposure. Biological parameters and parameters related to physico-chemical properties of investigated chemicals were considered separately (Tables 18-23).

The parameters considered for Tapes are reported in Table 18 and the results of sensitivity analysis (i.e., classification of considered parameters) are included in Table 19. Charts with examples of results of Morris sensitivity analysis for PCB180 and PCB126 are reported in Appendix B (Sensitivity analysis).

Parameter full name	**Parameter ID**
Fraction of assimilated food	Assimilated_food
Food transport coefficient	gamma_food
Allometric rate exponent	Kappa
Lipid fraction of animal	p_lipid
Lipid-layer permeation resistance	rho_lipid_layer
Water-layer diffusion resistance for uptake of chemicals from water	rho_water_layer
Water-layer diffusion resistance for uptake of chemicals from food	rho_water_layer_food

Table 18. Biological parameters considered in sensitivity analysis of *Tapes philippinarum*

Classification	**PCB 169**	**PCB 167**	**PCB 180**	**PCB 126**	**1,2,3,7,8-PeCDD**
Non sensitive parameters (μ,σ are low)	rho_water_layer, rho_lipid_layer	rho_water_layer, rho_lipid_layer	rho_lipid_layer, rho_water_layer	rho_lipid_layer,	rho_lipid_layer, rho_water_layer
Parameters with direct (linear) effects (μ is high, σ is low)	-	-	-	-	-
Parameters with interaction and/or non linear effects (σ is high regardless μ)	Assimilated_foodgamma_food, p_lipid, rho_water_layer_food, kappa	Assimilated_foodgamma_food, p_lipid, rho_water_layer_food, kappa	Assimilated_foodkappa, rho_water_layer_food, gamma_food, p_lipid,	Assimilated_food kappa, rho_water_layer_food, gamma_food, p_lipid, rho_water_layer	Assimilated_food kappa, rho_water_layer_food, gamma_food, p_lipid,

Table 19. Classification of biological input parameters for *Tapes philippinarum* based on Morris method.

Among the considered chemicals, PCB167 and PCB180 are those for which biological parameters have more effect (highest μ and σ values) on output (bioaccumulation in *Tapes philippinarum*). Parameters having non-linear effect or showing interactions are fraction of assimilated food (Assimilated_food), and water-layer diffusion resistance from food (rho_water_layer_food). μ and σ values of rho_water_layer_food change of several orders of magnitude when compared between PCB 180/167 and PeCDD. Similar behaviour can be observed also for other parameters. No parameter has been observed to have direct (linear)

effect on concentration of chemicals in *Tapes philippinarum*. For the remaining chemicals (PCB126, PCB169, 1,2,3,7,8-PeCDD) in *Tapes philippinarum*, bioaccumulation seems not to be siginificantly influenced by biological parameters (low μ and σ values).

The parameters considered for Chelon labrosus are reported in Table 20 and the results of sensitivity analysis (i.e., classification of considered parameters) are included in Table 21.

Parameter full name	**Parameter ID**
Intercept of weight-length relationship	a_W
Slope of weight-length relationship	b_W
Fraction of assimilated food	Assimilated_food
Food transport coefficient	gamma_food
Allometric rate exponent	Kappa
Animal length at maturity	L
Lipid fraction of animal	p_lipid
Age at maturity	time_life
Lipid-layer permeation resistance	rho_lipid_layer
Water-layer diffusion resistance for uptake of chemicals from water	rho_water_layer
Water-layer diffusion resistance for uptake of chemicals from food	rho_water_layer_food

Table 20. Biological parameters considered in sensitivity analysis of *Chelon labrosus*

Classification	**PCB 169**	**PCB 167**	**PCB 180**	**PCB 126**	**1,2,3,7,8-PeCDD**
Non sensitive parameters (μ,σ are low)	rho_lipid_layer, rho_water_layer	rho_lipid_layer, rho_water_layer	rho_lipid_layer, rho_water_layer	rho_lipid_layer,	rho_lipid_layer, rho_water_layer
Parameters with direct (linear) effects (μ is high, σ is low)	-	-	-	-	-
Parameters with interaction and/or non linear effects (σ is high regardless μ)	a_W, b_W, Assimilated_food, gamma_food, L, p_lipid, time_life, rho_water_layer_food	a_W, b_W, Assimilated_food, gamma_food, L, p_lipid, time_life, rho_water_layer_food	a_W, b_W, Assimilated_food, gamma_food, L, p_lipid, time_life, rho_water_layer_food	a_W, b_W, Assimilated_food, gamma_food, L, p_lipid, time_life, rho_water_layer_food, rho_water_layer	a_W, b_W, Assimilated_food, gamma_food, L, p_lipid, time_life, rho_water_layer_food

Table 21. Classification of biological input parameters for *Chelon labrosus* based on Morris method.

The most siginificant biological parameters specific to *Chelon labrosus* turned out to be: fish lenght at maturity (L) for PCB 167, water-layer diffusion resistance for uptake of chemicals from food (rho_water_layer_food) for 1,2,3,7,8-PeCDD, food transport coefficient (gamma_food) for PCB 169, age at maturity for PCB 180, and lipid fraction of animal (p_lipid) for PCB 126. No parameter is having direct linear effect on model outputs.

Physico-chemical parameters included in the Invertebrate and the Fish model for Morris sensitivity analysis are reported in Table 22, and classification of these parameters according to Morris sensitivity analysis are reported in Table 23.

Parameter full name	Parameter ID
Metabolic half-life of chemicals for organics	hl_metabolic_norm
Bioconcentration Factor for organics	log10_BCF_organic
Water-organic carbon partition coefficient	log10_K_oc
Octanol/water partition coefficient	log10_K_ow

Table 22. Physico-chemical parameters considered in sensitivity analysis of *Chelon labrosus*

Classification	PCB 169	PCB 167	PCB 180	PCB 126	1,2,3,7,8-PeCDD
Non sensitive parameters (μ,σ are low)	log10_BCF_organic	log10_BCF_organic	log10_BCF_organic	log10_BCF_organic	log10_BCF_organic
Parameters with direct (linear) effects (μ is high, σ is low)	-	-	-	-	-
Parameters with interaction and/or non linear effects (σ is high regardless μ)	hl_metabolic_norm, log10_K_oc, log10_K_ow	hl_metabolic_norm, log10_K_oc, log10_K_ow	hl_metabolic_norm, log10_K_oc, log10_K_ow	hl_metabolic_norm, log10_K_oc, log10_K_ow	hl_metabolic_norm, log10_K_oc, log10_K_ow

Table 23. Classification of phys-chem input parameters for *Tapes philippinarum* and *Chelon labrosus* based on Morris method.

For both *Tapes philippinarum* and *Chelon labrosus*, the metabolic half-life of chemicals for organics (hl_metabolic_norm) is the most influential parameter, showing non-linear effects and/or interactions, whereas bioconcentration factor for organics (log10_BCF_organic) is least significant. Physico-chemical parameters result to be more influential in estimating concentrations for the chemicals PCB 180 and PCB 167, in both *Tapes philippinarum* and *Chelon labrosus*, if compared to other considered chemicals. No parameters are directly (linearly) affecting the output.

Some general conclusions can be drawn from the results obtained from sensitivity analysis. Models predict accumulation of contaminants in aquatic species using first-order uptake and elimination kinetics. Main features of applied model include use of detailed phase partitioning, by taking into account resistances in lipid and water layers encountered by chemicals when passing across biological membranes, and different sorbing matrices where bioaccumulation can occur, that is organic carbon in phytoplankton and lipids in the remaining species. Then, individual models were used to create food web, where lipid and organic content together with estimated chemical concentration are used to link these models. It has been reported that replacing the equilibrium partitioning model (e.g. Mackay 1982, Gobas 1993) with kinetic bioaccumulation model betters model predictions (Arnot and Gobas 2004). Application of sensitivity analysis helped to identify parameters that have the largest influence on model outputs, namely: hl_metabolic_norm, log10_K_oc, log10_K_ow, a_W, b_W, Assimilated_food, gamma_food, p_lipid, rho_water_layer_food, rho_water_layer. Among these parameters K_{OW}, lipid content and food assimilation play an important role in

the variation of BAF, since they are directly used in calculating dietary uptake and elimination rates. Further, partial resistances, that are independent from lipid fraction and assimilated food, are main contributors to uncertainty in chemical uptake rate from water.

When testing model predictions against measured data it is important to consider the nature of samples and sampling process to explain variations between observed and modelled results, which in fact do not have to necessarily be interpreted as a failure of the model's predictive capabilities (Arnot et al., 2004). Further testing of the applied models on new environmental datasets, as well as refinement of the selected input data for the most sensitive parameters (through additional literature data or experimental activities) can support an improvement of model capability to reconstruct real bioaccumulation data.

3 Human exposure assessment

The accumulation of Persistent Organic Pollutants (POPs) along aquatic and terrestrial food web determines the exposure of human populations to these contaminants. Human exposure to POPs has been associated to several serious health effects, including cancer, birth defect, neurological impairment, sterility, endocrine disruption (ATSDR, 2000; Ritter et al., 2007; Schecter, 2012). POPs can enter human body trough different exposure route (i.e., inhalation, ingestion and dermal contact), but ingestion of contaminated food, especially of animal origin, is often the main route for non-occupational exposure (Kelly et al., 2007).

A dietary survey conducted in the municipality of Venice (Pedenzini, 1996) showed that the consumption of fish and seafood was significantly higher than the average regional and national consumption, in particular for individuals living in the lagoon islands and close to the sea, where, based on local traditions and geographical context, fishing activities are very important.

The INN-CA 1994-96 study (Turrini et al., 2001) on food consumption patterns in Italy indicates a mean consumption of fish and seafood (fresh and frozen) of 31.8 g/day at the national level, reduced to 24.9 g/day if only Northeastern Italian regions are considered. A survey in Veneto Region in 1993 reported a mean annual consumption of 11.6 kg of fish, corresponding to 31 g/day. However, this study was based on market data (not on individual questionnaires), and it did not include the quantity of fish and seafood which was not bought on the market (e.g., bought directly from fishermen or directly caught in the lagoon).

Instead, the study by Pedenzini (1996) highlighted that the consumption of local fish products is significant in the municipality of Venice, and most probably, it was higher in the past.

In this context, it is also worth mentioning that fishing and harvesting of molluscs has been regulated in the central lagoon area since the 90’s, after the discovery of high pollution levels in water and sediment due to industrial emissions from Porto Marghera industrial zone. However, there is evidence that illegal fishing continued after the ban, therefore the consumption of potentially contaminated organisms from this area has to be considered in a worst-case exposure assessment.

3.1 Available human biomonitoring data

Only few human biomonitoring studies have been performed in the Venice area in the last 20 years. The study of interest for this case study was performed 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). Chemical concentrations in serum samples were measured for PCDDs (10 congeners), PCDFs (8 congeners) and for three dioxin-like PCBs, specifically: PCB81, PCB126, and PCB169. 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). Moreover, for each

participant, data related to age, occupational history, health status, life-style (smoking, cooking habits) and diet habits (weekly frequency of consumption of different food items) have been collected through a questionnaire.

3.2 Study context and approach

After matching available biomarkers with the available monitoring data for individual congeners, in biota from the Venice lagoon (in order to support the verification of intermediate steps of the integrated exposure assessment), the following compounds have been selected for simulating human exposure with MERLIN-Expo:

- PCB126;
- 2,3,7,8-TCDD.

With the aim of estimating human exposure to POPs through the ingestion of local fish and seafood, the aquatic food web models applied for the ecological exposure assessment have been coupled with the Human Intake and with the Man model available in MERLIN-Expo library (the chain of models has been reported in Figure 5).

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.

The overall body burden of PCB and dioxins depends on toxico-kinetic processes (absorption, distribution, metabolism and excretion), influenced by age-dependent human physiology and physico-chemical properties of the chemicals, 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. Monitoring of PCBs and dioxins in food has been performed only in recent decades, and often congener-specific data are very scarce. In this context, the use of modelling approaches can effectively support the reconstruction of past exposure scenarios (e.g., Alcock et al., 2000; Ulaszweska et al., 2012).

As explained in the previous section on ecological exposure assessment, MERLIN-Expo aquatic food web models have been used to reconstruct time trend exposure of aquatic organism living in Venice lagoon, using water concentrations of contaminants provided by dated sediment cores.

Since some of the organisms included in the aquatic bioaccumulation modelling are edible species, commonly caught or harvested in the lagoon (such as clams, mullets, gobies), the results of the ecological exposure assessment can be used as input to the Human Intake model to simulate the daily dietary intake of the selected PBCs and 2,3,7,8-TCDD.

3.3 Parameterization of the Man model (PBPK)

Although chemicals absorbed from gut lumen enter the liver first, ingested TCDD and PCB 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” in MERLIN-Expo 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).

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.

The parameters values are reported in Table 24.

Parameters	**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

Table 24. Values of the PBPK model parameters

3.4 Input data

The most site-specific information on fish and seafood daily intakes for the municipality of Venice is available in the report “Fish production and diet habits of families in Venice” (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 is equal to 2.168 g/month, equivalent to 72.3 g/day. For individuals living in Venice lagoon islands and coastal villages, the average daily intake increases to 94.7 g/day. Percentage distribution of population according to daily intakes is reported in Figure 17.

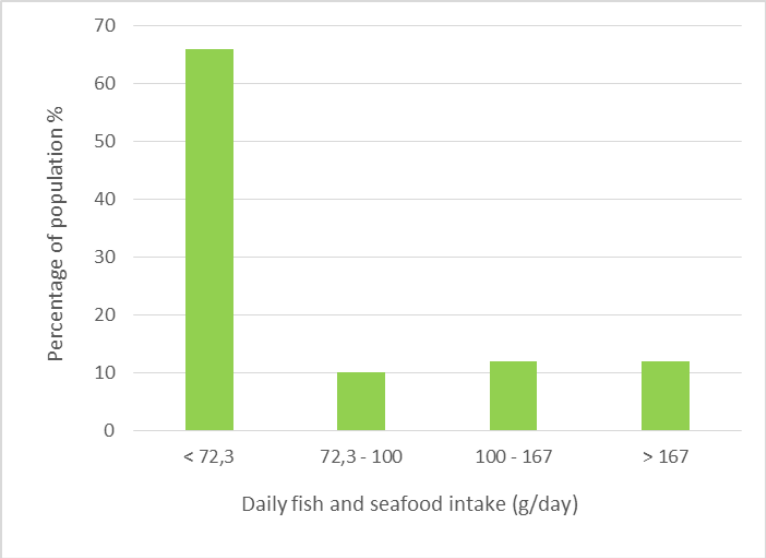


Figure 17. Distribution of fish daily intakes in Venice municipality (Pedenzini, 1996)

Typology and quantity of food intake vary depending on the age. Therefore it is important to consider age dependent food intakes when simulating life time exposure for the same individual.

Age dependent intake rates for Italian population are available 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 year old), adolescents (10 to 17 year old), adults (18 to 63 year old) and elderly people (more than 63 year old). 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 INN-CA survey has been used to scale Venice daily intake to different age group intakes to get site-specific age dependent intake values.

The selected dietary data date back to the 90’s and are representative of the same period when human biomonitoring data were collected (1998). 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 in Italy in general), mean daily intakes for different age groups have been assumed as constant.

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 25 for persons classified as high fish consumers.

	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 (crab)	0.008	0.011	0.012	0.010
Atherina boyeri (sand smelt)	0.003	0.004	0.004	0.004
Chelon labrosus (mullet)	0.006	0.008	0.009	0.008
Dicentrarcus labrax (seabass)	0.008	0.011	0.013	0.011
Sparus aurata (gilt-head bream)	0.008	0.011	0.013	0.011
Zosterisessor ophiocephalus (goby)	0.004	0.006	0.006	0.005

Table 25. Average daily intake of fish and food for different age groups.

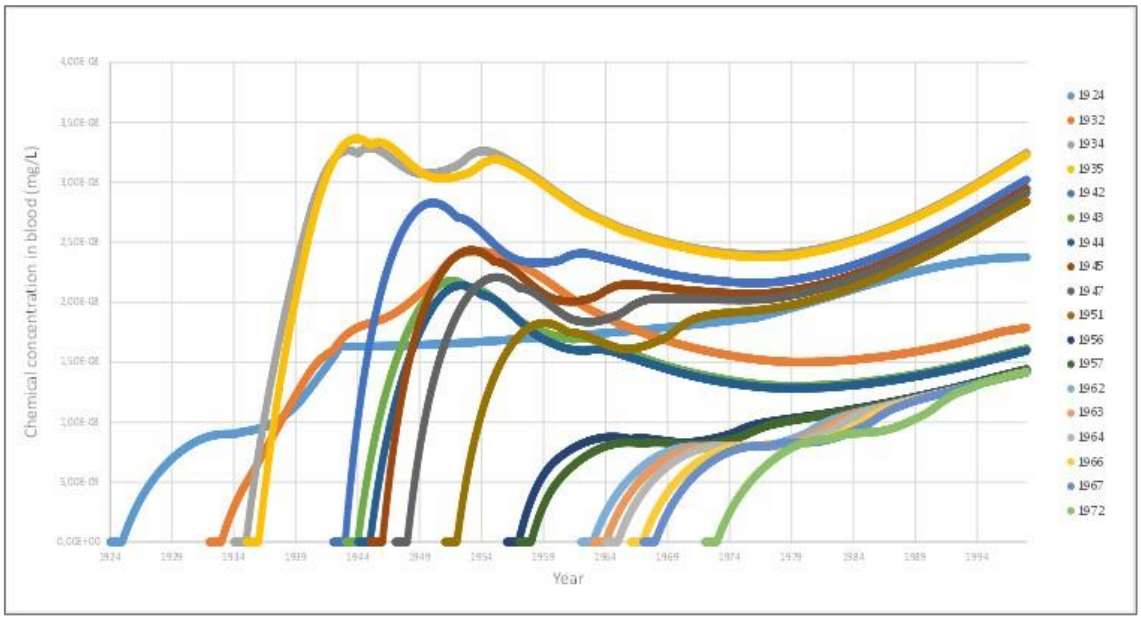
3.5 Results and discussion

Taking into account the available human biomonitoring data (Frangipane et al., 1999; see Paragraph 3.1), 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.

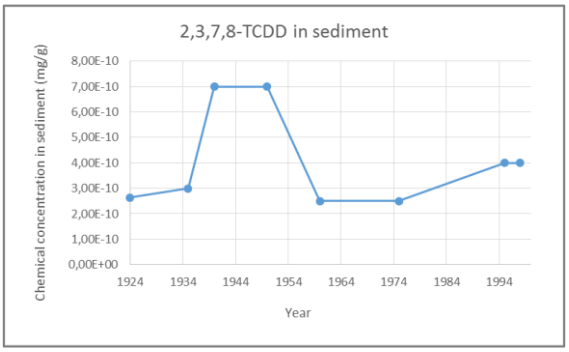
For this assessment, the full chain of models illustrated in Paragraph 2.2 and including the aquatic food web models (Phytoplankton, Invertebrate and Fish models), the Human Intake Model and the Man model has been applied. The final outputs provided by the Man model of interest for the present case study consist of time-dependent chemical concentrations in different human tissues and organs (e.g., blood, adipose tissue, brain, liver, etc.), but 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, etc.) which can support the understanding of exposure pathways and toxico-kinetic processes.

The Man model can simulate the change in blood concentrations throughout a lifetime, incorporating body growth (and change in weight of organs), metabolism, and evolution in dietary intakes (provided as inputs from the Human Intake model). Since we are considering a long term exposure scenario 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 is thus necessary to run separate simulations for different individuals born in different years (the tool does not allow to consider individuals born after the starting date of the simulation). Therefore, individual exposure simulations have been run separately with MERLIN-Expo, taking into account the year of birth of study participants (from 1924 to 1972). All simulations have been run until 1998, considering that available biomonitoring data in serum date back to that year.

Figure 18 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 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 19 illustrates the same results and data for PCB126.



(a)



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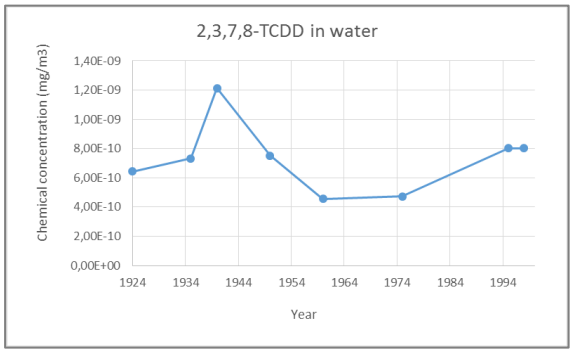
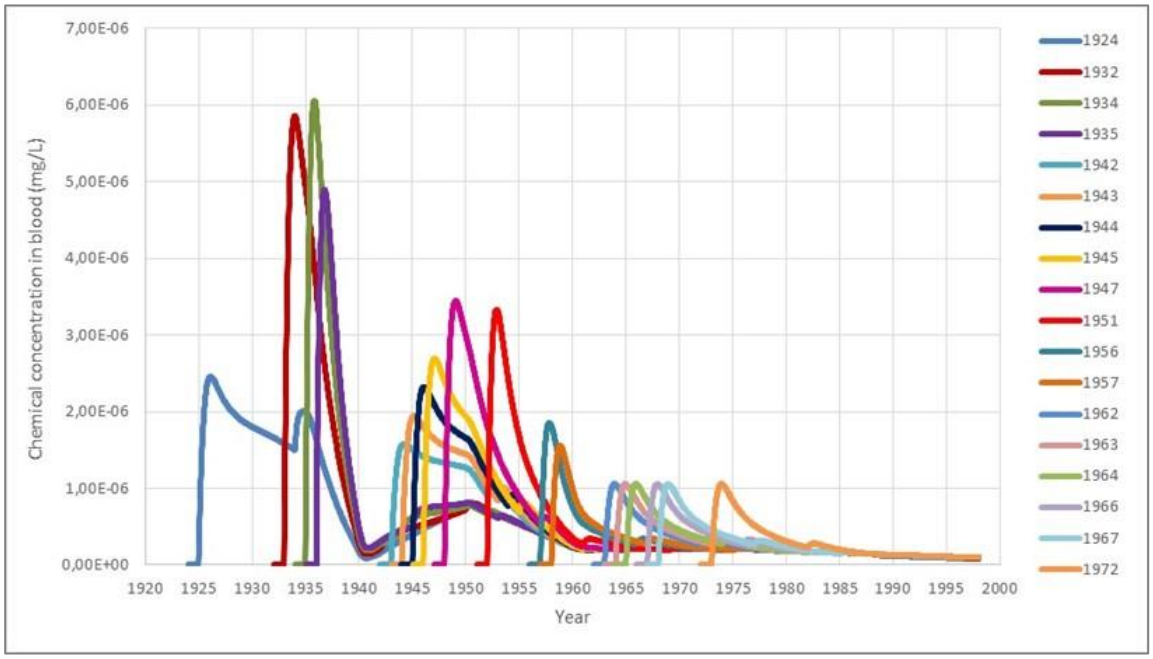
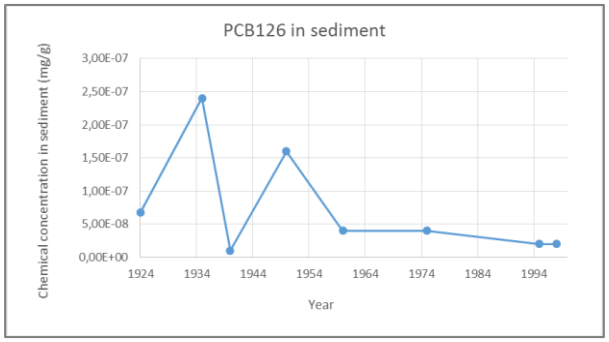


(c)

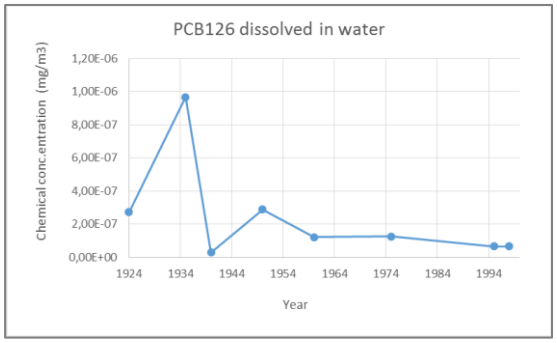
Figure 18. (a) Lifetime concentrations of 2,3,7,8-TCDD in blood of high fish consumers born between 1924 and 1972, (b) time trend of 2,3,7,8-TCDD in sediment; (c) time trend of 2,3,7,8-TCDD in dissolved water.



(a)



(b)



(c)

Figure 19. (a) Lifetime concentration of PCB126 in blood of high fish consumers born between 1924 and 1972; (b) time trend of PCB126 in sediment; (c) time trend of PCB126 in dissolved water.

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 Kow and metabolic half-life).

The chart in Figure 18 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 19 we can 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 19 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 (Figure 18), 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 fact that a unique average daily intake of fish and seafood was available for children between 1 and 9 years from the dietary survey. This low resolution in intake rates for toddlers combined with the low weight in early life stages can explain the observed peaks.

In order to test the performance of MERLIN-Expo model 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”.

To allow the comparison with results provided by MERLIN-Expo (chemical concentration in whole blood), 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 be distributed in blood serum (Schechter, 2012), the concentration in blood has been obtained by dividing by two the concentration measured in serum, according to the approach recommended by Health Canada (2003) 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.

As for deterministic simulations, available biomonitoring data have been compared with the simulated concentrations (22 persons) for year 1998. In Table 26 the comparison of statistics for measured and modelled blood concentrations of 2,3,7,8-TCDD and PCB126 is reported. 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). For sake of completeness in Table 26 different statistics are reported.

		Mean	SD	Min	Max	Geometric mean	Geom SD	Median
2,3,7,8-TCDD	Measured	9.06E-09	8.92E-09	1.28E-09	2.95E-08	4.98E-09	3.16E+00	4.60E-09
	Simulated	1.99E-08	7.18E-09	1.40E-08	3.22E-08	1.87E-08	1.00E+00	1.51E-08
PCB126	Measured	1.12E-06	1.13E-06	1.39E-07	3.97E-06	6.79E-07	2.73E+00	4.68E-07
	Simulated	1.81E-07	5.06E-09	1.61E-07	1.85E-07	1.81E-07	1.03E+00	1.81E-07

Table 26. Comparison of simulated and measured mean values of chemical blood concentrations for 2,3,7,8-TCDD and PCB126.

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 the same order of magnitude, the geometric mean (GM) of simulated 2,3,7,8-TCDD values in blood is about 3-time higher than the GM of measured values, while for PCB126 the geometric mean of simulated values is about 3-time lower than GM of measured data.

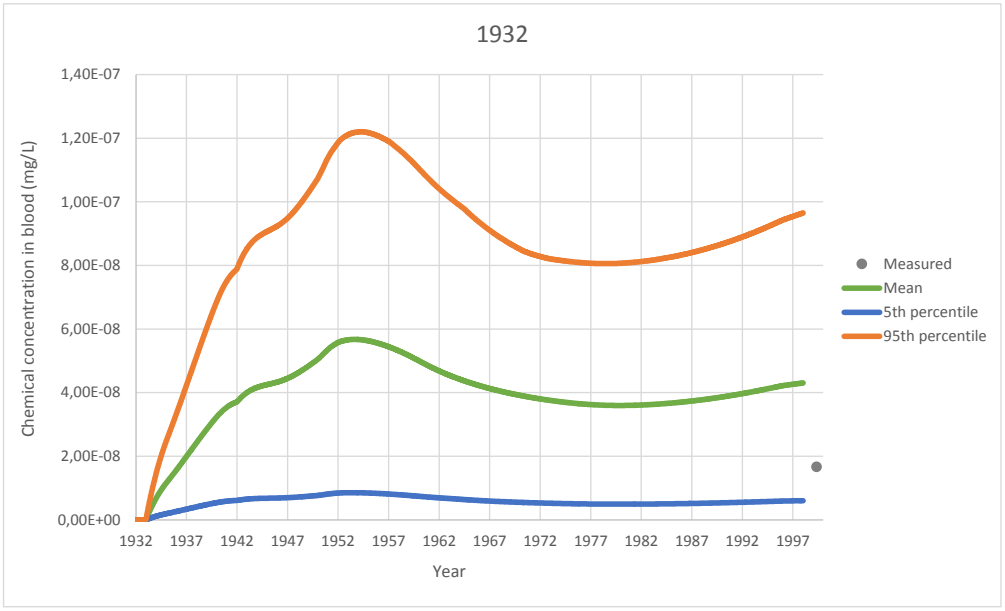
With the aim of exploring the impact of uncertainty and variability in model parameters on final model results, probabilistic simulations (Monte Carlo approach with 100 simulations) were run for selected birth years, specifically concentrations in blood were simulated for individuals born in 1932, 1956, and 1966 (years were chosen in order to cover different decades and according to the availability of measured blood concentrations above the detection limit).

Probability Density Functions (PDFs) (pre-defined in the model or defined for specific aquatic species based on literature data) has been used to describe specific parameters' values in different models of the applied modelling chain, namely:

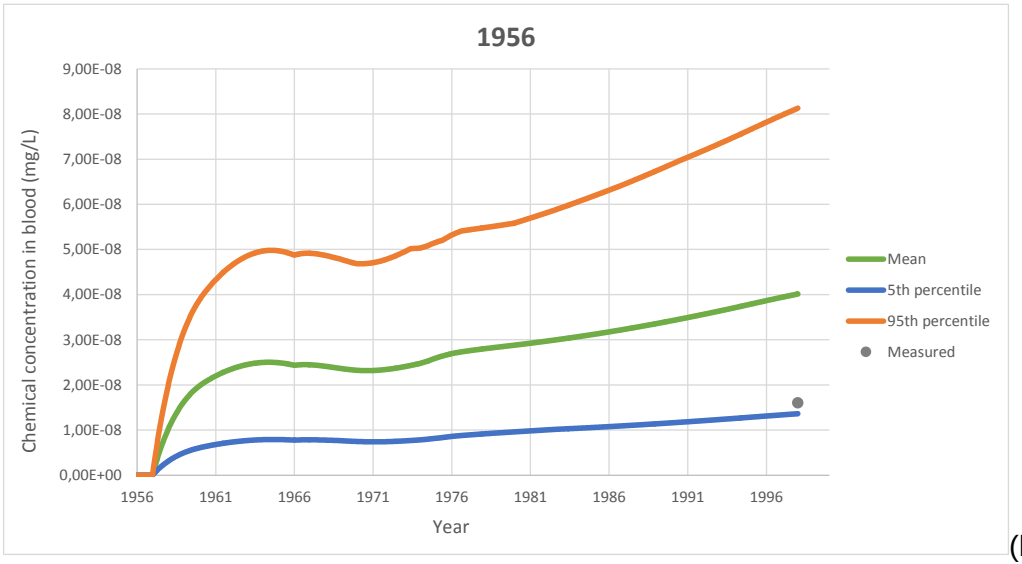
- biological parameters and physico-chemical parameters which resulted to be the most relevant from the sensitivity analysis for Invertebrate and Fish models (Paragraph 2.3.4); due to the complexity of the food web, PDFs have been considered only for *Tapes philippinarum* and *Chelon labrosus* (which are relevant components of human fish/seafood intake);
- as for the PBPK/Man model, PDF for describing the variability in the body weight and for tissue:blood partition coefficients were used.

The results of probabilistic simulations (mean, 5th percentile and 95th percentile of simulated chemical concentrations in blood) are illustrated in Figure 20 and Figure 21(for 2,3,7,8-TCDD and PCB126, respectively) for individuals born in 1932 (a), in 1956 (b) and in 1966 (c). In each chart, the measured concentration of target chemical in blood measured in 1998 for each individual is also reported.

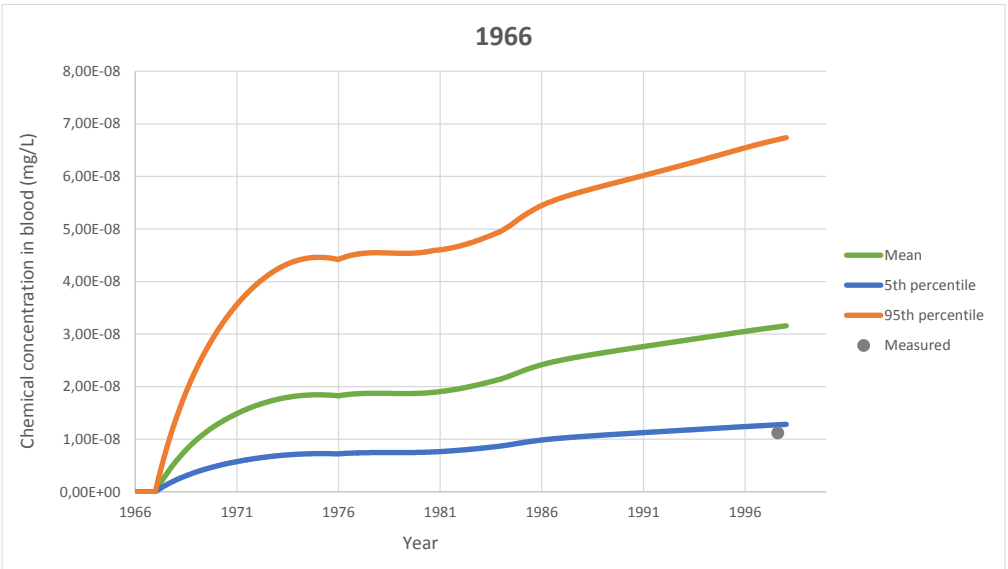
The outcomes of probabilistic application of MERLIN-Expo show how the tool can be effectively used to evaluate the interval of confidence in predicted results (and its variation at specific time points or different ages) in relation to uncertainty and/or variability associated to specific parameters of the applied models.



(a)

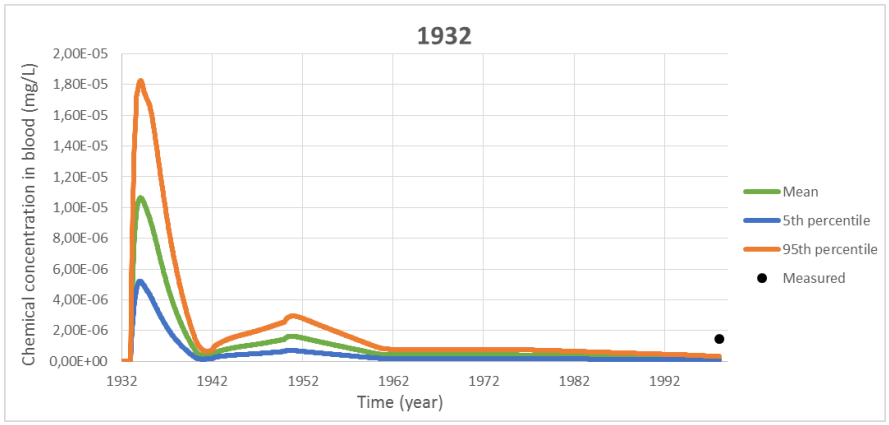


(b)

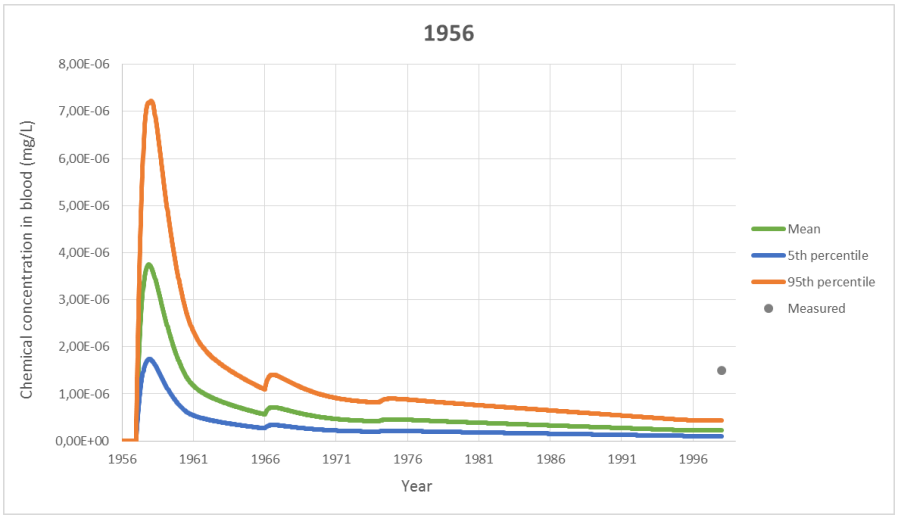


(c)

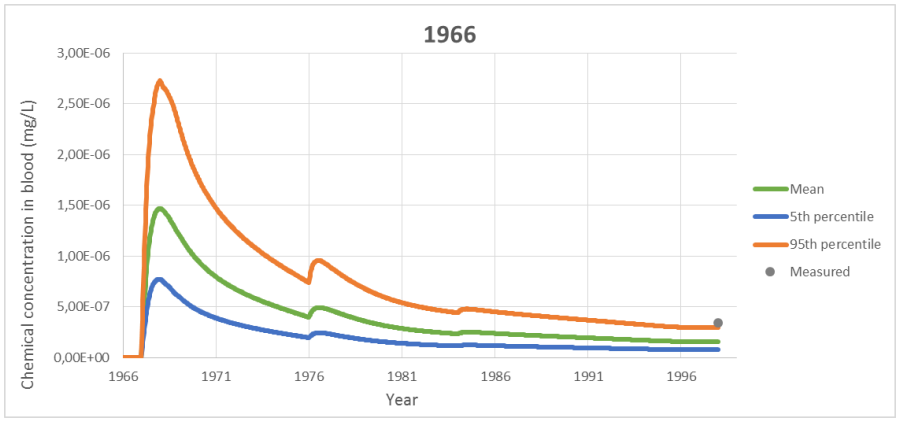
Figure 20. Probabilistic simulation of 2,3,7,8-TCDD concentration in blood for three individuals born in 1932 (a), 1956 (b) and 1966 (c).



(a)



(b)



(c)

Figure 21. Probabilistic simulation of PCB126 concentration in blood for three individuals born in 1932 (a), 1956 (b) and 1966 (c).

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 is assumed that all fish and seafood consumed by the population are 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 1990's). 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 leads to an underestimation of internal exposure.

Finally, it has to be remembered 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.

Despite the abovementioned uncertainties associated with the assessment framework and data availability, the results of the described application can already show how MERLIN-Expo can be used to reconstruct real biomonitoring data with a good approximation (comparable orders of magnitude between simulated and measured concentrations in blood). The tool is promising for higher tier exposure assessment and, 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.

4 Conclusions

4FUN Case Study 2 focused on the assessment of ecological and human exposure to PCBs and dioxins in the Venice lagoon. MERLIN-Expo has been applied to a complex exposure scenario, with the aim of assessing the bioaccumulation and bio-magnification of target chemicals in the aquatic food web and the exposure of local population (high fish consumers sub-group) through the intake of contaminated fish and seafood caught in the Venice lagoon. For these purposes, five models from MERLIN-Expo library were combined (Phytoplankton, Invertebrate, Fish, Human Intake and Man models) and deterministic and probabilistic simulations were run for a time period of several decades (from 1934 to 1998).

The application demonstrated the feasibility of reconstructing with MERLIN-Expo 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 allowed reconstructing a rather complex aquatic food web, representative of 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. This feature allows to easily compare the behaviour of chemicals with different physico-chemical characteristics and to explore their potential for bioaccumulation and/or bio-magnification in a straightforward way.

The outcomes of ecological exposure assessment (chemical concentrations in aquatic species) were evaluated against monitoring data for five species, finding an appreciable agreement, with some differences depending on the species and the target chemicals. Also the results of human exposure assessment (concentrations of PCB126 and 2,3,7,8-TCDD in blood) were compared to real human biomonitoring data measured in local population (adult men) in 1998. Despite many assumptions were needed in the assessment framework, simulated concentrations resulted close to measured data (i.e., the same order of magnitude or one order of magnitude of difference).

The sensitivity analysis functionalities implemented in MERLIN-Expo demonstrated to be easy to apply to identify the parameters, which mostly influence the final outcomes of bioaccumulation modelling. The possibility to run sensitivity analysis (e.g. Morris method in the case of case study 2) without the need to apply a separate software represents a strength of MERLIN-Expo tool and can support end-users in a better understanding of model behaviour under default or site-specific conditions.

Application of MERLIN-Expo tool to case study 2 also allowed identifying aspects that can be further improved in the tool in the next future, such as the possibility to include uncertainty/variability also on environmental input data (e.g., chemical concentrations in sediment, water, food, etc.) and not only on model parameters, or the possibility to run contemporary simulations for individuals of different ages.

As general conclusion of the work presented in this report, it is possible to state that MERLIN-Expo proved to be flexible and suitable to support integrated exposure assessment where both ecological and human targets are considered, even for long term scenarios, and may constitute a useful tool to support the detailed assessment of exposure in higher tier risk assessment procedures.

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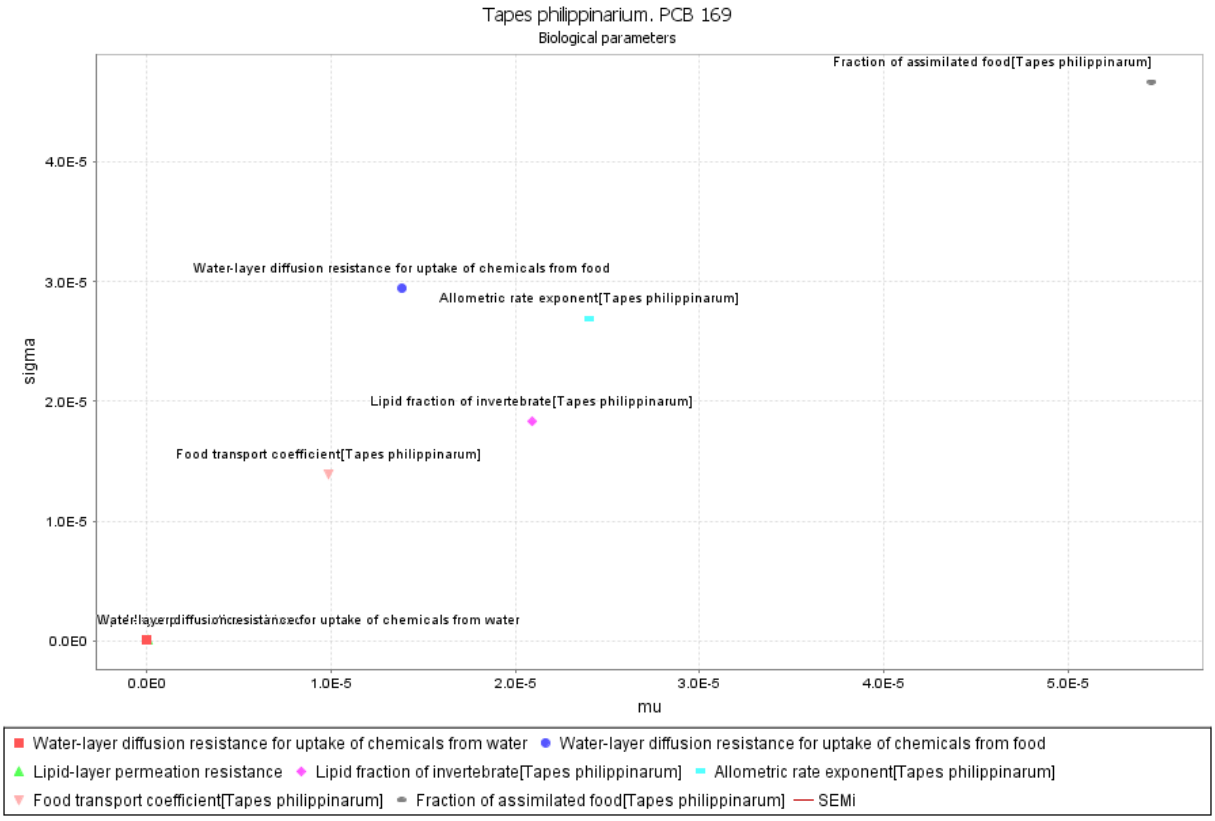
Appendices

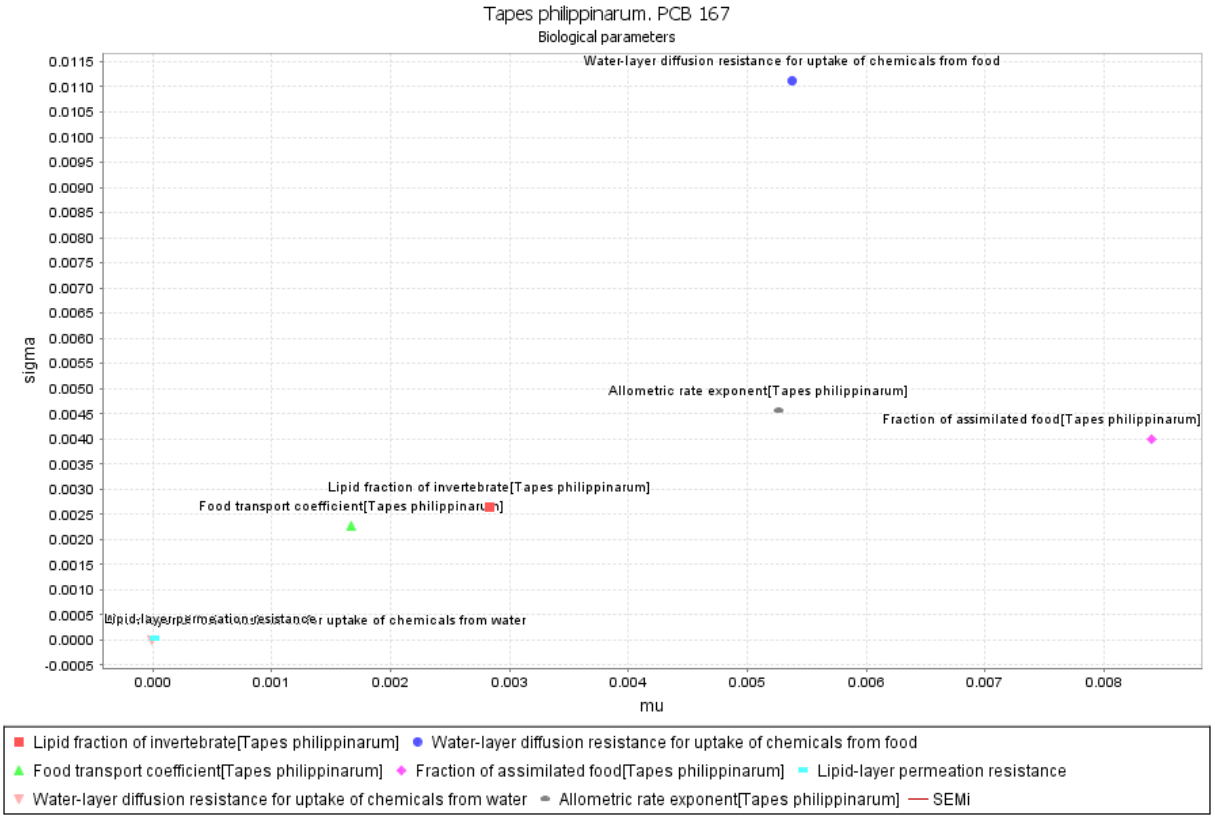
Appendix A: List of parameters used in aquatic food web models and references used for the selection of parameter values

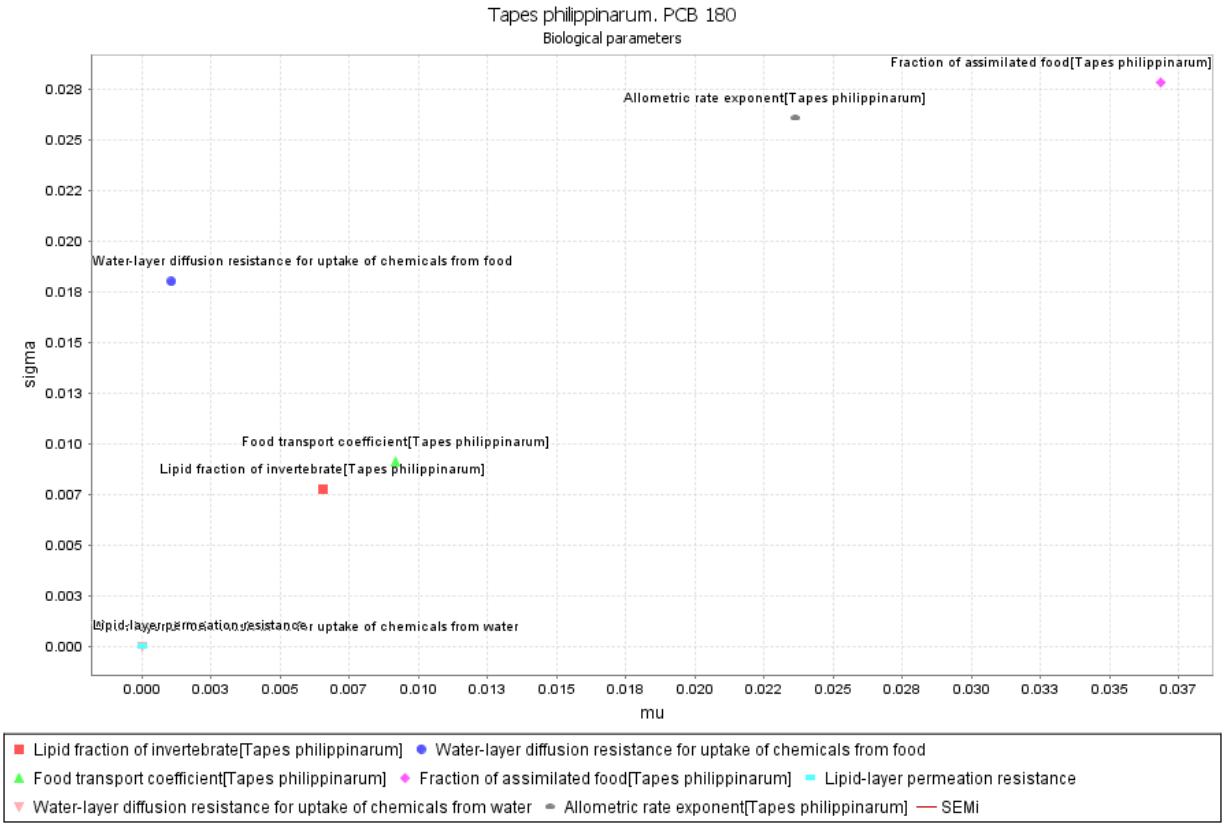
Full name	id	Reference
Metabolic half-life of chemicals for organics*	hl_metabolic_norm	J.A. Arnot et al., 2008; J.A. Arnot et al., 2009
Bioconcentration Factor for organics*	log10_BCF_organic	J.A. Arnot. et al., 2003; J.A. Arnot et al., 2006
Water-organic carbon partition coefficient* (Phytoplankton)	log10_K_oc	G. Schuurmann et al., 2006
Octanol/water partition coefficient*	log10_K_ow	W.M. Meylan et al., 1995
Lipid-layer permeation resistance	rho_lipid_layer	M. Hauck et al., 2011
Water-layer diffusion resistance for uptake of chemicals from water	rho_water_layer	M. Hauck et al., 2011
Water-layer diffusion resistance for uptake of chemicals from food	rho_water_layer_food	M. Hauck et al., 2011
Octanol/water partition coefficient	log10_K_ow	W.M. Meylan et al., 1995
Lipid-layer permeation resistance	rho_lipid_layer	M. Hauck et al., 2011
Water-layer diffusion resistance for uptake of chemicals from water	rho_water_layer	M. Hauck et al., 2011
Intercept of weight-length relationship (Fish)	a_W	R. Froese et al., 2014 http://www.fishbase.org/
Slope of weight-length relationship (Fish)	b_W	R. Froese et al., 2014 http://www.fishbase.org/
Fraction of assimilated food	Assimilated_food	M. Hauck et al., 2011
Food transport coefficient	gamma_food	M. Hauck et al., 2011
Allometric rate exponent	kappa	M. Hauck et al., 2011
Animal length at maturity (Fish)	L	R. Froese et al., 2014 http://www.fishbase.org/
Lipid fraction of animal	p_lipid	R. Froese et al., 2014 http://www.fishbase.org/; M. Hauck et al., 2011; C. Micheletti et al., 2008
Age at maturity	time_life	R. Froese et al., 2014 http://www.fishbase.org/
Weight at maturity (invrtebrates)	W_invertebrate	C. Micheletti et al., 2008; E. G. Durbin and A. G. Durbin 1978; P. Palmerini et al., 1994; L. A. Robinson et al., 2010
Intercept of phytoplankton growth rate	a_growth	E. Marañón et al., 2013
Slope of phytoplankton growth rate	b_growth	E. Marañón et al., 2013
Organic carbon fraction of phytoplankton	p_carbon_phytoplankton	R. S. Skoglund et al., 1999; I. Olenina et al., 2006
Lipid fraction of phytoplankton	p_lipid_phytoplankton	R. S. Skoglund et al., 1999; I. Olenina et al., 2006
Phytoplankton cell volume	V_cell	I. Olenina et al., 2006

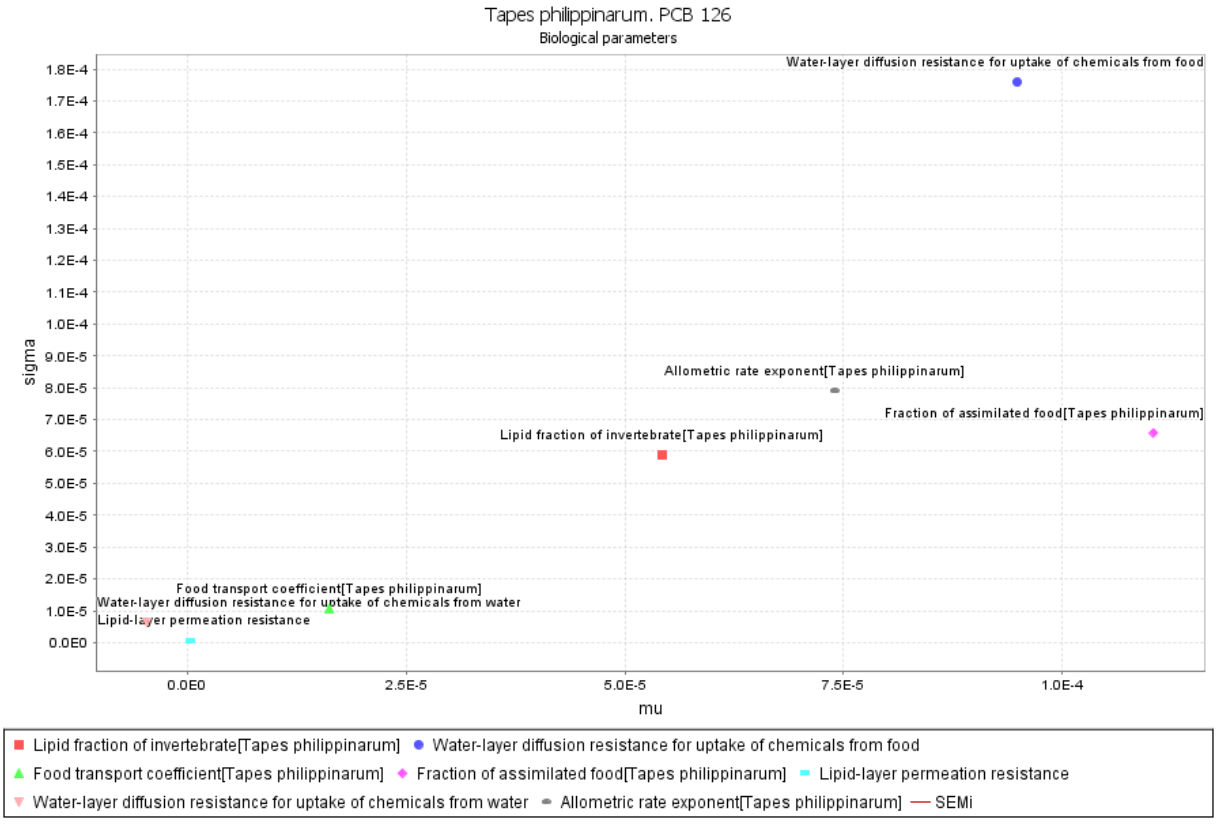
*US EPA. 2012. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11. United States Environmental Protection Agency, Washington, DC, USA.

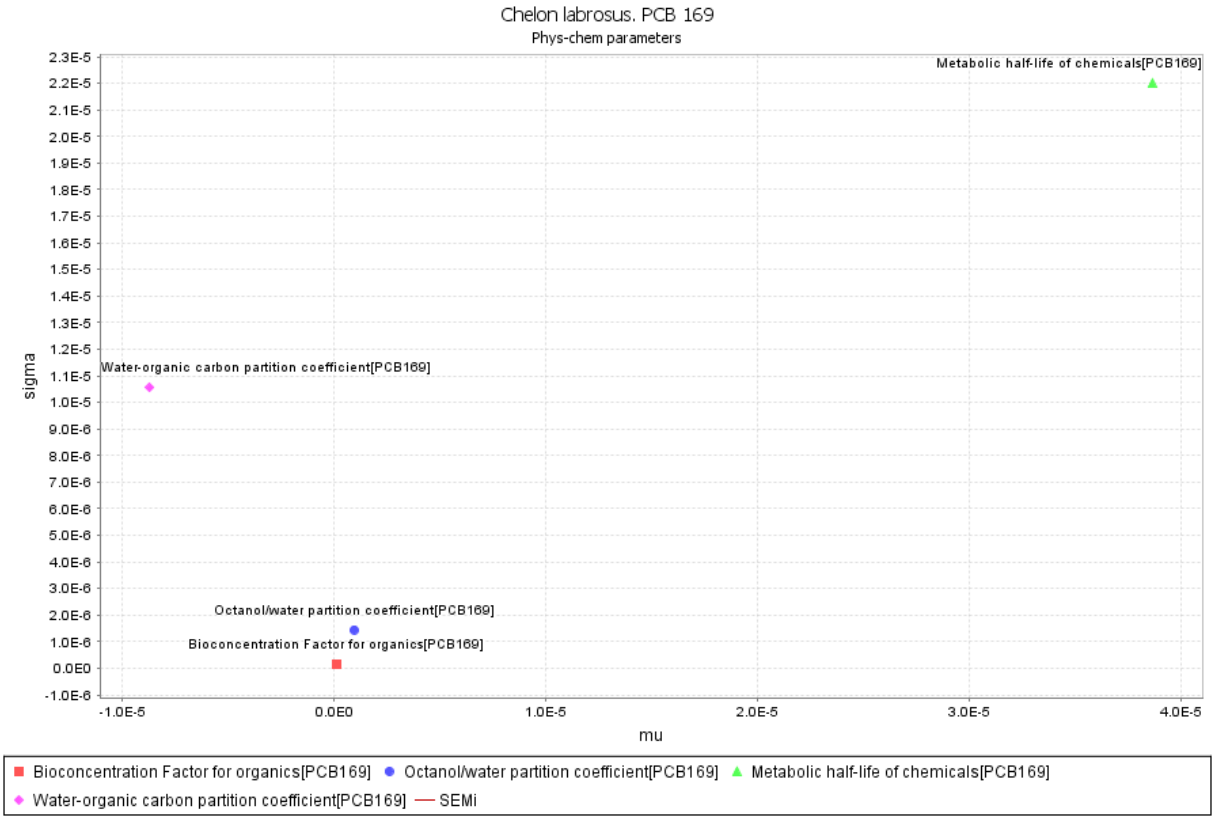
Appendix B: Results of sensitivity analysis for *Tapes philippinarum*

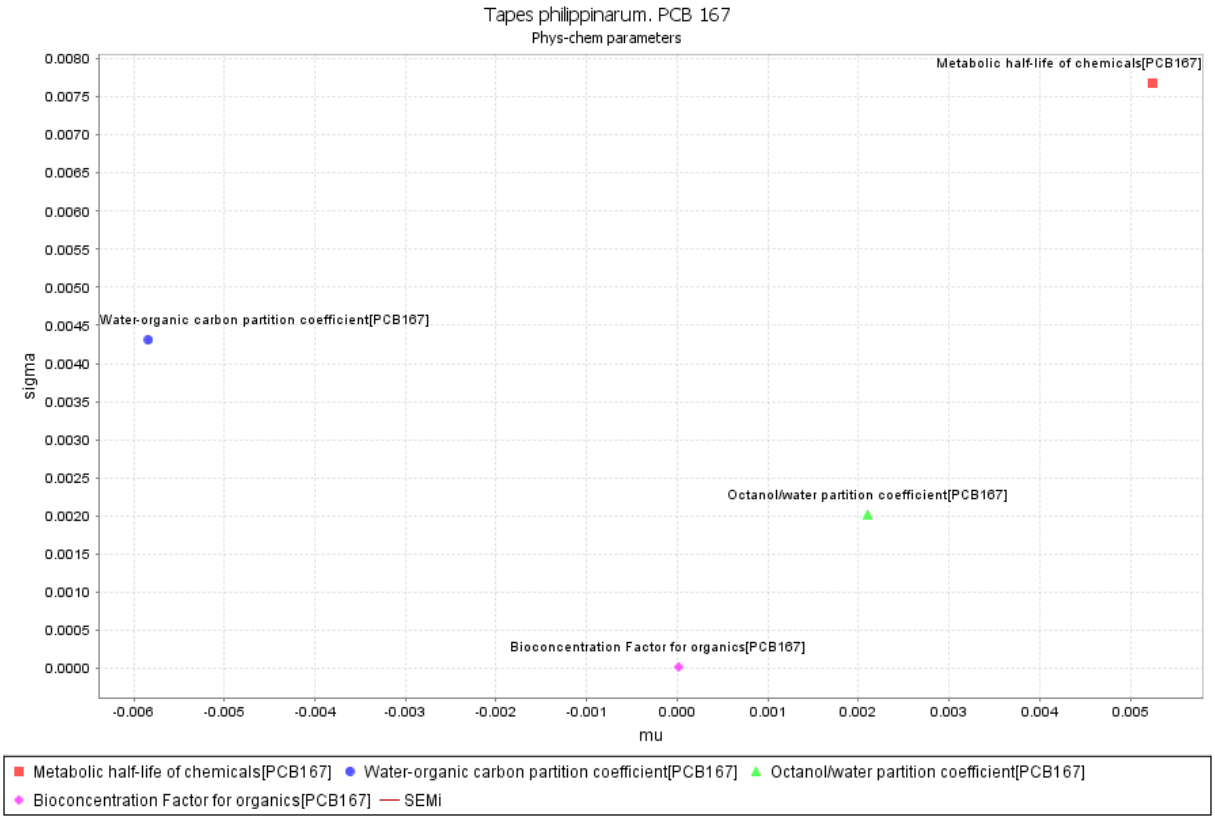


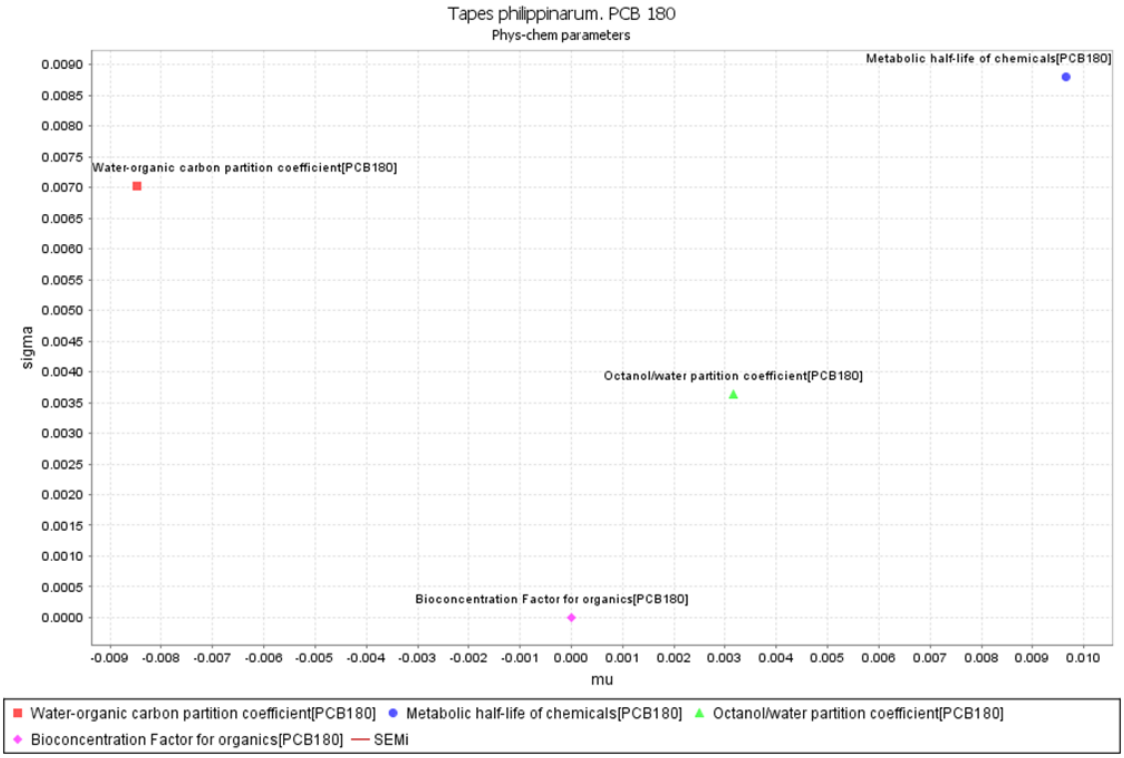


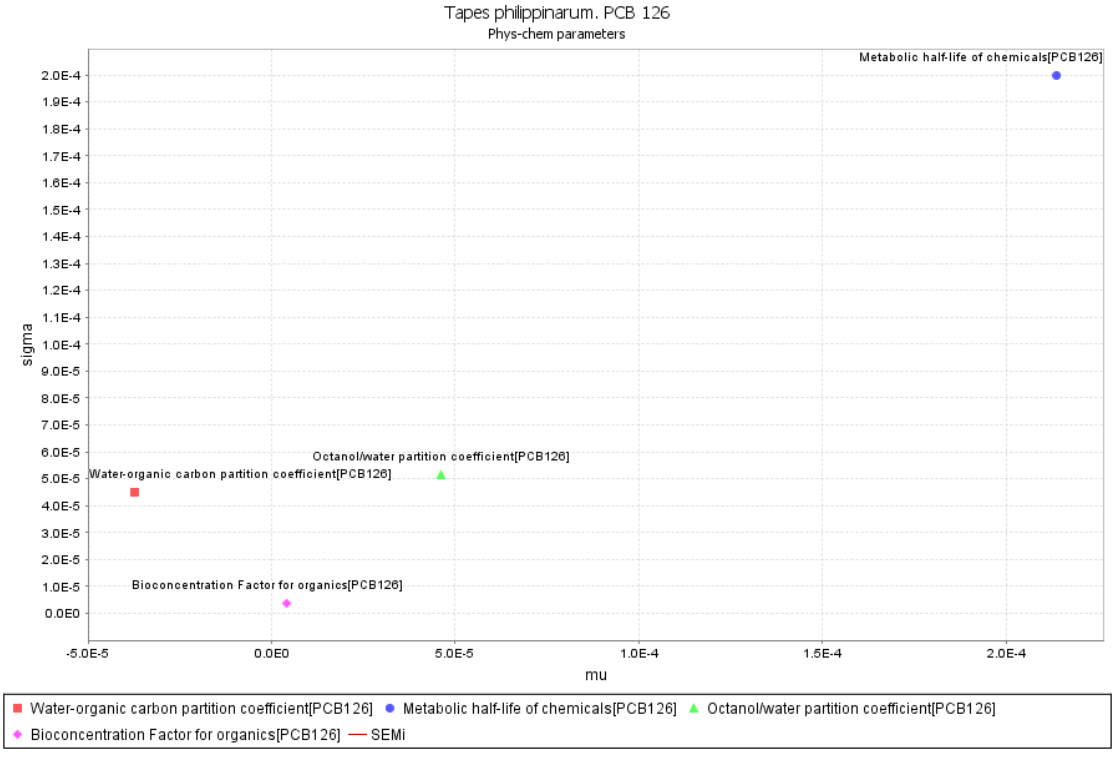

Tapes philippinarum. PCB 180
Biological parameters
Fraction of assimilated food[Tapes philippinarum]
Allometric rate exponent[Tapes philippinarum]
Water-layer diffusion resistance for uptake of chemicals from food
Food transport coefficient[Tapes philippinarum]
Lipid fraction of invertebrate[Tapes philippinarum]
Lipid-layer permeation resistance
sigma
mu
Lipid fraction of invertebrate[Tapes philippinarum]
Water-layer diffusion resistance for uptake of chemicals from food
Food transport coefficient[Tapes philippinarum]
Fraction of assimilated food[Tapes philippinarum]
Lipid-layer permeation resistance
Water-layer diffusion resistance for uptake of chemicals from water
Allometric rate exponent[Tapes philippinarum]
SEMi


Tapes philippinarum. PCB 126
Biological parameters
Water-layer diffusion resistance for uptake of chemicals from food
Allometric rate exponent[Tapes philippinarum]
Fraction of assimilated food[Tapes philippinarum]
Lipid fraction of invertebrate[Tapes philippinarum]
Food transport coefficient[Tapes philippinarum]
Water-layer diffusion resistance for uptake of chemicals from water
Lipid-layer permeation resistance
sigma
mu
Lipid fraction of invertebrate[Tapes philippinarum]
Water-layer diffusion resistance for uptake of chemicals from food
Food transport coefficient[Tapes philippinarum]
Fraction of assimilated food[Tapes philippinarum]
Lipid-layer permeation resistance
Water-layer diffusion resistance for uptake of chemicals from water
Allometric rate exponent[Tapes philippinarum]
SEMi


Chelon labrosus. PCB 169
Phys-chem parameters
Metabolic half-life of chemicals[PCB169]
Water-organic carbon partition coefficient[PCB169]
Octanol/water partition coefficient[PCB169]
Bioconcentration Factor for organics[PCB169]
sigma
mu
Bioconcentration Factor for organics[PCB169]
Octanol/water partition coefficient[PCB169]
Metabolic half-life of chemicals[PCB169]
Water-organic carbon partition coefficient[PCB169]
SEMi


Tapes philippinarum. PCB 167
Phys-chem parameters
Metabolic half-life of chemicals[PCB167]
Water-organic carbon partition coefficient[PCB167]
Octanol/water partition coefficient[PCB167]
Bioconcentration Factor for organics[PCB167]
sigma
mu
Metabolic half-life of chemicals[PCB167]
Water-organic carbon partition coefficient[PCB167]
Octanol/water partition coefficient[PCB167]
Bioconcentration Factor for organics[PCB167]
SEMi


Tapes philippinarum. PCB 180
Phys-chem parameters
Metabolic half-life of chemicals[PCB180]
Water-organic carbon partition coefficient[PCB180]
Octanol/water partition coefficient[PCB180]
Bioconcentration Factor for organics[PCB180]
sigma
mu
Water-organic carbon partition coefficient[PCB180]
Metabolic half-life of chemicals[PCB180]
Octanol/water partition coefficient[PCB180]
Bioconcentration Factor for organics[PCB180]
SEMi


Tapes philippinarum. PCB 126
Phys-chem parameters
Metabolic half-life of chemicals[PCB126]
Octanol/water partition coefficient[PCB126]
Water-organic carbon partition coefficient[PCB126]
Bioconcentration Factor for organics[PCB126]
sigma
mu
Water-organic carbon partition coefficient[PCB126]
Metabolic half-life of chemicals[PCB126]
Octanol/water partition coefficient[PCB126]
Bioconcentration Factor for organics[PCB126]
SEMi