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Modelling ecological and human exposure to POPs in Venice lagoon – Part II: Quantitative uncertainty and sensitivity analysis in coupled exposure models

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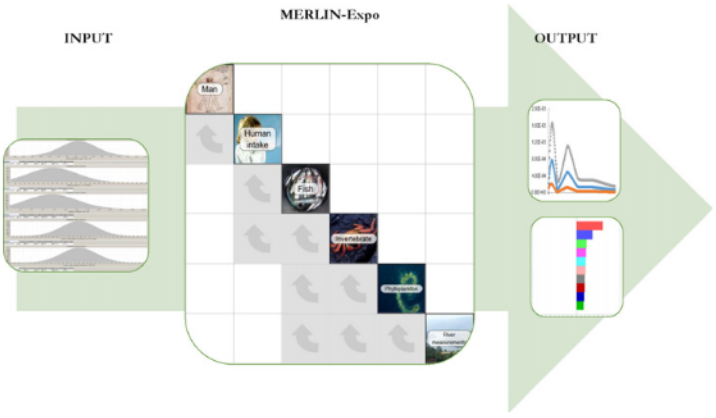
HIGHLIGHTS

- Uncertainty in integrated ecological and human long term exposure modelling was investigated.
- Sensitivity analysis of food web and PBPK models was performed.
- Various local and global sensitivity analysis methods were tested.
- Levels of uncertainty in internal exposure estimates and sensitive parameters were identified.
- Time evolution of total sensitivity index of important parameters is presented.

GRAPHICAL ABSTRACT



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ABSTRACT

The study is focused on applying uncertainty and sensitivity analysis to support the application and evaluation of large exposure models where a significant number of parameters and complex exposure scenarios might be involved. The recently developed MERLIN-Expo exposure modelling tool was applied to probabilistically assess the ecological and human exposure to PCB 126 and 2,3,7,8-TCDD in the Venice lagoon (Italy). The 'Phytoplankton', 'Aquatic Invertebrate', 'Fish', 'Human intake' and PBPK models available in MERLIN-Expo library were integrated to create a specific food web to dynamically simulate bioaccumulation in various aquatic species and in the human body over individual lifetimes from 1932 until 1998. MERLIN-Expo is a high tier exposure modelling tool allowing propagation of uncertainty on the model predictions through Monte Carlo simulation. Uncertainty in model output can be further apportioned between parameters by applying built-in sensitivity analysis tools. In this study, uncertainty has been extensively addressed in the distribution functions to describe the data input and the effect on model results by applying sensitivity analysis techniques (screening Morris method, regression analysis, and variance-based method EFAST). In the exposure scenario developed for the Lagoon of Venice, the concentrations of 2,3,7,8-TCDD and PCB 126 in human blood turned out to be mainly influenced by a combination of parameters (half-lives of the chemicals, body weight variability, lipid fraction, food assimilation efficiency),

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physiological processes (uptake/elimination rates), environmental exposure concentrations (sediment, water, food) and eating behaviours (amount of food eaten). In conclusion, this case study demonstrated feasibility of MERLIN-Expo to be successfully employed in integrated, high tier exposure assessment.

1. Introduction

The need for advancement in risk assessment by improving exposure assessment with innovative approaches has been recently recognised by three independent EC Scientific Committees. The Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risk (SCHER), and the Scientific Committee on Emerging and Newly Identified Risks (SCENIHR) have provided European Commission with scientific advice regarding consumer safety, public health, and environment policy making (European Commission and Directorate General for Health & Consumers, 2013a, 2013b). The Committees' opinion addresses new challenges in environmental and human risk assessment, such as development of realistic scenarios to predict temporal and spatial variations, incorporation of specific organism parameters, and food web path of chemicals, and application of physiologically-based pharmacokinetic (PBPK) modelling to refine exposure assessment. In addition, it has been underlined that the quantification of exposure should be conducted based on an integrated (coupled) external and internal dynamic exposure modelling helping to address more complex exposure situations (Science for Environment Policy, 2015).

Ecological and human exposure assessment is a process of estimating magnitude, frequency and duration of individual or population exposure to an agent (Järup et al., 2001). The assessment process consists of three elements: scenario, model, and parameters. An exposure scenario as defined by WHO is a combination of facts and assumptions that define a discrete situation where potential exposure may occur. Conceptual and mathematical representation of exposure processes for a given exposure scenario are described by the exposure model, and parameters which, in general, can refer to input to the exposure model (Bundesinstitut für Risikobewertung, 2015). WHO/IPCS Guideline on uncertainty in the exposure assessment distinguishes these three elements as sources of uncertainty: exposure scenario uncertainty, model uncertainty, and parameters (IPCS/WHO, 2008). Uncertainty in exposure scenario arises from the identification of the sources of chemicals, routes of exposure, target organism/population, geographical location, frequency and duration of exposure. Other authors (Linkov & Burmistrov, 2003) propose an additional source of uncertainty originating from subjective treatment of the exposure problem, which can be seen as an extra dimension in scenario uncertainty. Uncertainties in a model reflect gaps in scientific knowledge and can be attributed to modelling errors, for instance the exclusion of some parameters, or relation errors due to incorrect conclusions from correlations. Imperfections in numerical values used to determine exposure factors can be associated with measurement errors, sample uncertainty, type of data (e.g., default values, measurement data), and uncertainty in the statistical distributions.

A clear understanding of the aforementioned uncertainties in exposure assessment is needed to aid decision makers in judging how probable it is that risks will be overestimated or underestimated for every member of the exposed population in order to balance costs and benefits of risk mitigation (NRC, 1994). Disregarding uncertainty may lead to incomplete risk assessments, poor decision-making and poor risk communication, therefore the following aspects need to be taken into account: (i) clear separation between uncertainty and variability, (ii) clarification of which uncertainties and variabilities are included in the assessment, and (iii) application of statistical tools, such as uncertainty and sensitivity analysis, for a quantitative assessment of uncertainty to characterize the confidence bounds in a model output (Özkaynak et al., 2008). Application of sensitivity analysis to exposure assessment can help in identifying factors or groups of factors responsible for the uncertainty in the prediction.

Uncertainty in estimating bioaccumulation of organic contaminants by higher trophic level organisms including man represents a significant contribution to the overall uncertainty in chemical risk assessment (Von Stackelberg et al., 2002). Understanding variability and uncertainty in environmental, physicochemical, and physiological model input factors is pivotal in estimating uncertainty in exposure assessment. Additionally, exposed individuals may have different behavioural patterns and physiological characteristics, which change depending on where and when exposure event occurs. These differences accounts for inter- and intra-variability in exposure levels of individuals. Thus application of uncertainty and sensitivity analysis can help to account for the uncertain factors, increasing credibility in risk analysis by providing assessment of the output uncertainty and relationship between the uncertain input parameters and output.

Publicly open list of current environmental and human exposure models, databases and reference materials has been made recently accessible through searchable US EPA-Expo-Box, a toolbox aiding individuals in selecting resources needed for exposure assessment (http://www.epa.gov/expobox). Indeed, various exposure models are readily available, however no tool offers comprehensive, high tier approach to chemical fate modelling and biota/human exposure assessment that includes also advanced functionalities for uncertainty and sensitivity analysis (Ciffroy et al., 2015), in line with the proposed approach by IPCS/WHO (2008).

Recently, a new tool for environmental and human exposure assessment has been proposed in the framework of the EU FP7 project '4FUN' (http://merlin-expo.eu/). Based on an extended library of environmental, biota and human exposure models, it incorporates several functionalities for performing probabilistic simulations as well as for tiered sensitivity analysis. MERLIN-Expo tool was demonstrated to carry out integrated exposure assessment on a real life case study, using coupled ecological and human exposure models to dynamically simulate historical exposure to environmental contaminants, followed by quantitative uncertainty and sensitivity analysis. The deterministic exposure assessment was described in Giubilato et al. (2016), where the case study context, the conceptual framework and the available data were presented in detail. The main objective of the present work was to characterize and quantify uncertainty in a chain of exposure models through probabilistic assessment of ecological and human exposure to two environmental persistent contaminants, 2,3,7,8-TCDD and PCB 126, in the Lagoon of Venice (Italy). The subsequent step was to identify sensitive environmental (e.g., fraction of organic carbon in sediments), physiological (e.g., assimilation efficiencies, age, weight), ecological (e.g., diet preferences), and PBPK parameters, supported by the evaluation of their impact on the magnitude of uncertainty of modelled outputs.

We demonstrated MERLIN-Expo tool and its uncertainty/variability analysis, and sensitivity analysis functionalities in order to provide confidence in the model structure and predictions, and also to improve our understanding about bioaccumulation phenomena along the complex food webs including exposure of a man as a final receptor. Large exposure models require numerous input factors, therefore, it is important to know which contribute mostly to model output uncertainty. Exercising sensitivity analysis can be beneficial to exposure modellers with regard to scaling down initial number of model factors to the most influential ones and upgrading their quality by additional research. This study is aimed at gaining insight into contaminant transport

phenomena in food webs including human receptors, with the final objective of identifying possible further improvements of the proposed modelling framework.

2. Materials and methods

2.1. Case study

The Lagoon of Venice bears characteristics of a coastal, and transitional ecosystem, supporting many human activities such as tourism or fishery. However, its environmental quality has been affected over many years by a densely populated catchment area, industrial settlings, oil refining plants, wastewaters and waste incineration plants. The pollution sources have been affecting various environmental compartments, through the release of a range of important environmental contaminants to the lagoon including organic (e.g. PCBs, dioxin-like PCBs, PCDD/Fs, PAHs) and inorganic (e.g. Cd, Pb, As, Cr, Zn, Ni) chemicals (Micheletti et al., 2007, 2008). Persistent organic pollutants tend to accumulate and magnify in aquatic organisms, causing a potential significant long term human dietary exposure (Von Stackelberg et al., 2002).

The exposure modelling exercise was performed on bioaccumulation aquatic food web and PBPK models targeting two persistent organic contaminants, namely 2,3,7,8-TCDD and PCB 126. Exposure to these compounds among the general human population involves continuously low-level exposures predominantly through gastrointestinal absorption via diet (Diliberto et al., 2001), hence they are important environmental contaminants potentially leading to adverse ecological and human effects.

The exposure scenario and model input factors were defined as characteristic for the Lagoon of Venice to reflect real exposure conditions for both ecological and human targets. The considered aquatic food web has been specifically defined to capture the characteristics of local aquatic ecosystem and it is described in Giubilato et al. (2016), where a more detailed description of the case study is also provided.

In the presented study, five models from MERLIN-Expo library were used, i.e. models simulating the fate of chemicals in Phytoplankton, Invertebrates, Fish, Human intake and Man. These models are briefly described in the following paragraph.

3. Description of the models and models' coupling

Conceptual models for 'Fish', 'Invertebrate' and 'Phytoplankton', shown in Fig. 1, are based on 'Optimal Modeling for EcotoxicoloGical Applications' (OMEGA) modelling approach proposed by Hendriks and colleagues (Hendriks et al., 2001; Hendriks and Heikens, 2001). Mass balance equations describing accumulation of organic chemicals in aquatic species are given by Eq. S1–S3 in SI.

The 'Fish' and 'Invertebrate' models include two media that correspond to two input/output pathways for chemical accumulation in fresh weight (fw) whole organism, i.e. the respiratory system and the gastro intestinal tract (GIT) system, while the 'Phytoplankton' model is represented by single compartment. The considered media and processes are represented in Fig. 1. The uptake of chemicals by aquatic invertebrates and fish species is based on allometric scaling and assumption that physicochemical properties of respiratory surfaces (e.g., gills, cell membrane) are essential for uptake of chemicals (Flynn and Yalkowsky, 1972; Gobas et al., 1986). Dietary uptake is considered to be the same for invertebrates and fishes. It is assumed that chemical exchanges across the GIT are driven by diffusion gradients, i.e. the concentration differences between phases within the organism and its food/feces (Fisk et al., 1998). Dietary uptake is driven also by ecological factors such as animal's trophic level and diet composition. Specific diet preferences were assigned to each animal reflecting its position and interactions with other animals in the food web (Micheletti et al., 2008). Loss of chemicals is described by elimination kinetics excretion, egestion, growth and metabolism being an important factor affecting chemicals that entered animals' body (Papa et al., 2014). A detailed description of the models can be found in dedicated documentation available on MERLIN-Expo website (http://merlin-expo.eu/learn/documentation/model-documentation/).

The 'Human intake' model consists of several equations for calculating the total human daily intake of target chemicals through different exposure pathways. For the case at hand, the model was applied to estimate the total daily ingested quantity from different food items (only dietary exposure is considered). The amount of consumed contaminated seafood is derived from age-dependent dietary data and is expressed as ingestion rate for each food item (kg_{fw}/d).

The 'Man' model is a PBPK model, applied to predict time-dependent concentrations of 2,3,7,8-TCDD and PCB126 in the blood of individual human males. The tissues and organs are represented by one compartment in which the compound is rapidly and homogeneously distributed and the distribution is flow-limited (Beaudouin et al., 2010). Each organ or tissue can receive different doses of the compound and the compound can remain in the organs or tissues for a varying amount of time. The compound can move from the plasma to the tissue until the equilibrium is established. The distribution in the tissues or organs depends on factors related to the physiology of the individual (e.g., vascular permeability, regional blood flow, cardiac output and

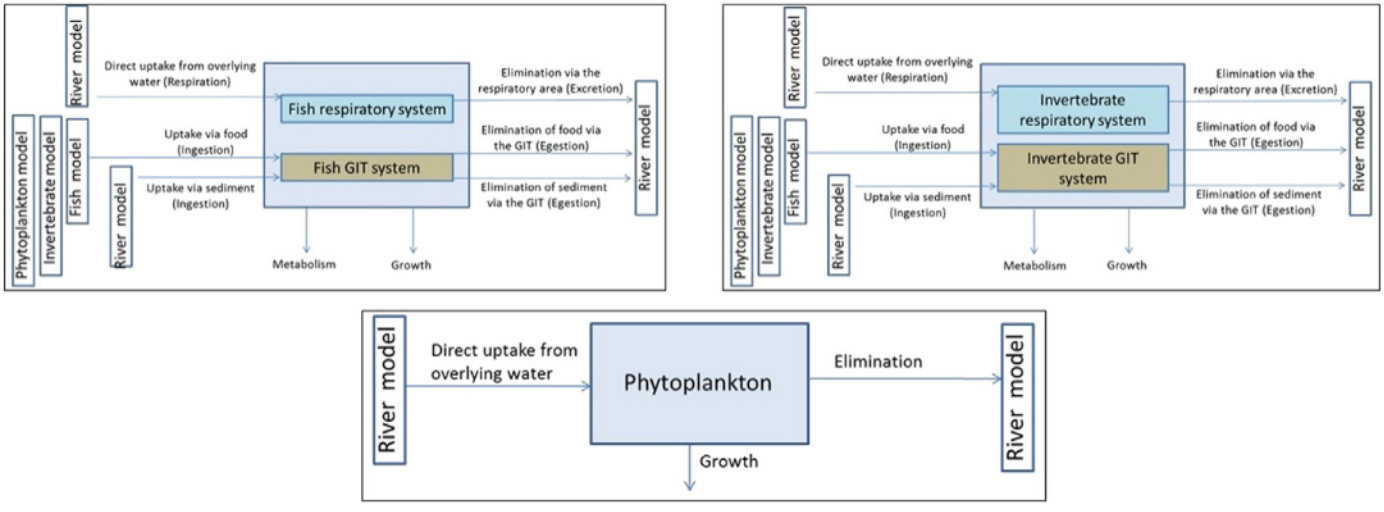


Fig. 1. Media considered + Loading inputs + Losses + Exchanges + Coupled models in the Fish, Invertebrate and Phytoplankton model (4FUN Fish, Invertebrate and Phytoplankton model Documentations).

perfusion rate of the tissue) and factors related to the compound (e.g., molecular size, lipid solubility, pKa, affinity to bind tissue and plasma proteins) (Brochot et al., 2007). The blood is represented in the model by two compartments: the arterial and venous blood. The arterial blood is distributed into all tissue compartments and the venous blood collects blood at the exit of most of the tissue compartments. Mass balance equations for calculating concentration in blood are described by Eq. S4 and S5 in SI.

Finally, to recreate the required exposure scenario, all the models can be coupled in a model chain in MERLIN-Expo, by defining output(s) of one model as input(s) to another one. This can be done using Graph or Matrix design. Here we built model chain using the matrix feature, where models selected from the software library are pictured as boxes placed on matrix diagonal and connected by off-diagonal 'Connectors' (Fig. 2). The 'River measurement' module is used to input data on chemical concentration in dissolved water, concentration in sediments and water temperature. The 'Connectors' (grey arrows) are used to feed this information to 'Phytoplankton', 'Invertebrates', and 'Fish' models. All aquatic organisms included in the Venice lagoon food web are grouped into these three categories and linked by concentration of accumulated contaminant and lipid content in their diet through input/output system. Chemicals accumulated in seafood are transferred to the top consumer, i.e. human population, described by the 'Man' model, through the intermediate model 'Human intake', responsible for calculating age-dependent chemical daily intakes.

3.1. Uncertainty analysis and parameter selection

In addition to deterministic assessment, MERLIN-Expo enables an assessor to include uncertainty on input parameters by specifying probabilistic distribution functions (PDFs) for parameter values. For probabilistic analysis, parameters that have been assigned PDFs were selected. 5000 probabilistic simulations of the full model chain were run using Monte Carlo sampling scheme for the period of 24,091 days at 100-day time step. As a human target, a high-fish consumer individual (see Giubilato et al., 2016) born in 1932 was selected, so the time period represents 66 years of this individual's lifetime. Environmental and biomonitoring measurement data are available for year 1998, hence it was considered as a final time in all modelling exercises. Considering the five MERLIN-Expo models selected and coupled for this case study, in total 156 parameters were included in the probabilistic assessment.

3.1.1. Aquatic food web

Two invertebrate species, *Tapes philippinarum* (Manila clam), *Carcinus mediterraneus* (green crab), and two fishes, *Chelon labrosus* (mullet), and *Zosterisessor ophiocephalus* (goby), were included in the probabilistic assessment of time-varying whole body internal concertation of 2,3,7,8-TCDD and PCB126 over the period 1932–1998.

The uncertain input parameters used to estimate accumulated contaminant concentration in aquatic biota are represented as probability distributions based on literature review or analysis of available datasets

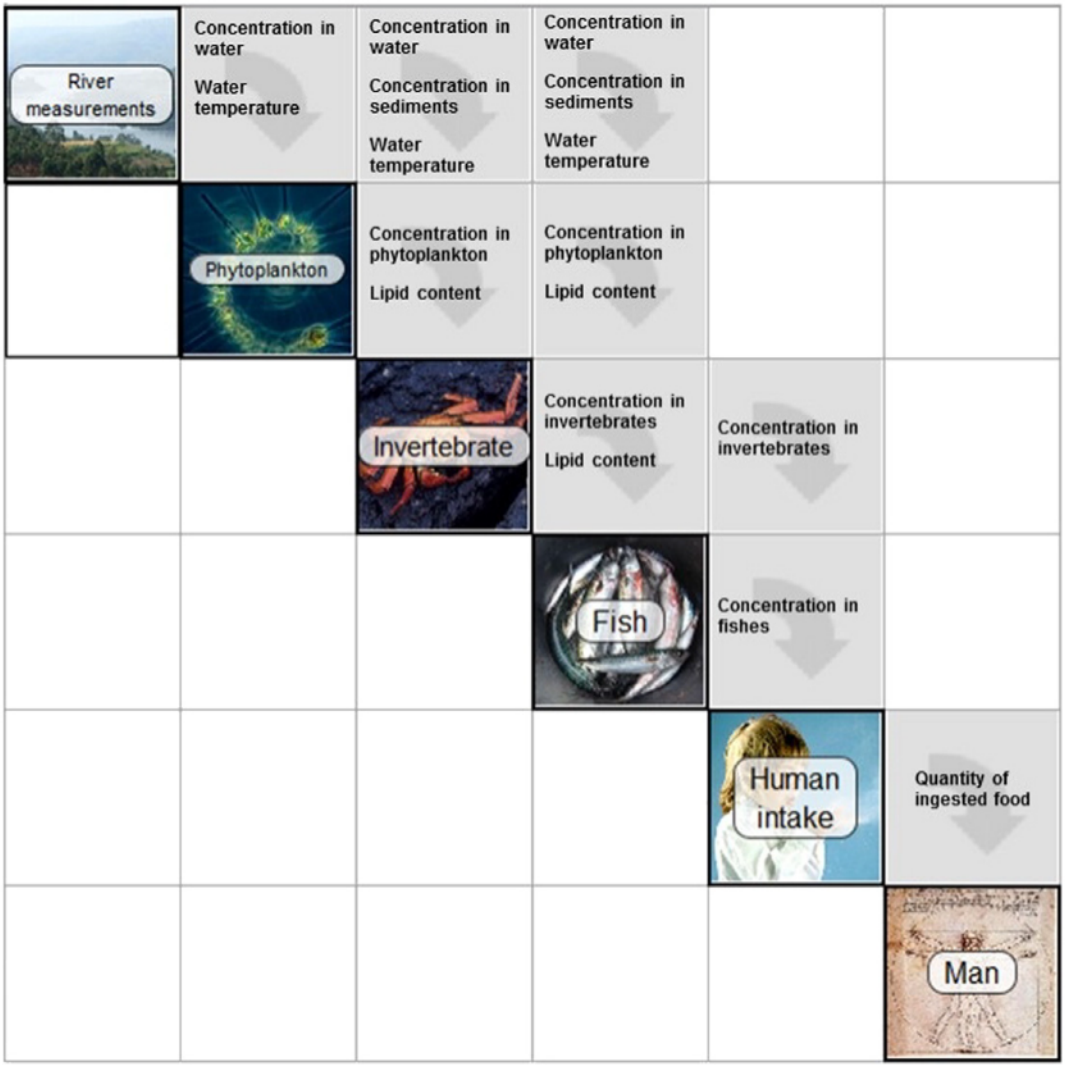


Fig. 2. Models chained on diagonal of the matrix, and connectors allowing flow of information between the models.

(e.g., phytoplankton lipid content and cell volume) (Hauck et al., 2007, 2011; Hendriks, 1999, 2007; Olenina et al., 2006; Seth et al., 1999). Parameters representing generic trophic levels (fish, invertebrates, and phytoplankton) were probabilistically estimated, providing uncertainty in calibration data, by Hauck et al. (2011), by comparing observed and estimated rate constants for physiological and chemical uptake and elimination. Parameters were originally grouped into three categories reflecting different approaches to their estimation. The first group includes independent parameters whose values can be determined independently from transport coefficients and partial resistances. These parameters are: lipid fraction of organism and its food, fraction of food assimilated, and allometric rate exponent. However, we used organism specific data to parameterize the lagoon food web (Excel file provided as SI). The second group was defined as transport coefficients and consists of transport of water through the organism, transport of food through an organism, and the production of biomass – their values were estimated from allometric data. The third group of parameters include partial resistances, which were derived by comparing the measured and estimated chemical rate constants and minimizing the differences by maximum likelihood estimation (Hauck et al., 2011; Hendriks, 2007).

Contaminant-specific parameter values were derived using QSAR models implemented in EPI Suite software (US EPA, 2012): metabolic half-life of chemicals for organics (Arnot et al., 2008, 2009), bioconcentration factor for organics (Arnot and Gobas, 2003, 2006), water-organic carbon partition coefficient (Schüürmann et al., 2007), and octanol/water partition coefficient (Meylan and Howard, 1995). PDFs were estimated based on the primary source where the given QSAR was developed. Assuming identical, independent and normally distributed errors, the uncertainty in a QSAR prediction (X_p) was deduced from reported predicted mean ($\overline{X_p}$), standard error of prediction ($SE(\overline{X_p})$), and estimated student t-distribution based on reported number of data in a training set n and a number of descriptors k used originally in QSAR development (t_{n-k-1}). Described method is given by Eq. 1:

$$X_p \sim \bar{X}_p + t_{n-k-1} \times SE(\bar{X}_p) \tag{1}$$

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

3.1.2. Human intake and PBPK

The 'Human intake' model is used to calculate the total ingested quantity of contaminants from contaminated food, i.e., 'Ingestion rate for food'. It is a step function of age used to assign different rates for different age groups as 'Age group ingestion rate for food' time series. MERLIN-Expo does not allow to assign uncertainty to time-dependent inputs such as age-dependent quantity of ingested food, therefore the 'Human Intake' model was not included in the uncertainty assessment.

As for the 'Man' model, body weight and tissue-blood partition coefficients have been included in uncertainty analysis. Individual's bodyweight is calculated as a function of age to allow inter-individual bodyweight variability among same aged persons. Body weight is normally distributed, described by mean and standard deviation (Beaudouin et al., 2010).

A tissue:blood partition coefficient is defined as the equilibrium factor represented by ratio of the concentration in a tissue to the concentration in blood. The partition coefficient is a normally distributed variable with mean and standard deviation. Statistics were obtained from reported values in Plowchalk et al. (1992); Shin et al. (2009); Björkman et al. (1996); Ishizaki et al. (1991); Björkman et al., 1990, 1994; Csanády et al. (2002); Gearhart et al. (1993). Several normal probability distribution functions have been parameterised for different tissues (Excel file provided as SI), separately for 2,3,7,8-TCDD and PCB126.

3.2. Sensitivity analysis experiment design

MERLIN-Expo allows to select among several tools to perform sensitivity analysis, including local sensitivity analysis methods, screening methods based on optimized experimental designs, global regression methods, global variance-based methods.

Sensitivity analysis is the study of how the variation in the output of a model (numerical or otherwise) can be apportioned, qualitatively or quantitatively, to different sources of variation. Sensitivity analysis highlights the inputs that have the greatest influence on the results of a model, therefore, it provides useful insights for model builders and users. Insights from sensitivity analysis can be used for: (i) identification of key sources of uncertainty, (ii) identification of key controllable sources of variability, and (iii) model refinement, verification, and validation.

SA methods available in MERLIN-Expo were organised in a stepwise structured approach, by starting from computationally 'inexpensive' Morris method to most costly variance-based methods. Since higher-tier methods are targeted on those uncertainties that have most influence on the assessment outcome, one can use the screening step (Morris method) to narrow number of input factors to those that are most influential, so that time needed to run final step (e.g., FAST, EFAST, and Sobol methods) can be shortened. The Morris screening method followed by regression based method and EFAST were applied in order to first reduce the number of parameters and then produce three sensitivity measures standardised regression coefficient β_i, first order sensitivity and total order sensitivity indices (S_i and TS_i).

3.2.1. The Morris screening method

Only a summary of the Morris method is presented in this paragraph, the complete description can be found in Morris (1991). The Morris method is a one-factor-at-a-time (OAT) method where the impact of changing the values of each factor (input parameter) is evaluated one by one in each run. It is a qualitative method providing a ranking of input parameters in order of importance but not a decomposition of the output variance. The Morris method is categorized as a global sensitivity analysis because the method covers the entire ranges over which the factors may vary. In the method based on OAT, each input factor may assume a discrete number of values which are selected within the factor's range of variation, and only one input parameter (x_i) is modified by a fixed factor. Each parameter uncertainty interval is first divided into p equally large layers, due to generating a hyperspace Ω, identified by a n-dimensional p-level grid, where n is the number of parameters. In the Morris method uncertain parameters are considered to be uniformly distributed, subsequently transformed to the original distribution, which will be used in the model. Model simulation is performed based on a selection of parameters randomly sampled from the previously defined grid. Next, a single parameter is randomly selected and modified by a fix factor Δ, and a second simulation is performed. Δ is a value in $\{1/(p-1), \ldots, 1-1/(p-1)\}$, but a more economical design is suggested with $\Delta = p/[2(p-1)]$. The model is evaluated for r trajectories within the parameter space. The starting point of a trajectory is selected randomly. For each trajectory, every single parameter is changed separately, whereas the new point of this trajectory is an element of the parameter space (Specka et al., 2015). Morris proposed a measure called elementary effects $EE_y(x_i)$ based on calculating for each input $X = (x_1, \ldots, x_n)$ a number of incremental ratios (Eq. 2) from which basic statistics are computed to derive sensitivity information.

$$EE_y(x_i) = \frac{y(x_1, \ldots, x_i + \Delta, \ldots, x_n) - y(x_1, \ldots, x_i, \ldots, x_n)}{\Delta} \tag{2}$$

This procedure is repeated r times, which is equal to the sampling number, providing r elementary effects for each parameter. The cost of running the screening test is based on the following relation $r(n+1)$. The method can distinguish between factors with negligible effects,

linear and additive effects, and factors with non-linear or interaction effects. For each elementary effect $EE_y(x_i)$ two sensitivity measures are computed: μ_i, which assesses the overall influence of the factor on the output, and σ_i, which estimates the non-linear effect and/or the interaction effect with other factors. To classify parameter sensitivity, μ_i values must always be considered together with $\sigma_i$values. Campolongo et al. (2007) suggested the use of mean of the absolute elementary effects μ_i^* as to avoid cancelling of positive and negative effects. In general, Morris proposed a method, which is particularly well-suited when the number of uncertain factors is high and/or the model is expensive to compute.

In our case study, the parameter ranking based on Morris method was applied as first step of sensitivity analysis. The number of realisations r was set to 10 and number of levels p to 4. This settings has been reported to be optimal for the Morris method (Ciric et al., 2012; Campolongo et al., 2007).

3.2.2. Regression-based methods

Regression-based sensitivity analysis is performed on probabilistic simulation outputs, in order to calculate regression coefficients. The space of the input factors is sampled via the Monte Carlo method and a linear regression model is built from the model output values. The standard regression coefficient (β_i) quantifies the contribution of the variance of each input factor to the overall output variance and is more attractive than local derivatives as it offers a measure of the effect of each given factor on the output, which is averaged over a sample of possible values, as opposed to being computed at the fixed point. Hence β_i^2 is a global sensitivity measure (Hall et al., 2009). β_i values can be used to verify linear model when the following relation holds: $\sum_i \beta_i^2 = 1$.

The standardised regression coefficient is derived from Eq. 3:

$$\beta_i = \left(\frac{\sigma_{X_i}}{\sigma_Y}\right)/b_i \tag{3}$$

where σ_{xi} is the standard deviation of the the input, σ_y is the standard deviation of the output, and b_i is the estimate of the regression coefficient.

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

The regression method complemented by the coefficient of determination (denoted by R^2) can be used to indicate whether linearity assumption for the model is appropriate. R^2 is interpreted as the proportion of the model variance explained by the regression model. R^2 can take a positive number between (0, 1).

Calculated according to Eq. 4, it can be used for instance to identify non-monotonic relationships between input and output.

$$R_Y^2 = \sum_{i-1}^{N} \frac{(Y_i{}^* - \mu_Y)^2}{(Y_i - \mu_Y)^2} \tag{4}$$

where N is the number of simulations, Y_i is the simulation results, Y_i^* is the Y_i derived from regression model, and μ_Y is the mean of the output Y.

3.2.3. EFAST

Extended Fourier Amplitude Sensitivity Test (EFAST) (A. Saltelli et al., 1999) is a variance-based global sensitivity analysis method, which computes both first-order (S_i) (Eq. 5) and total sensitivity indices (TS_i) (Eq. 6). The S_i measures the main (first order) effect of each individual or a group of inputs on the model output, while Total Sensitivity Index (TS_i) measures all higher order effects (i.e., considering interactions) that can be attributed to that parameter.

$$S_i = \frac{V_{x_i}\left(E_{x_{-i}}(y|x_i)\right)}{V(y)} \tag{5}$$

where S_i is the first order sensitivity index,V_{Xi} is the variance of output due to parameter x_i, $V(y)$ is the total variance of output y, and E_{x-i} is the expectation value.

$$TS_i = \sum_{k\#i} S_k \tag{6}$$

where TS_i is the total sensitivity index, $\#i$ represents all of the sets containing index i.

For assessing model linearity, all first order sensitivity indices should follow the relation $\sum_i S_i = 1$. The EFAST method is based on monodimensional decomposition of the model along the search curve in the n-dimensional parameter space. The search curve is defined by a set of parametric equations. The range of variation in EFAST is explored for all parameters simultaneously.

EFAST is independent of any assumptions regarding the relationship between input parameters and outputs. It provides the fraction of the output variance due to each input parameter. Uncertain input factors with small first order indices but high total sensitivity indices affect the model output mainly through interactions. Such an observation suggests redundancy in the model parameterization.

4. Results and discussion

4.1. Probabilistic analysis

4.1.1. Ecological exposure assessment

Accumulation of 2,3,7,8-TCDD and PCB 126 in biota soft tissue were simulated over the time period 1932–1998 with a time step of 100 days. Mean concentrations and 5th and 95th percentile confidence interval are shown in Fig. 3. Curve evolution is specific for the contaminant in question. Accumulated PCB 126 reaches different levels in different species but the concentration trend is similar for all four species. The same observation can be made for 2,3,7,8-TCDD: all species share similar internal concentration trend, however they differ in the level of accumulated chemical.

Two concentration peaks are observed for PCB 126 in all reported species, the first high peak in 1935 and the second, smaller one in 1952. After the second peak the concentration decreases rapidly until 1960s, and steadily continues to decline for the rest of the simulated period until 1998 (Table 1).

Mean 2,3,7,8-TCDD concentration reaches its maximum in the early 1940′s, reaching a plateau lasting until early 1950s, when a sudden decrease can be observed continuing until early 1960s. Afterwards concentration is maintained at the same level, and starts building up slowly from mid-1970s until 1998 (Table 1). Overall, PCB 126 accumulates in organisms to higher concentrations than 2,3,7,8-TCDD (Table 1). Estimated whole body mean concentration of 2,3,7,8-TCDD and PCB 126 is higher for invertebrates in *Tapes philippinarum* (clam) comparing to *Carcinus mediterraneus* (green crab), and, among fishes, concentration in *Chelon labrosus* (mullet) is higher than in *Zosterisessor ophiocephalus* (goby) (Fig. 3).

Accumulation of PCB126 is burdened with lower uncertainty than uncertainty on accumulated 2,3,7,8-TCDD. Confidence interval in the case of PCB126, after reaching the second concentration peak, tends to diminish towards the end of the simulation, while uncertainty on accumulated 2,3,7,8-TCDD after mid-1970′s shows growth when approaching 1998. In general, uncertainty varies along the simulated concentration and follows the same behaviour. Difference between uncertainty ranges (95th–5th percentile) are always between 2 and 3×

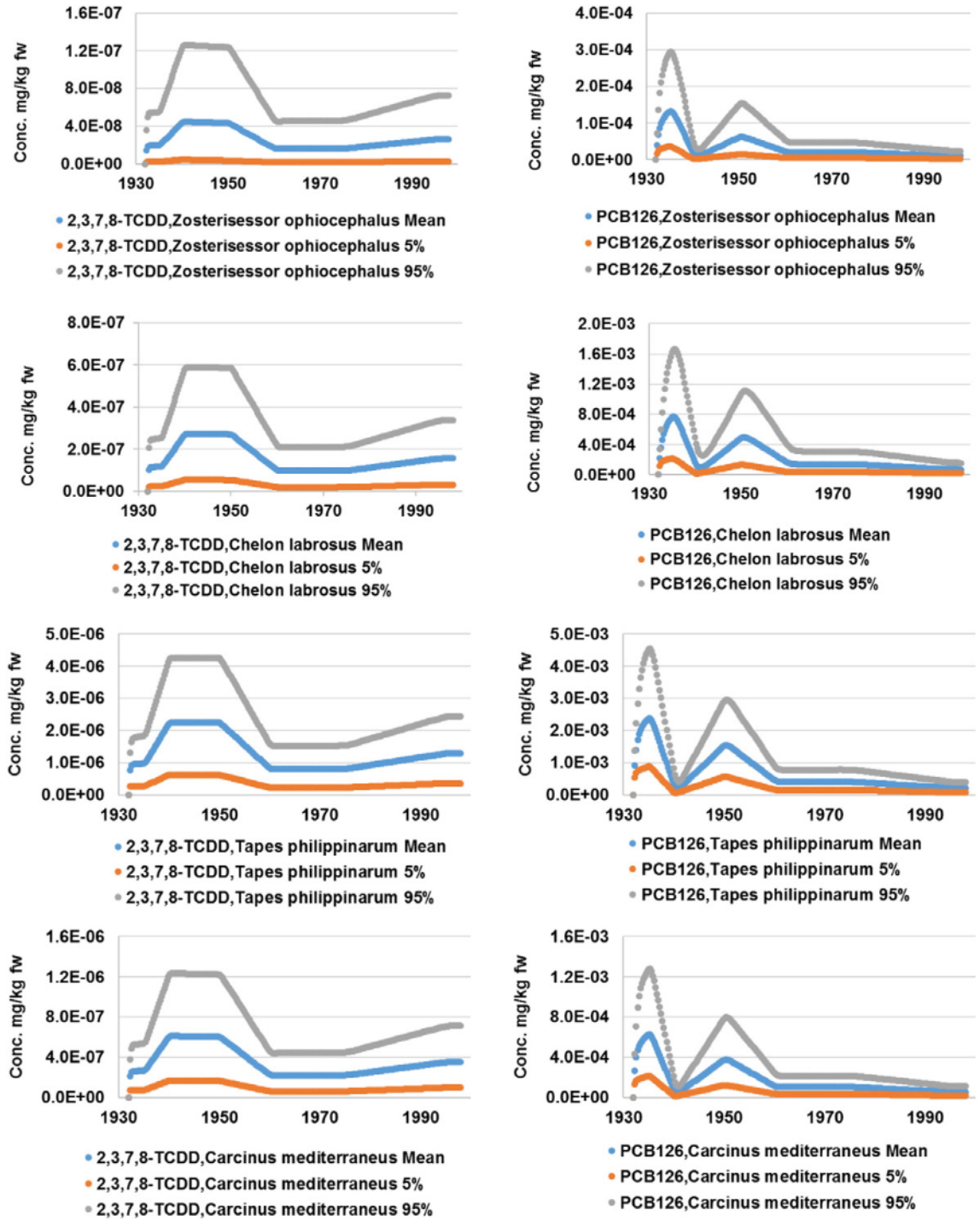


Fig. 3. Simulated concentration of PCB126 (mg/kg fw) and 2,3,7,8-TCDD (mg/kg fw) including all routes of exposure and uncertainty ranges of internal concentration (95th–5th %ile interval) in: *Tapes philippinarum*, *Carcinus mediterraneus*, *Chelon labrosus*, and *Zosterisessor ophiocephalus*, over period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

the mean value and invariable between species when all routes of exposure are considered.

4.1.2. Human exposure assessment

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

Amount of 2,3,7,8-TCDD in blood slowly increases arriving to a maximum simulated value of 1.3×10^{-7} (mg/L) in 1950s. On the contrary, 2,3,7,8-TCDD accumulates in blood in higher concentration than PCB 126 at the final time of the simulation. In fact, Ruiz et al. (2014) noted that the concentrations of 2,3,7,8-TCDD in serum increases with age due to higher environmental dioxin levels in past exposure, the number of years of past exposure, and slower elimination among older persons. Results reported by Giubilato et al. (2016) on 2,3,7,8-TCDD levels in blood among 18 age groups are in accordance with Ruiz observations. Similarly to results from ecological exposure assessment, PCB 126 have tendency to accumulate to greater level than 2,3,7,8-TCDD (Table 2).

By comparing concentration trends in biota and environmental (Figs. 3 and 5) one can arrive to the conclusion that temporal evolution

Table 1
Mean concentration and lower and upper confidence intervals for PCB 126 and 2,3,7,8-TCDD at maximum concentration predicted in 1935 and 1940 and concentrations for both chemicals simulated in 1998.

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

of internal concentration in aquatic organism is shaped mainly by the chemical concentration in exposure media, albeit more by concentration of contaminants in sediments than in water (Table S1, Fig. S1—S3). We observed huge drop in accumulated concentration over simulation period in aquatic species and consequently in human blood, with regard to concentration calculated when all food web bioaccumulation routes are active (2,3,7,8-TCDD down by 98% and PCB126 by 94%). Furthermore, exposure concentration in diet (seafood) affects computed temporal variation of concentration levels of the contaminants in blood (Fig. S3).

Cross-correlation function (CCF) is used to show potential influence of the environmental concentration time series on the concentration in blood (Fig. 5, right pane). In order to apply cross-correlation function, concentrations in water and sediments were used as input time series and computed concentration in blood as output time series. Negative line segments correspond to events that are not correlated. Positive relationship with positive time lag is characteristic for the dioxin time trend in sediments and blood. Nevertheless, the correlation is weak, largest value at lag −10 reaches 0.49 (Fig. 5). Concentration of 2,3,7,8-TCDD in water poorly correlates with concentration in blood too. It weakly correlates at lag −10 (0.24), but mostly lack of relationship is predominant with highest negative values at lag 10 (−0.78). Interestingly, it takes roughly 15 years for 2,3,7,8-TCDD to reach peak concentration in blood after the occurrence of the environmental peak exposure.

Simulated PCB126 concentration response is immediate with respect to concentration in water and sediments. This is noticeable by pronounced strong positive correlation over simulation period between both environmental concentration time trends and concertation computed in blood. High peak 0.93 points out strong correlation and it occurrence and lag −2.0 signifies that PCB's concentration in water and sediments slightly leads concentration in blood.

Several studies inform about environmental concentrations as a critical source of uncertainty in modelling bioaccumulation in aquatic food webs (De Laender et al., 2010; Ciavatta et al., 2009; Nfon and Cousins, 2007). It is also well recognised that human dietary exposure concentration together with information on food consumption are one of the most important sources of variability and uncertainty in dietary exposure assessment (Kettler et al., 2015; Kennedy and Hart, 2009). We stress that uncertainties in our reconstructed historical concentration trends are high and remain unquantified. Details on measurements of environmental input concentration and the method applied to calculate historical exposure concentration in water is described by Giubilato et al., (2016).

4.2. Sensitivity analysis

In order to account for all important model parameters and their effects, sensitivity analysis was performed as a sequence of methods, as illustrated in Paragraph 2.4. For the sake of simplicity, the presented results were narrowed down to show most influential parameters only with regard to 2,3,7,8-TCDD and PCB 126 concentration in man's blood as model outputs.

4.2.1. Results of Morris method

The model was run for 24,091 days. 1790 model evaluations were run including 156 parameters in the analysis of PCB 126 and 2,3,7,8-TCDD in blood. Results presented in Fig. 6 allowed to screen parameters for the most influential ones observed in 1998 (that is 24,091st day of the simulation). The results were used to reduce number of parameters

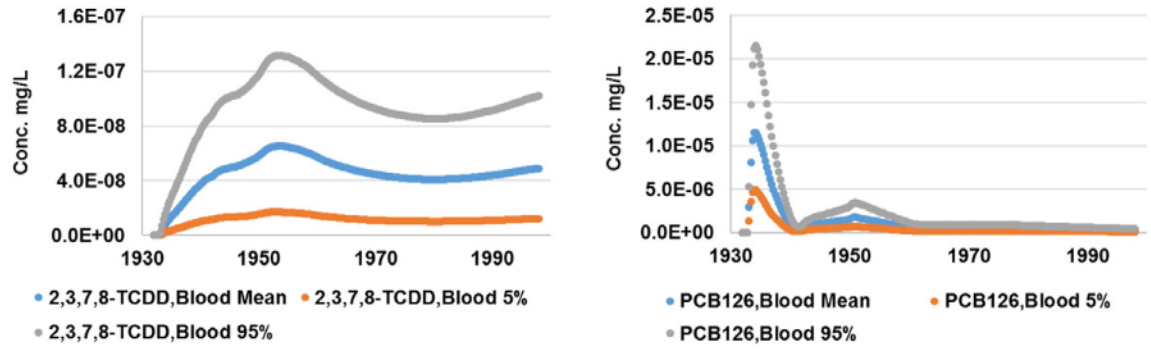


Fig. 4. Simulated concentration of PCB126 (mg/L) and 2,3,7,8-TCDD (mg/L) and uncertainty ranges of internal concentration (95th–5th percentile interval) in man's blood over period 1932–1998.

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

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

to be included in further sensitivity analysis steps. Parameters with μ_i^* (mu*) higher than 2.0×10^{-8} and σ_i (sigma) higher than 1.0×10^{-8} were considered important for calculating concentration of 2,3,7,8-TCDD in blood. Factors deemed as significant for modelling PCB 126 in blood were restricted to those characterised by μ_i^* higher 5.0×10^{-8} and with σ_i higher than 7.0×10^{-8}. Regardless of the considered compound, chemical metabolic half-life and man's body weight were found to be the most important parameters. There are differences in the two sets of influential parameters, for instance tissue-blood partition coefficient for adipose for 2,3,7,8-TCDD was noted as important but not for PCB 126. On the other hand, lipid content in zooplankton and phytoplankton seems to be more important in case of PCB 126 than for 2,3,7,8-TCDD. Overall, results of the Morris screening method imply that parameters used in 'Invertebrates' bioaccumulation model for *Tapes philippinarum* (lipid fraction, food assimilation efficiency, water-layer diffusion resistance for uptake of chemicals from food, metabolic half-life of chemicals, allometric rate exponent, food transport coefficient) are predominant among influential parameters and matter most in calculating concentration in blood for both contaminants in question. Parameters identified as important in the Morris methods (Fig. 6) were used in further steps of the sensitivity analysis.

4.2.2. Results of regression-based analysis

Regression-based analysis to assess influence of uncertain input factors on model output variance was performed using Monte Carlo sampling scheme by drawing 2000 samples. Correlations between 4 uncertain input parameters and probabilistically simulated 2,3,7,8-TCDD and PCB 126 concentration in man's blood are visualised on scatterplots (Fig. 7). Parameters included in scatterplots were selected for each contaminant based on the highest mu* and sigma scores indicated in the Morris method. Examination of scatter plots reveals various patterns between selected input parameters and model output, hence informing about various relationship. Scatter plots reveal metabolic half-lives and lipid content in Manila clam to be positively correlated with computed output, and negative correlation in case of variability in bodyweight and liver-blood partition coefficient with concentration of contaminants in blood. The standardised regression coefficient β_i^2, decomposed according to 10 input factors, captures 73% of the model output variance in case of computed 2,3,7,8-TCDD concentration in blood (Table S2). Table S3 shows individual β_i^2 values for 12 input parameters accounting for 71% of variation in computed PCB 126 concentration in blood. The quality of regression model is assessed by the R^2 which for both computed chemicals in blood is above 0.7, indicating that the linear regression fits well model output and that an appreciable fraction of output variance can be apportioned to linear component of the model (Manache and Melching, 2008). Nevertheless, 25% and 29% of the variation in computed concentration of 2,3,7,8-TCDD and PCB 126, respectively, remains unexplained by the β_i^2. Therefore, further analysis was applied to understand the contribution of uncertain parameters to model output variance.

4.2.3. EFAST

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

4.2.3.1. 2,3,7,8-TCDD in blood. Sum of first order sensitivity indices (S_i) explains 76% of the model output variance and implies that remaining 24% is due to higher order interactions taking place among the uncertain factors. Two parameters with the highest S_i, metabolic half-life of 2,3,7,8-TCDD and variability in the bodyweight, account for 50% of the variation in the computed 2,3,7,8-TCDD concentration in blood.

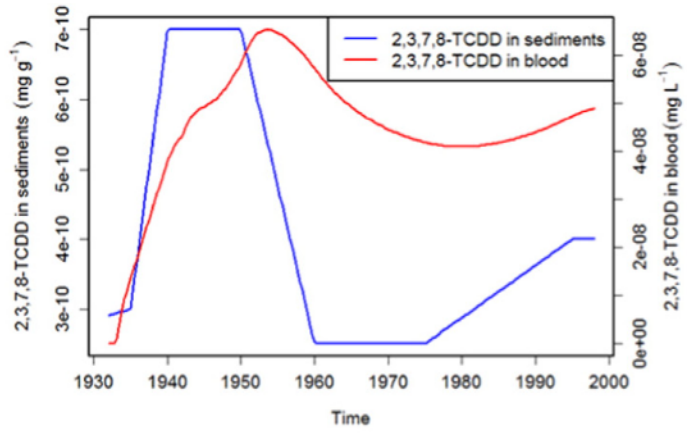
The total sensitivity indices (TS_i) inform that the most important parameter for computing 2,3,7,8-TCDD concentration in blood is its metabolic half-life, which is used as an input parameter to 'Invertebrate' bioaccumulation model, and turned out to be responsible for 47% of the output variance. The second most important parameter is the inter-individual variability of the body weight, accounting for 24% of output's variation (Table S2). TS_i values computed for metabolic half-life and variability in body weight are respectively 15% and 6% higher than S_i indices, indicating small interaction among the parameters. All global SA methods consistently show metabolic half-life of 2,3,7,8-TCDD and variability in the bodyweight as the most influential parameters for computing 2,3,7,8-TCDD concentration in blood.

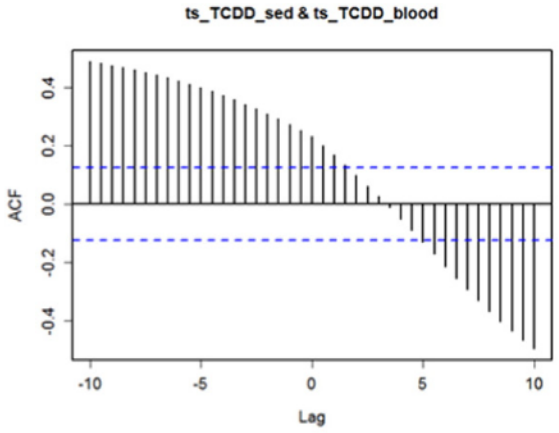
The time evolution of the total sensitivity index for a set of parameters is plotted in Fig. 8. The key relations are the decreasing index for adipose tissue-blood partitioning coefficient and the increasing index for body weight. Also interesting is the increase in liver tissue-blood partition coefficient total sensitivity index: its importance begins to grow only starting from 1950s. Among parameters specific to aquatic biota, the allometric scaling parameter (kappa), used to model bioaccumulation in Manila clam, shows a significant drop from the beginning of the simulation.

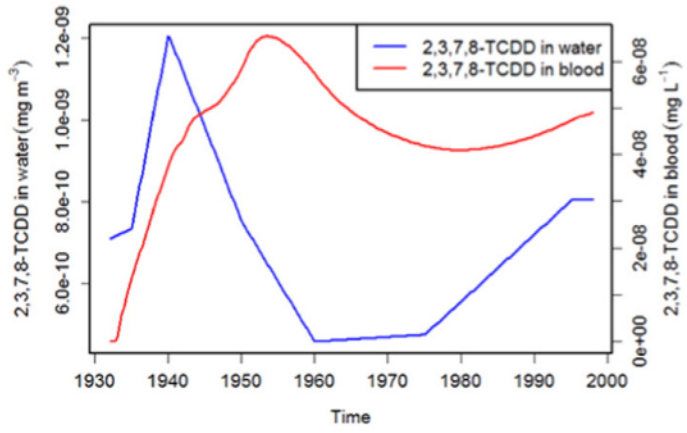
Biotransformation half-lives of organic chemicals are known to affect exposure estimates in aquatic food webs (Arnot et al., 2010). Metabolic half-lives of 2,3,7,8-TCDD is burden with uncertainty attributable to QSAR modelling, which was originally intended to provide screening level predictions of the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

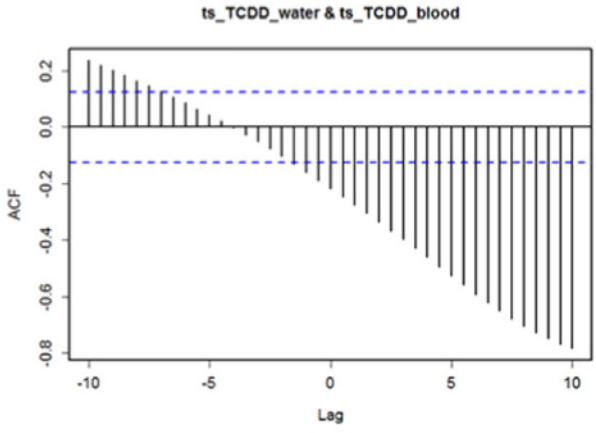
4.2.3.2. PCB126 in blood. The most important parameters detected by the three computed global sensitivity indices are lipid content and fraction of assimilated food specific to Manila clam (Table S3). S_i calculated for 12 input parameters arrives at 81% of variation leaving 19% to be explained by interaction between parameters. First order effects computed for lipid content and fraction of assimilated food capture together 35% of output variance. Total effects show that 28% percent of variation in the simulated internal concentration of PCB 126 is explained by uncertainty in lipid content of Manila clam (*Tapes philippinarum*) and 22% is due to the fraction of assimilated food. It is interesting to note that contribution of these factors to the output variance through interaction is weak as the difference between TS_i and S_i is small, respectively 8% and 7% for each of the parameter (Table S3). Estimated TS_i for the top three parameters reported in Table S2 captures 86% of the variation in concentration of 2,3,7,8-TCDD, while 66% of variation in concentration of PCB 126 is explained by the three most influential parameters (Table S3). Overall, the fractions of variation in PCB 126 concentration in blood given parameters are responsible for, are less discernible than in the case of 2,3,7,8-TCDD.

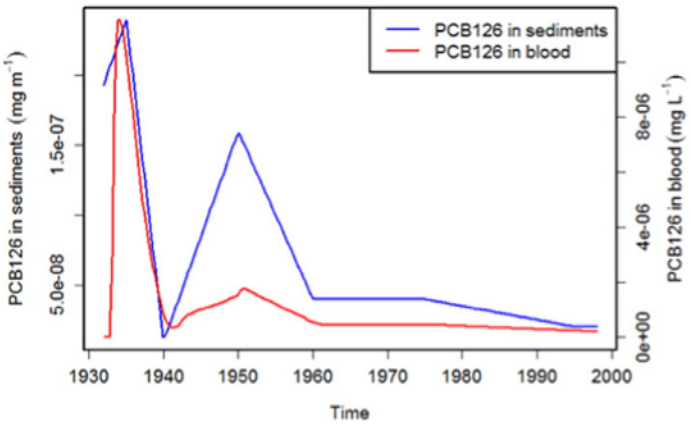
The time evolution of total sensitivity index for the most influential parameters for accumulation of PCB 126 in blood does not show any

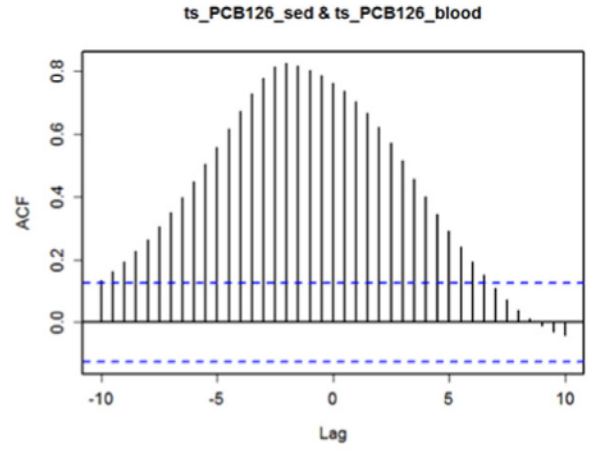

2,3,7,8-TCDD in sediments
2,3,7,8-TCDD in blood
2,3,7,8-TCDD in sediments (mg g-1)
2,3,7,8-TCDD in blood (mg L-1)
Time

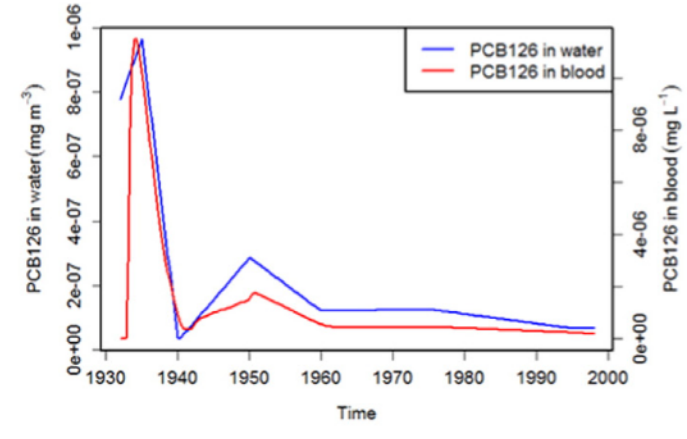

ts_TCDD_sed & ts_TCDD_blood
ACF
Lag

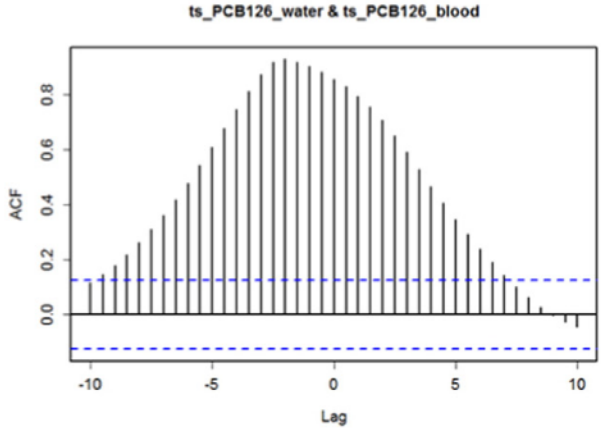

2,3,7,8-TCDD in water
2,3,7,8-TCDD in blood
2,3,7,8-TCDD in water (mg m-3)
2,3,7,8-TCDD in blood (mg L-1)
Time


ts_TCDD_water & ts_TCDD_blood
ACF
Lag


PCB126 in sediments
PCB126 in blood
PCB126 in sediments (mg m-1)
PCB126 in blood (mg L-1)
Time


ts_PCB126_sed & ts_PCB126_blood
ACF
Lag


PCB126 in water
PCB126 in blood
PCB126 in water (mg m-3)
PCB126 in blood (mg L-1)
Time


ts_PCB126_water & ts_PCB126_blood
ACF
Lag

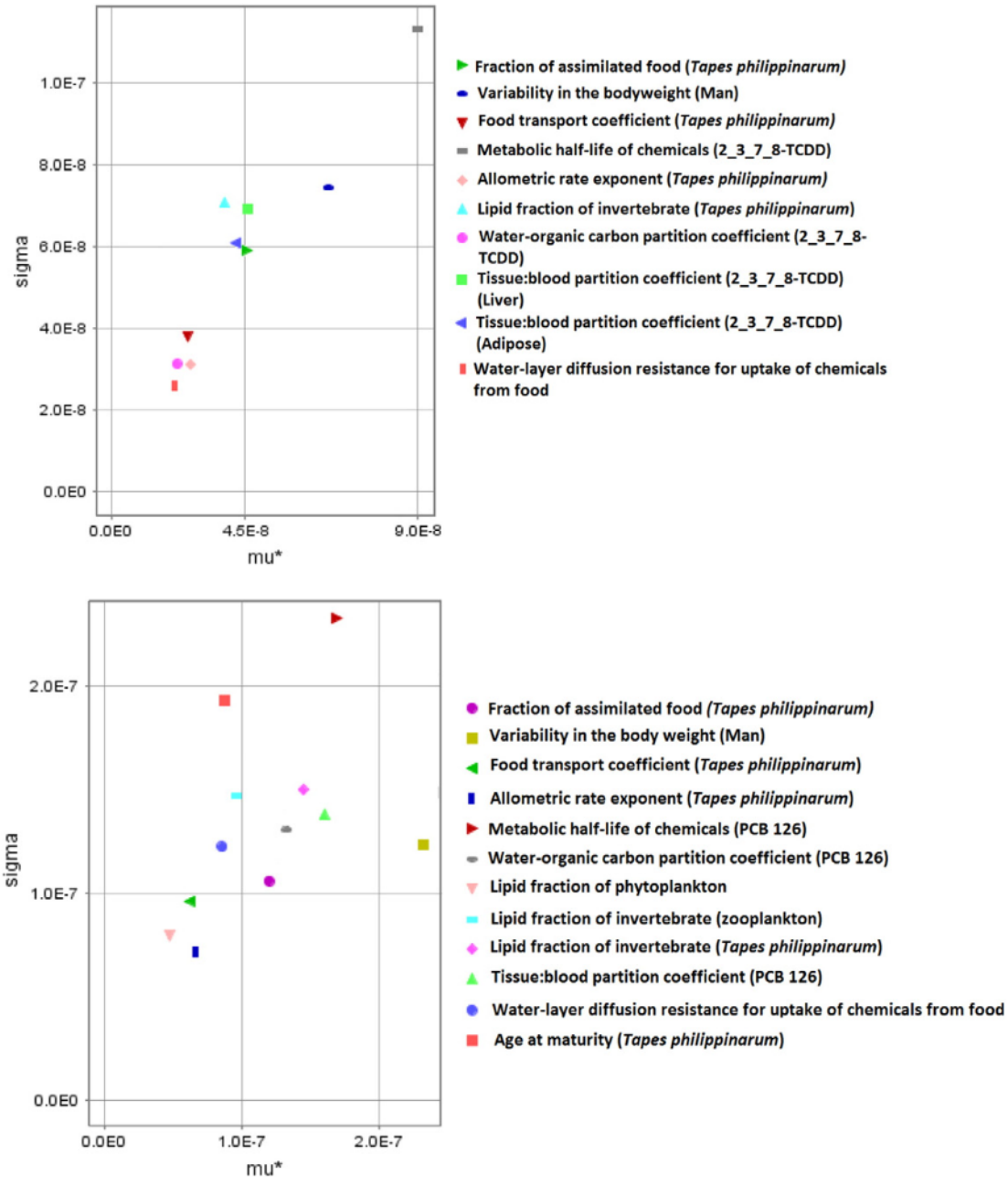


Fig. 6. Most influential parameters for calculating 2,3,7,8-TCDD concentration and PCB126 concentration in human blood PCB 126 based on Morris method.

significant changes over time, as depicted in Fig. 9, possibly due to weak interactions between parameters. Two bumps, one in 1940s and the other one smaller in 1960s, for several parameters (Fig. 9) seem to be related to two distinctive spikes in environmental concentration of PCB126 in sediments and water (Fig. 5).

The calculation of respiratory and dietary uptake and elimination kinetics of organic chemicals in aquatic species is based on parameters related to animal physiology, such as food assimilation efficiency, and partition of hydrophobic organic chemicals to lipid content, hence these factors are expected to have an effect on bioaccumulation and human exposure estimates. This is confirmed in our study of sensitive parameters where, indeed, parameters representing lipid content and food assimilation efficiency are the most important ones. The estimation of PDF assigned to assimilation efficiency for Manila clam should attract more attention then, given that it was originally estimated as a generic factor describing efficiency of assimilation in aquatic invertebrates (and not specifically for clam).

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

One significant factor having the potential to strongly influence obtained results is the food intake rate for man, which for Manila clam is the highest among the considered seafood items (Fig. 10).

Fig. 5. Left pane: Concentration of 2,3,7,8-TCDD and PCB 126 in dissolved water (mg/m^3) and human blood (mg/L); Right pane: Cross-correlograms showing influence of chemical concentration in water on concentration in human blood. ACF is defined as autocorrelation function.

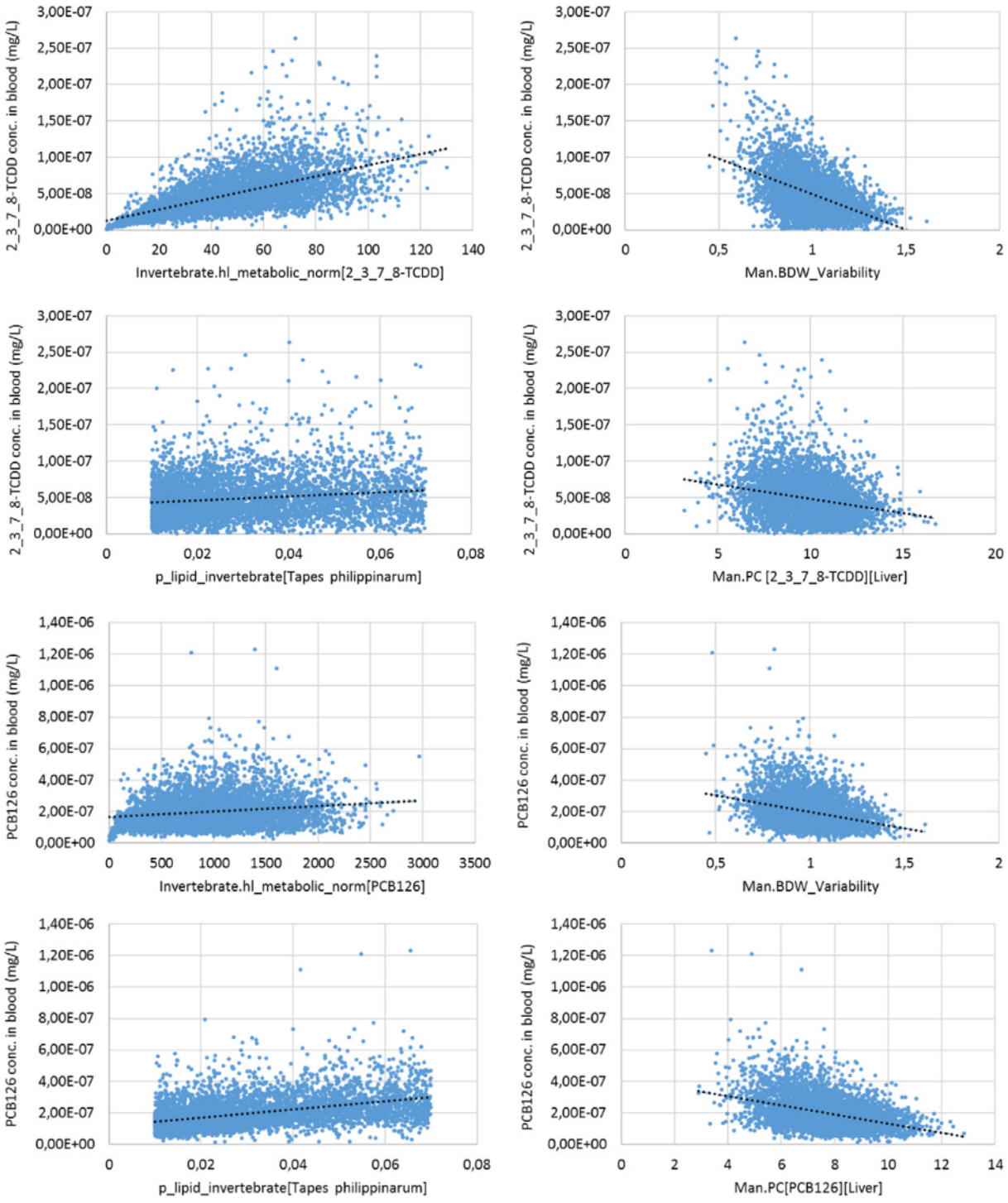


Fig. 7. Scatter plots of concentration distribution of 2,3,7,8-TCDD (two upper rows) and PCB 126 (two bottom rows) in blood versus uncertain input parameters at simulation time 24,091 (1998).

This may be the reason why five parameters directly related to the clam are relevant for estimating PCB 126 in man's blood and four for estimating 2,3,7,8-TCDD. Both chemicals are highly lipophilic ($K_{OW,2,3,7,8\text{-}TCDD} = 6.9$, $K_{OW,PCB126} = 6.8$). 2,3,7,8-TCDD tends to concentrate in lipid-rich tissues, as discussed by Diliberto et al. (2001) and that its lipid solubility is particularly important at low doses. However, despite high lipophilicity of PCB126, higher concentration was found in liver than in fat due to most likely protein binding (Lohitnavy et al., 2008). This difference between the two chemicals could be addressed in the PBPK model by additional data collection and parameterisation.

5. Conclusions

We demonstrated the application of MERLIN-Expo tool in studying uncertainty and sensitivity of exposure models on a real life case study in Venice lagoon (Giubilato et al., 2016), having the potential to aid in understanding chronic historical and future dietary human exposure to organic contaminants in foodstuffs. The scope of exposure assessment was to provide transparency and credibility to historical lifetime exposure assessment of a human individual and of a food web, through application of different uncertainty and sensitivity analysis tools offered by the software.

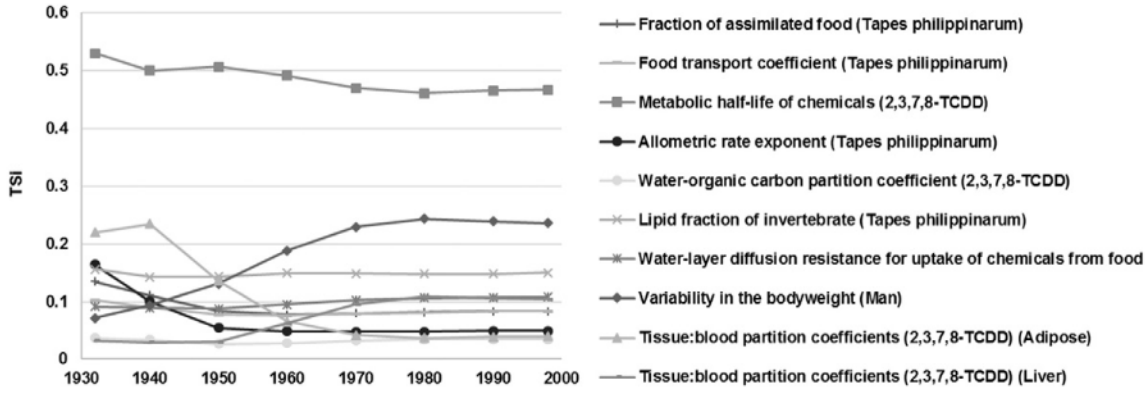


Fig. 8. Change of total sensitivity index of 10 parameters over simulation time 1932–1998, considering as output the 2,3,7,8-TCDD concentration in blood.

Historical environmental exposure concentrations were used. The source of past concentration are sediment cores which are known to be a good source of information about past contamination trends of organic contaminants. The scarcity of data on historical contamination is a drawback in our study, nevertheless it gives the idea about the general contamination trend over several decades, which in fact agrees well with pollution emissions in the Lagoon of Venice (contamination peaks in 1940s and 1950s).

The general conclusion with regard to the obtained results after first two steps of SA (i.e., application of Morris method and regression-based analysis) is that model is quasilinear. With regard to uncertain model parameters related to PBPK model, EFAST yielded body weight and liver tissue-blood partition coefficient, additionally, adipose tissue-blood partition coefficient in the case of exposure to 2,3,7,8-TCDD were distinguished, but the indices values are low, suggesting that other more dominant sources of uncertainty exist.

The main driver for ecological exposure to POPs resulted to be environmental concentration, especially in sediments. After disabling the consumption of sediments by the considered aquatic species, we found that this exposure route is the most important one (sediment is a part of diet items but its ingestion is modelled separately due to water-organic carbon partitioning and organic carbon content used specifically in the sediment uptake model). Even though 2,3,7,8-TCDD concentration in water and sediments is not well correlated with blood concentration, we noticed a huge decrease in biota and human blood concentration after shutting down the sediment ingestion exposure route.

Human exposure to POPs depends on a significant number of parameters, processes and behaviours. Results from SA are spread across many model parameters and do not clearly identify a reduced number of influential factors. However, for 2,3,7,8-TCDD there is still some contribution from metabolic half-life used in invertebrate model (when human body weight variability and clam lipid content are considered, this contribution goes up to almost 90%). These factors and the high ingested quantity of seafood with major presence of clam in the daily intake would add up to factors strongly affecting concentration of 2,3,7,8-TCDD in human blood. The environmental concentration of the dioxin shows, however, very weak correlation with concentration in human blood. PCB126 in blood, on the other hand, is noticeably more correlated to environmental concentration both in sediments and water. Also PCB's contribution from seafood intake is larger than that of 2,3,7,8-TCDD. SA does not show any major driver of PCB126 concentration in blood among model parameters. The obtained results suggest that environmental concentrations and eating behaviours should be scrutinized better in order to elucidate contribution of uncertainty to model outputs and also encourage to include functionalities in MERLIN-Expo for considering uncertainty in time series inputs in UA/SA.

While ecological parameters affect the level of accumulated concentration in biota, and should be better considered in order to obtain more accurate bioaccumulation estimates, for human exposure to POPs they do not play such an important role, what is confirmed by rather low values of the SA measures.

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

Overall, the study allowed to conclude that MERLIN-Expo freeware developed in 4FUN project can be effectively used in integrated human and ecological exposure modelling. Thanks to the availability of uncertainty and sensitivity analysis tools, MERLIN-Expo becomes a versatile tool offering set of features for comprehensive ecological and human exposure assessment. Considering also that MERLIN-Expo is an open platform where new environmental, biota or human models can be included (by following model development steps described in details

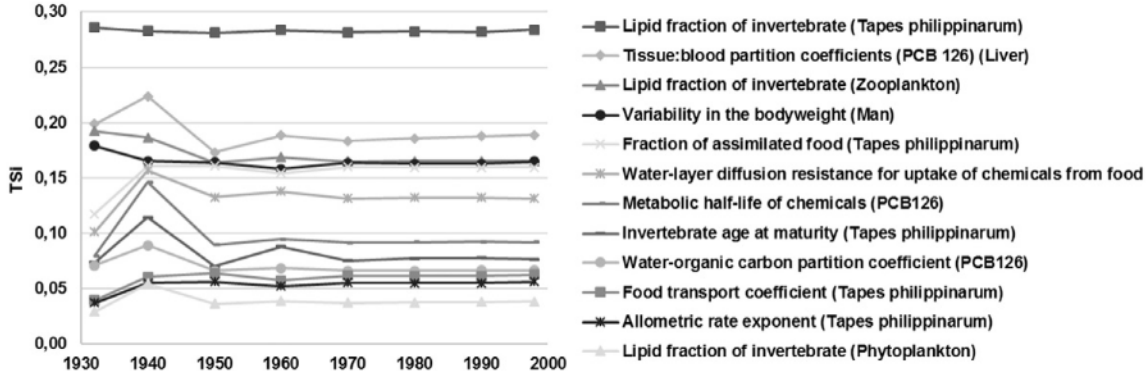


Fig. 9. Change of total sensitivity index of 12 parameters over simulation time 1932–1998 considering as output PCB 126 concentration in blood.

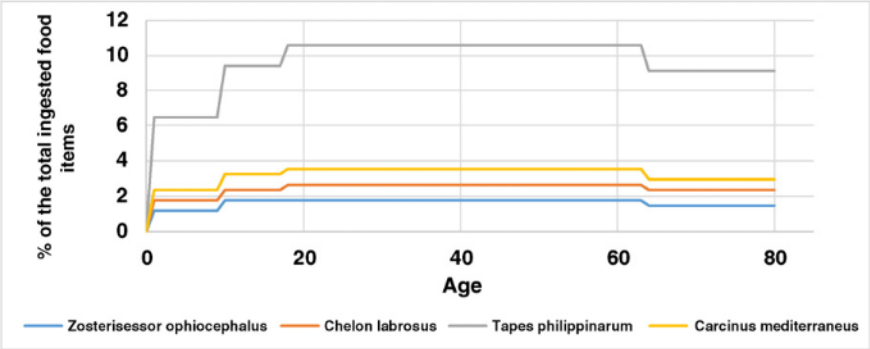


Fig. 10. % contribution of the four selected species to the total ingested seafood by human as a function of age (years). Full list of considered animals (8 species) and specific human age dependent ingestion rates can be found in Giubilato et al. (2016).

in model standard documentation), the potentialities offered by this tool for advanced exposure assessment modelling are very promising.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.07.057.

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