4FUN

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PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

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Abbreviations

EFAST	Extended Fourier Amplitude Sensitivity Testing
EFSA	European Food Safety Authority
ENCAT	Evaluation of Nutritional Status in Catalonia
FAST	Fourier Amplitude Sensitivity Testing
PBPK	Physiologically Based Pharmacokinetic Modelling
PDF	Probability Density Function
PFCs	Perfluorinated compounds
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonate
SA	Sensitivity Analysis

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

This report aims to present the achievements obtained in the third case study of the 4FUN project (Task 5.3). The objective of the three case studies is to increase the confidence in the applicability of MERLIN-Expo through targeted demonstration activities based on complex realistic case studies. As described in the document of work of the 4FUN project, the process of model demonstration applied in each case study entails the same following steps:

- Parameterisation of the multi-media and PBPK models to run a site-specific assessment. For each case study, the selected modules of MERLIN-Expo will be parameterised according to the substances of interest and the characteristics of the investigated context,
- Comparison between the model outcomes and the actual monitoring data for the full chain of models,
- Propagation of uncertainty and variability in the full chain of model to evaluate their impacts on model outputs,
- Sensitivity analysis in order to identify the key parameters of the exposure and human models, and to assess the contribution of the different relative pathways, sources and routes of exposure on a model outcome.

1.1 Context

Case study 3 focuses on halogenated emerging pollutants classified as priority chemicals due to their toxicological effects, and in particular on the perfluorinated compounds (PFCs). PFCs have been manufactured since 1940s. Because of their properties, these compounds are employed in a wide variety of industrial and consumer products. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) have been two of the most used and studied PFCs, but due to their resistance to degradation, widespread in the environment, bioaccumulation and toxicological properties, these two compounds are limited in use and production practically worldwide. However due to their highly persistence and because they are the degradation product of other PFCs currently in use, PFOS and PFOA are two of the more frequently compounds that are found in the environment and they have been detected in environmental and biological samples widespread around the world (even in remote areas) including water, soils and sediments, and/or human samples. Recently, PFOS has been included as a persistent organic pollutant (POP) under the Stockholm Convention for global regulation of production and use as well as in the list of priority substances of the Water Framework Directive (Directive 2013/39/EU). PFCs are also prime candidates for chemicals that will need authorisation within the REACH regulation.

In case study 3 we studied the contamination of the Ebro River Basin in Spain by PFOS and PFOA. The Ebro river is one of the most important rivers in Spain, 928 km in length and with a drainage basin of 85,550 km². It also generates the Ebro Delta, one of the largest wetland areas (320 km²) in the western Mediterranean region (Figure 1). The following high contaminated areas have been identified along the Ebro basin: Monzón, downstream to the cities Pamplona, Zarragoza and Lleida, and the reservoir of Flix.

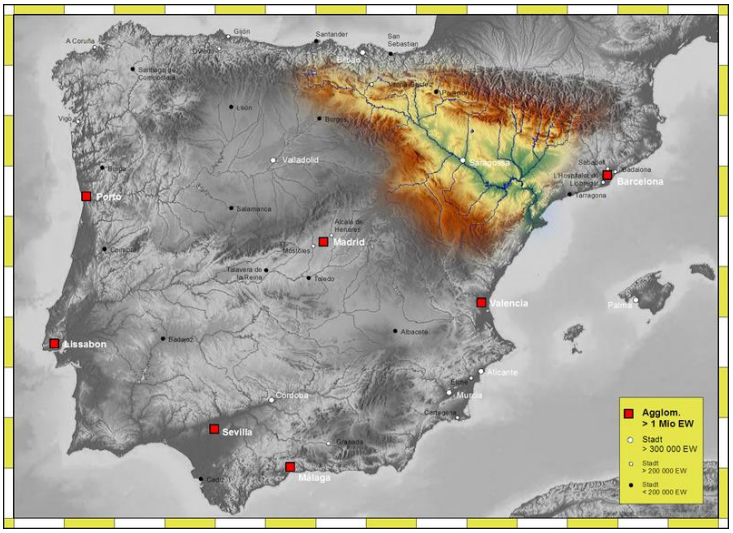

Figure 1: Location of the Ebro River basin in Spain

1.2 Objectives

Because the environmental and the human data are not well correlated, we proposed to define two exposure scenarios: the first one related to the environment and the second one to the human population living in the Ebro river basin. These scenarios aim at estimating:

- the contamination of the environmental media in the different zones of the Ebro river basin,
- the exposure of the human population via food intake.

2 The environmental exposure scenario

The objective of the environmental exposure scenario is to simulate the fate of two perfluorinated compounds (PFOS and PFOA) in the Ebro River.

2.1 The conceptual model

Sampling campaigns were carried out under the frame of different research projects, as the European project AQUATERRA in different zones at potential risk along the Ebro River basin (4 sampling campaigns), and the Spanish project Consolider-Ingenio 2010 CSD2009-00065 Scarce (2 sampling campaigns); data collected during these campaigns were used for this case study. In total data were collected in 47 sampling points[1]. For the purpose of this case study data were analyzed and selected according to availability and compatibility of data needed for the simulation purposes. All relevant hydrological data are obtained from the Hydrographical Confederation of Ebro (CHE)[2]. For the comparison between simulated and measured values, we considered only river stretches with measured pollutant concentrations, measured flow and concentration in biota. Taking that into account the number of relevant sampling point was considerably smaller and was equal to 3.

The environmental model was then assembled from 3 river stretches (Oca, Miranda del Ebro and Tudela) and their corresponding fish species and sediments (Figure 2). The number of stretches (3) selected for the modeling was depending on the data availability. We chose to model only the stretches where all data were available (river flow, measured concentrations in river water, sediments and fish).

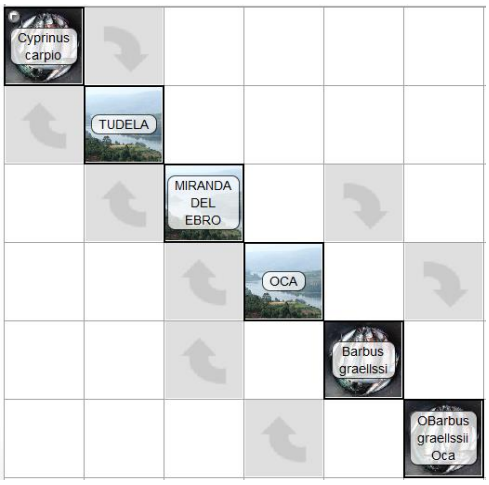

Figure 2: The Environmental conceptual model

2.2 Environmental data

Monitoring data have very good spatial distribution but rather poor time distribution. The monitoring data for fish were collected in 2011. Concentration in fish for both contaminants was measured in all stretches throughout the Ebro River. For this case study we chose to use data measured in species Barbus Graellsi and Cyprinus Carpio. For Tudela only one value of fish concentration measurement was above the limit of detection. Modeling was conducted for 3 river stretches since the input data set for the MERLIN-Expo was completed

[1] CSD2009-00065, S.C.p.C.-I., Database of the project.
[2] Ebro, H.C., http://iber.chebro.es/geoportal/.

only for them (pollutant concentrations in upstream river stretch, sediments and biota, daily flow measurements, irrigation, water temperature and emission from the industry). While the concentration measurements were provided by the SCARCE Consolider project. The irrigation data, water temperature and daily flow measurements were provided by the water authority CHE (Confederación Hidrologica Ebro). The emission from industry is estimated from bibliography data (Pistocchi and Loos, 2009).

The existence of only one concentration measurement (September 2011) of the pollutants in the monitoring campaign was the reason we had to construct the concentration profile based on the daily flow measurements. It is important to mention that the stretches are not adjacent therefore the flow rate is not the same. Since the flow influences the concentrations to a great extent (Osorio et al, 2012) we used the following formula:

$$Q * C = Q' * C'$$

$$C = (Q' * C)/Q$$

Q-river flow in the previous stretch

Q'-river flow in the current stretch

C-concentration of the pollutant in the previous stretch

C'-concentration of the pollutant in the current stretch

2.3 Parameterization of the environmental models

Data have been collected to set site-specific values for forcing variables and some parameters required by the river and fish models. These data include:

- the river flow in the different stretches obtained from the CHE (Hydrological Conference Ebro),
- data on the geography of the region to define the length of the river boxes (CHE),
- the irrigation rate using water from the Ebro river, 5.75 $hm^3/year^2$ (15753 m^3/day) (Causape and Aragues 2006),
- the SPM (solid particulate matter) parameters were estimated by fitting the model (using the least squares regression on logarithms) to measurements of flow rates (Flow_river) and SPM,
- Physical and chemical properties of PFOS and PFOA[3], such as the Henrys law constant, the water organic carbon partition coefficient, the molar mass, the global degradation rate in water, the global degradation rate in sediments (Kutsuna and Hori, 2008).

Parameters were calculated using the documentation of the "River model" module of MERLIN-Expo. In this document, the parameterization was written following the most reliable sources available at the time of the project. Some parameters were left as default depending from their physico-chemical properties.

[3] Environment Canada, http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=370AB13311

Table 4: Parameters of the environmental model

Parameter	PFOA	Source	PFOS	Source	Unit
Henry's law constant	2.4* 10^{-5}	Et al Catherine Arundel Barton 2008	3.4*10^{-9}	EPA (2015)	Pa m^3 mol^{-1}
Molar mass	414	EPA (2015)	538	EPA (2015)	g/mol
Water-organic carbon partition coefficient (logKOC)	2.06	EPA (2015)	2.57	EPA (2015)	-
Bioconcentration factor for organics	3.1	ENV agency UK	2796	ENV agency UK	L $kg.fw^{-1}$
Fish age at maturity	1460				day
Fish length at maturity	17.5				Cm
Metabolic half life	3	EPA (2015)	100	EPA (2015)	day
Global degradation rate in sediments	0.19	EPA (2015)	0.10	EPA (2015)	day^{-1}
Global degradation rate in water	3*10^{-5}	EPA (2015)	6.6*10^{-5}	EPA (2015)	day^{-1}

2.4. Results

Model gives as an output the predicted environmental concentration (PEC) for every stretch. Since the monitoring data exist for 1 measurement (monitoring campaign conducted in 2011 on 1 day in September), we only compare the PEC with this measurement (also called MEC for measured environmental concentration). Deterministic simulations were run to predict the concentrations of PFOS and PFOA in river water (Figure 3), fish (Figure 4) and sediments (Figure 5).

PEC values for PFOA are in the same order of magnitude as the MECs in river water for two stretches (MEC for Oca is 1×10^{-3} mg/m^3 and for Miranda del Ebro 3.2×10^{-3} mg/m^3). Results of the deterministic simulation are in the same order of magnitude as the measured results (MECs) for the 2 river stretches: Miranda del Ebro and Oca while the results for Tudela were higher than the measured results (MEC=4×10^{-6} mg/m^3). In the case of the PFOS PECs are in the same order of magnitude with MECs for Oca and Miranda del Ebro while the modeling results for Tudela are a bit less correlated with the measured values.

Deterministic simulation in fish and sediments shows that results are in the same order of magnitude as the measured results in all river stretches.

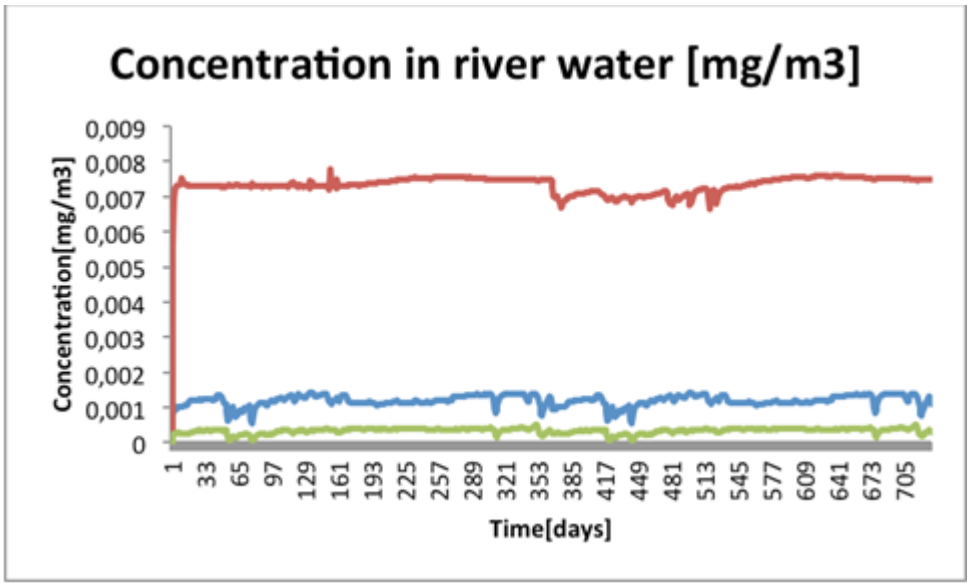

Figure 3: Deterministic simulation of the environmental scenario to predict the concentrations of PFOA in river water for three stretches (Miranda in blue, Oca in red, and Tuleda in green)

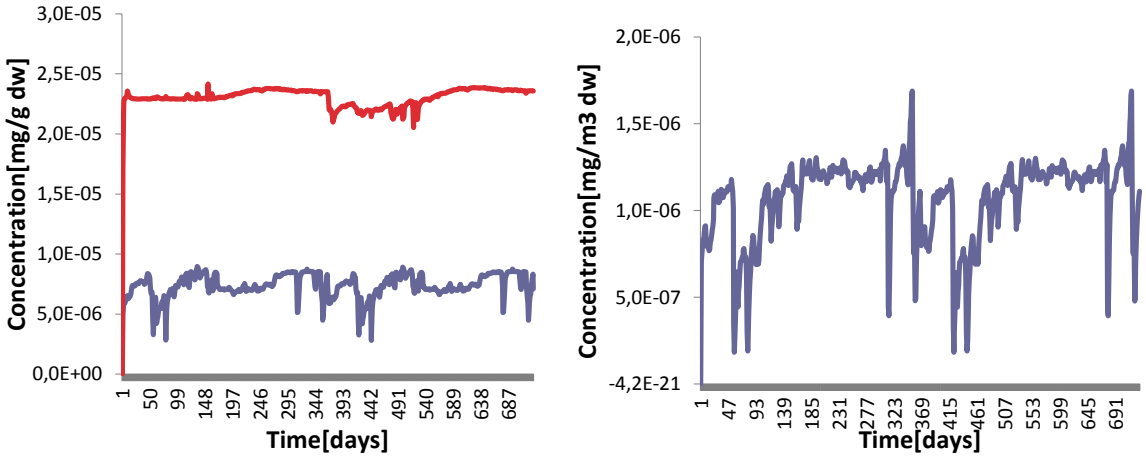

Figure 4: Prediction of the concentrations of PFOA in fish (Cyprinus Carpio and Barbus Graellsii) obtained from deterministic simulations. Left: in Barbus Graellsii for the stretches Miranda (blue) and Oca (red). Right: in Cyprinus carpio for the stretch Tudela (blue).

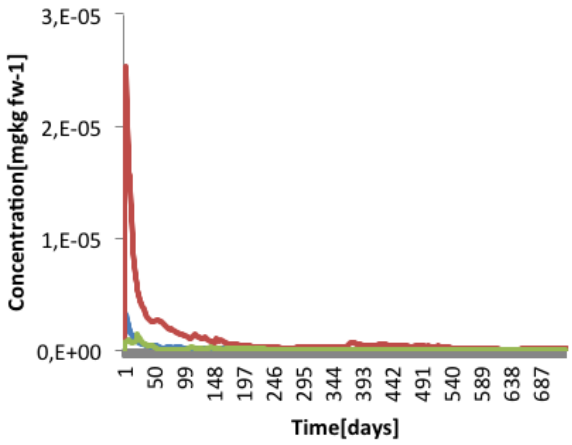

Figure 5: Predicted concentrations of PFOA in sediments for the stretches Miranda (blue), Oca (red) and Tudela (green) from deterministic simulation.

Predicting the environmental fate and occurrence of PFOA and PFOS is a very difficult task taking into account their behaviour in river bed which is a result of their physico-chemical properties. Unlike the situation with most other hydrocarbons, hydrophobic and hydrophilic interactions are not the primary partitioning mechanisms, but electrostatic interactions may be more important. It has been suggested that PFOS adsorbs via chemisorption (Hekster *et al.* 2002). A soil adsorption/desorption study using various soil, sediment and sludge matrices found that PFOS adsorbed to all matrices tested (USEPA OPPT AR226-1107). River sediments displayed the most desorption, at 39% after 48 hours, whereas sludge samples did not desorb detectable amounts of PFOS. If PFOS does bind to particulate matter in the water column, then it may settle and reside in sediment. However, as noted, desorption may also occur. Figure 5 shows the concentrations of PFOA in sediments where it is obvious that after just a couple of days sediment concentration drops drastically. However, the peak at the starting time followed by a long decay is not easily explainable.

On the other hand, we are facing a continuous phenomenon driven by the river flow, not an accidental spill or discharge that takes place at a certain moment and then decays slowly on time as the figure suggests. Time profile should be quite steady (with some random fluctuations) as in the case of water or fish concentrations decrease significantly.

Monte Carlo probabilistic simulations were performed for a period of 1 year in order to avoid overestimation of the predicted concentrations. Model was set up to perform 1000 runs for a probabilistic simulation of all model parameters that have defined pdf (probability density function) were selected as the output. The results are presented in Figure 6, Figure 7, Figure 8, Figure 9, Figure 10 and Figure 11.

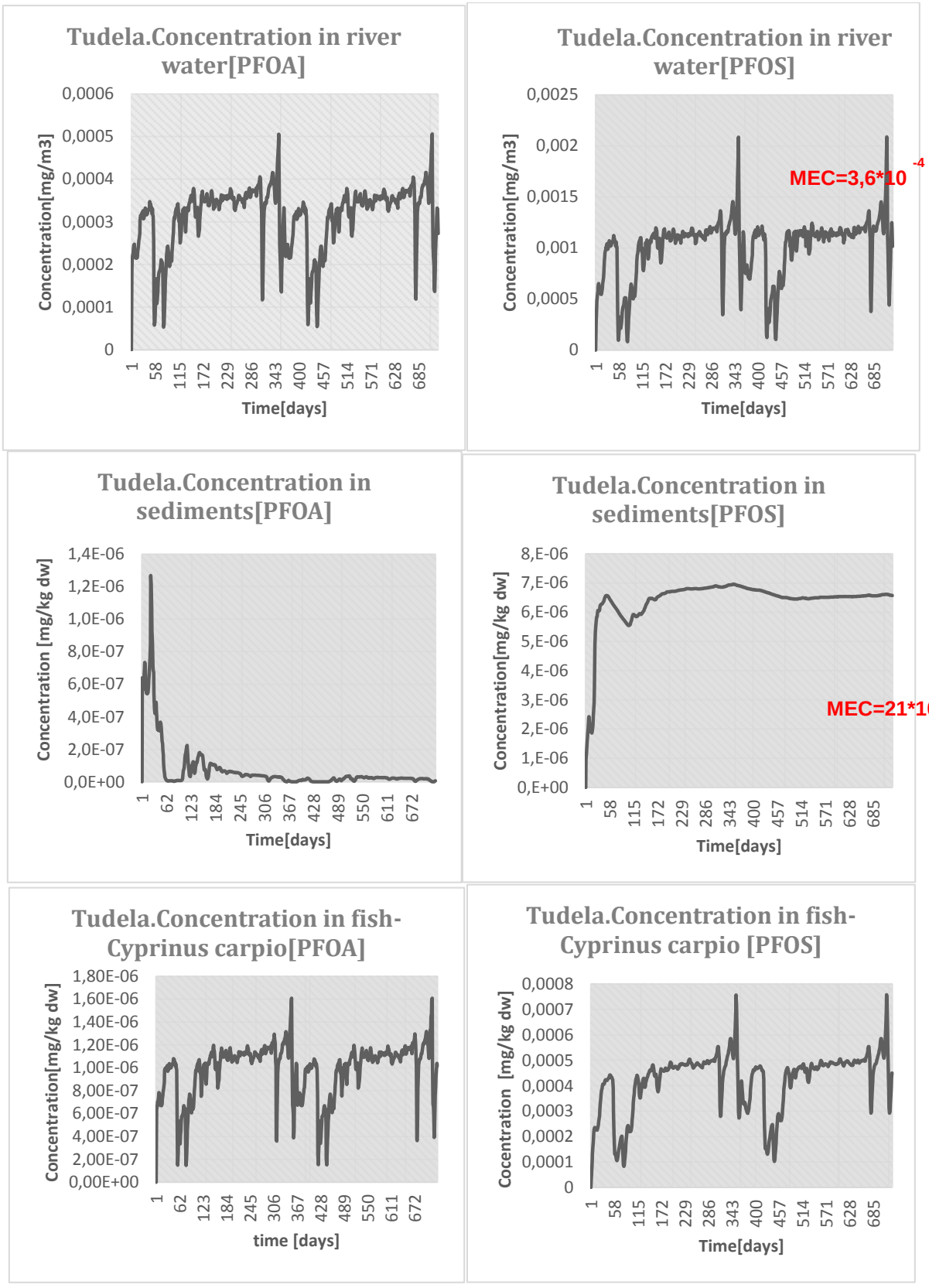

Figure 6: Predicted concentrations of PFOA and PFOS for the stretch Tudela from probabilistic simulations ((only the mean is represented)

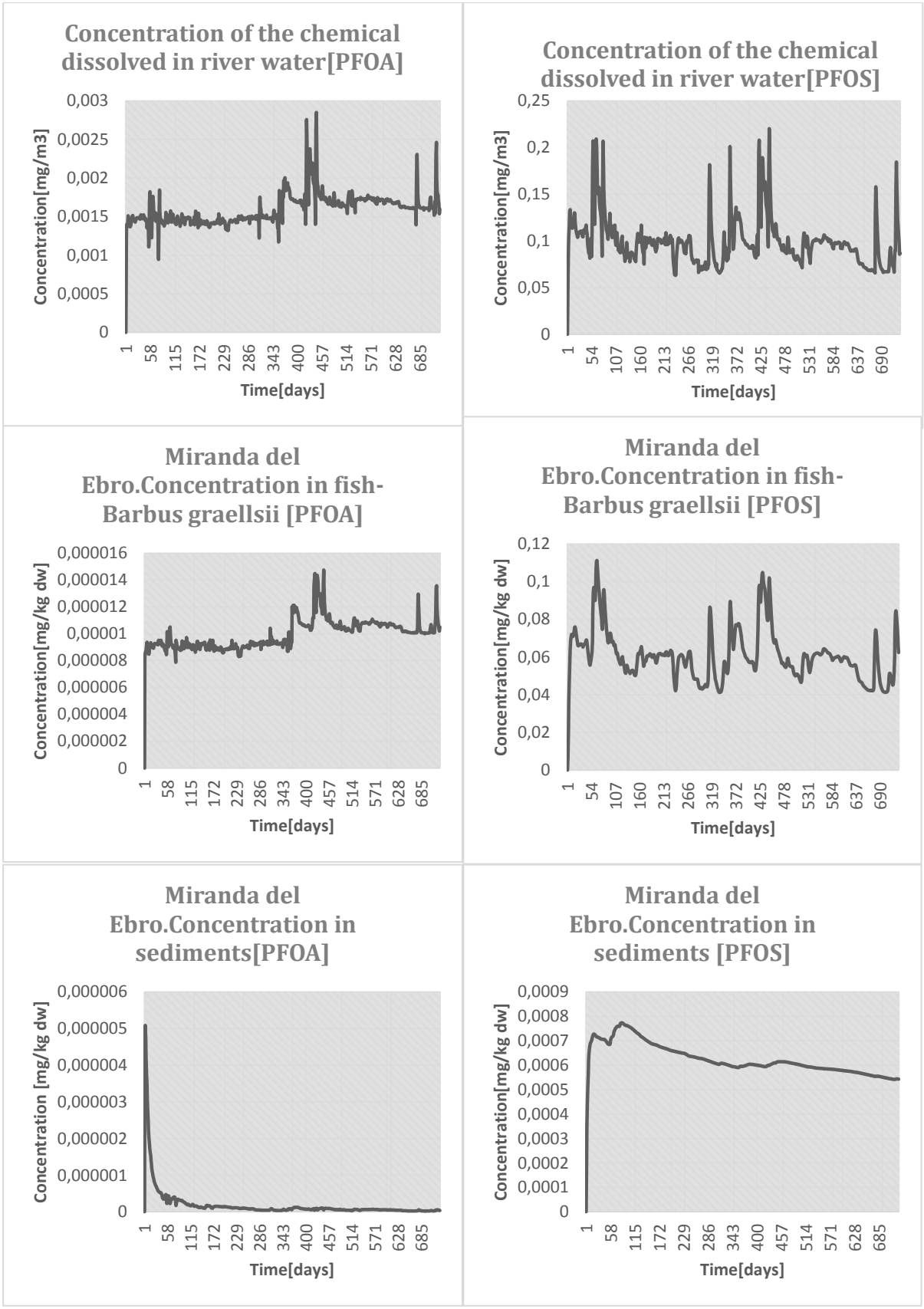

Figure 7: Predicted concentrations of PFOA and PFOS for the stretch Miranda del Ebro from probabilistic simulations (only the mean is represented)

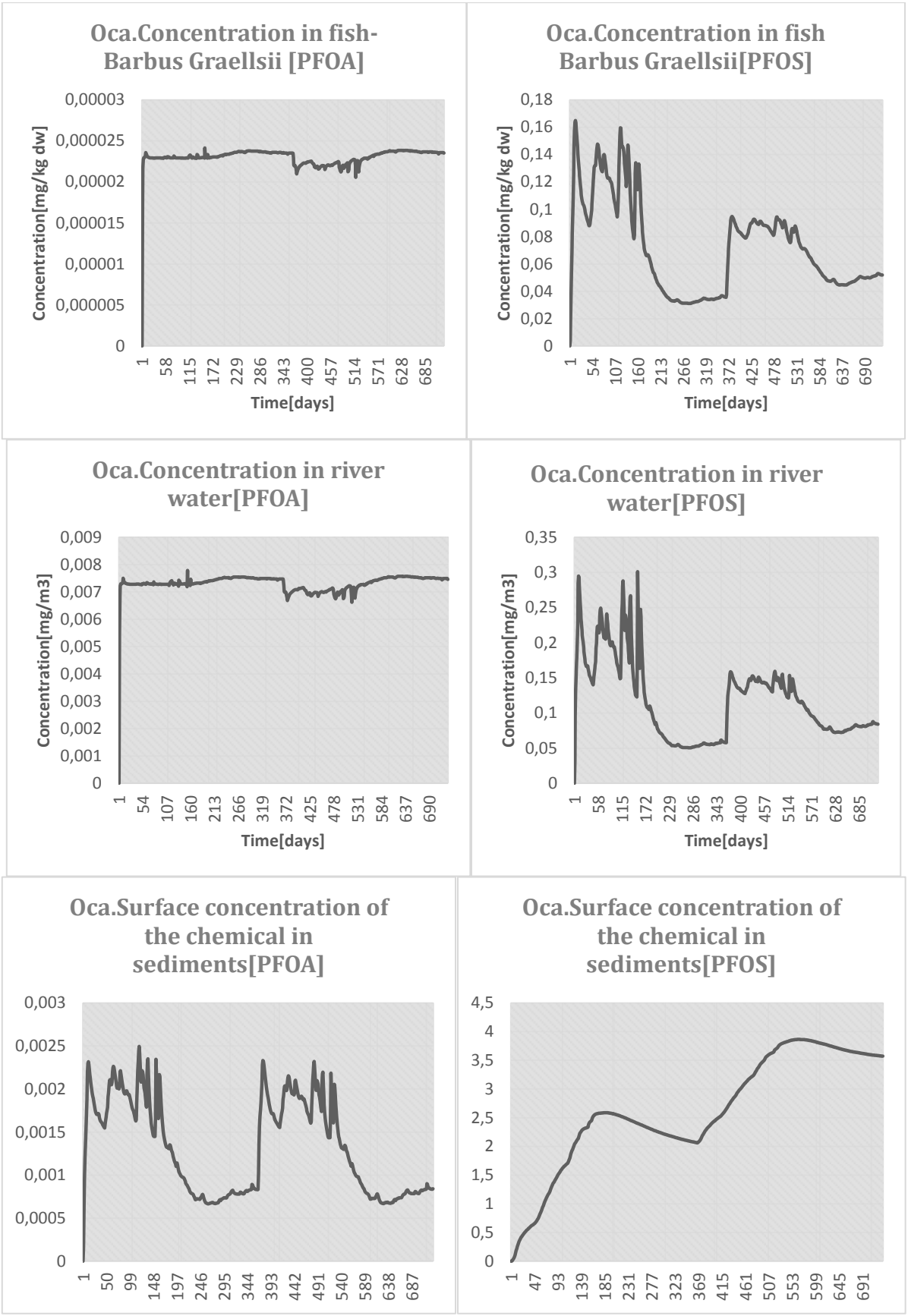

Figure 8: Predicted concentrations of PFOA and PFOS for the stretch Oca from probabilistic simulations (only the mean is represented)

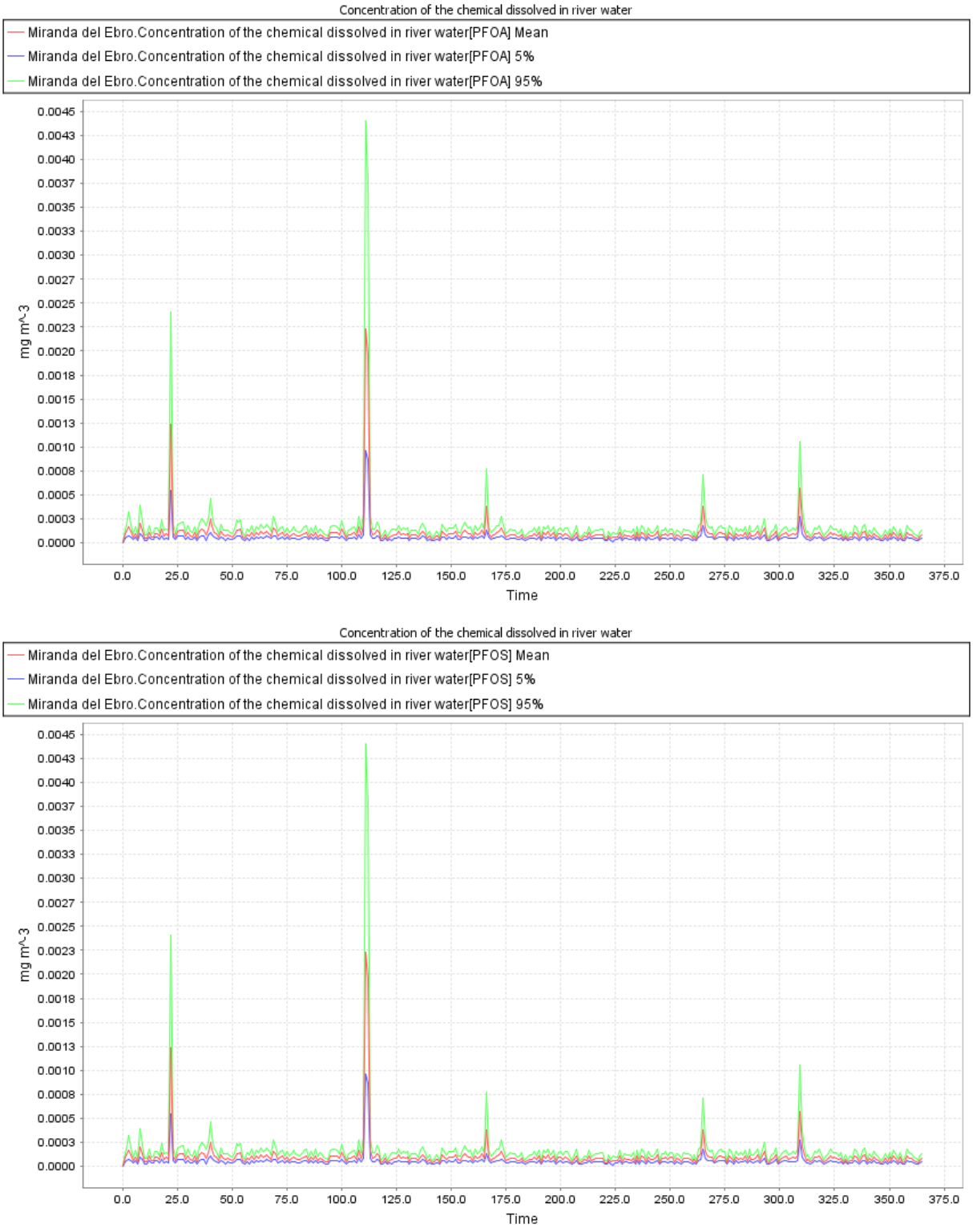

Figure 9: Confidence intervals for the concentration of the chemical(PFOA and PFOS) in dissolved river water, Miranda del Ebro

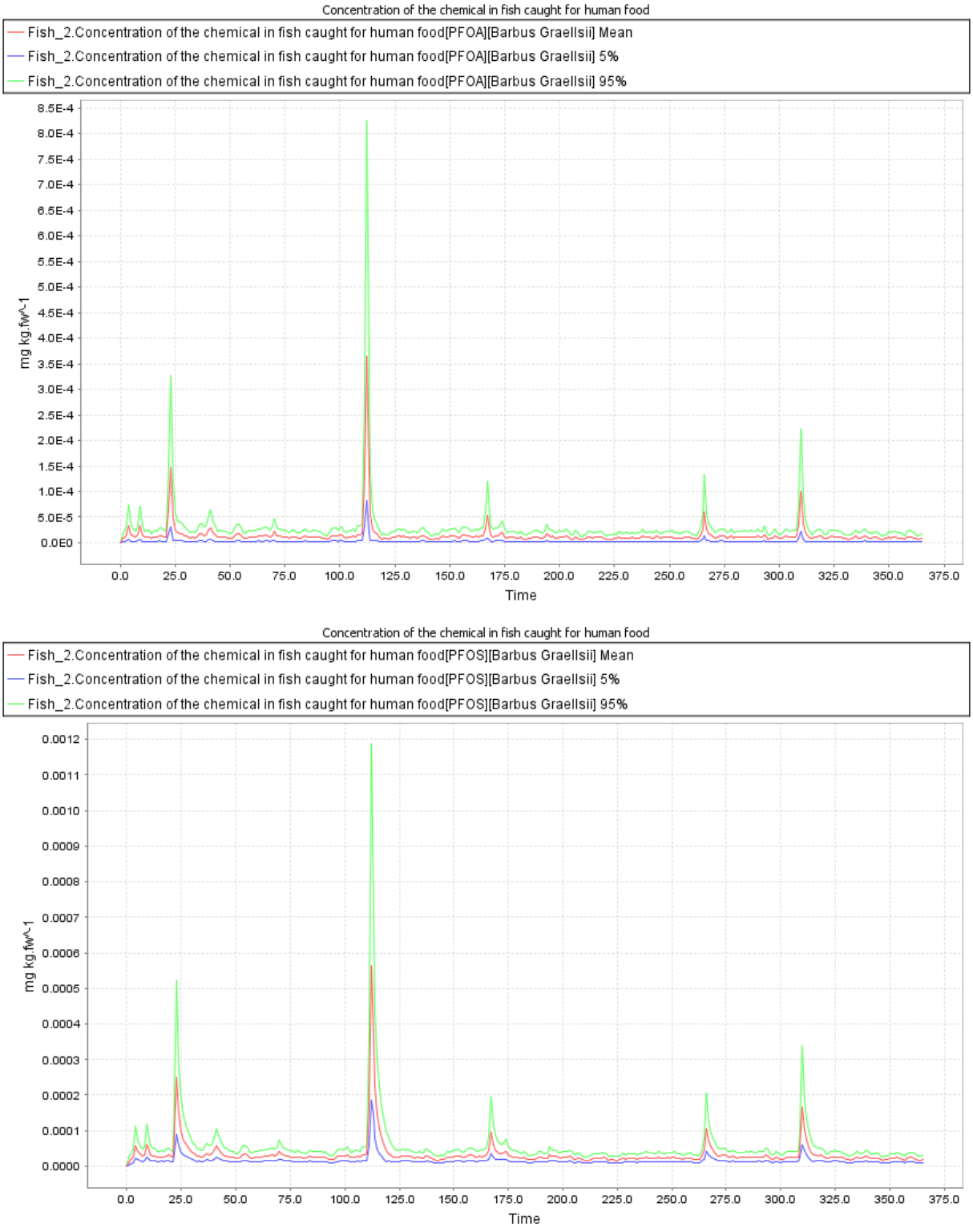

Figure 10: Confidence intervals for the contaminants (PFOA and PFOS) in fish, Miranda del Ebro

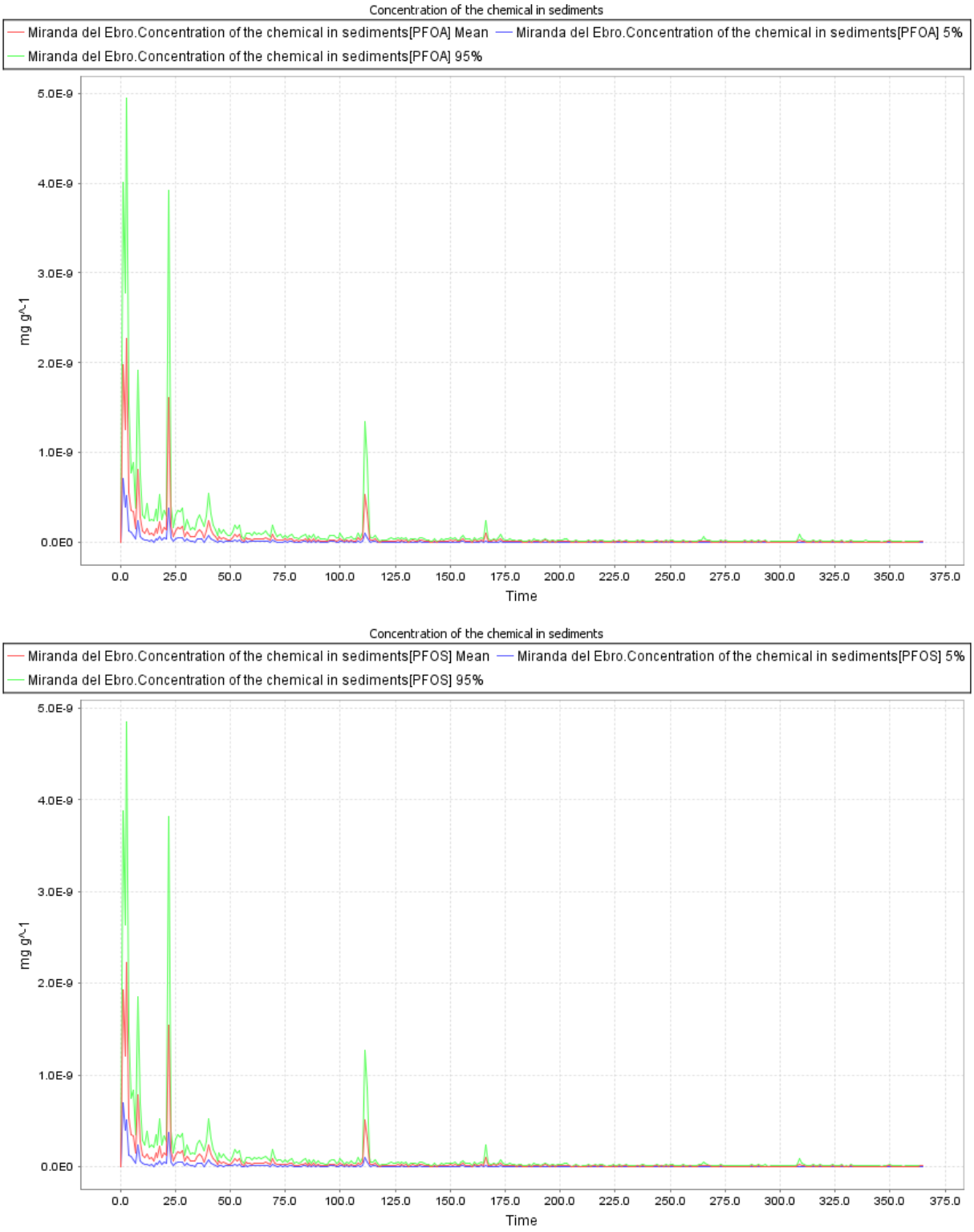

Figure 11: Confidence intervals for the contaminants (PFOA and PFOS) in sediments, Miranda del Ebro

Tornado charts for the probabilistic simulation show the percentage of how much a certain parameter influences the entire process of modeling. In this case the first order sensitivity analysis was conducted. In the case of fish its lipid layer and the river bed volume (length and width) are the most abundant parameters. While in the case of sediments the river dimensions and SPM (suspended particulate matter) were the most sensitive parameters (Figure 12 and Figure 13).

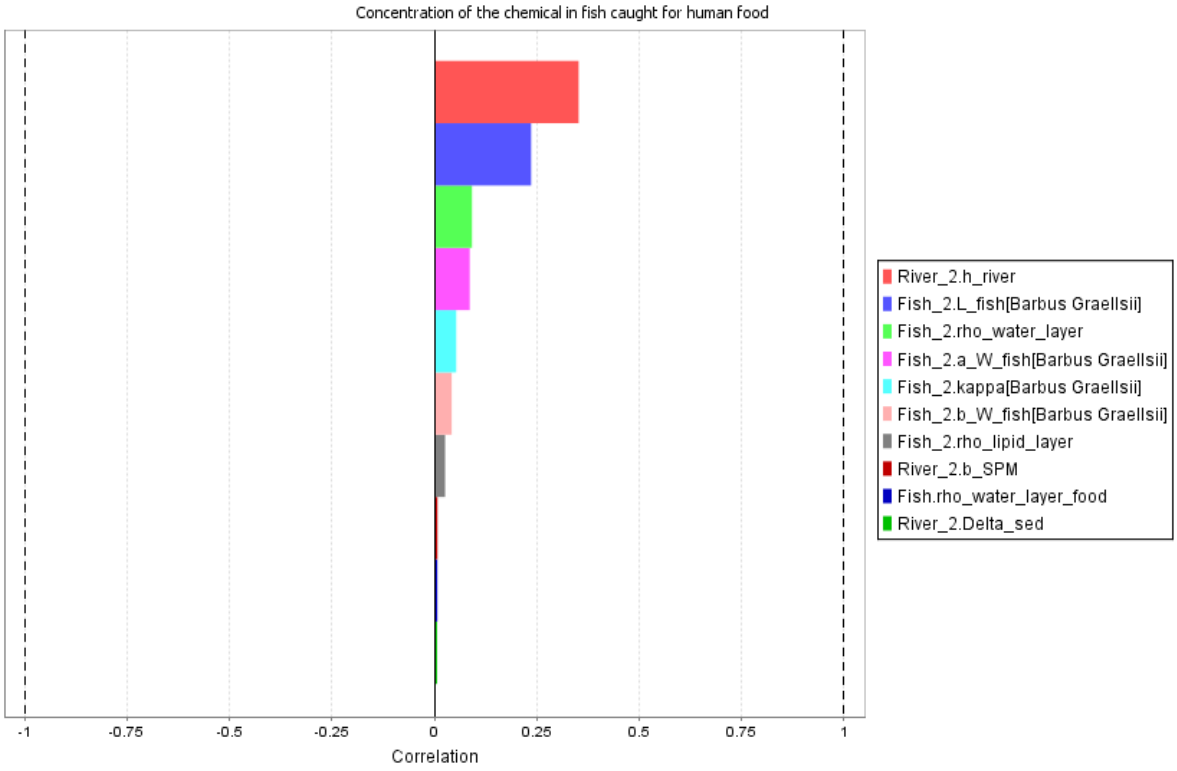

Figure 12: First order sensitivity analysis of modeling parameters-fish

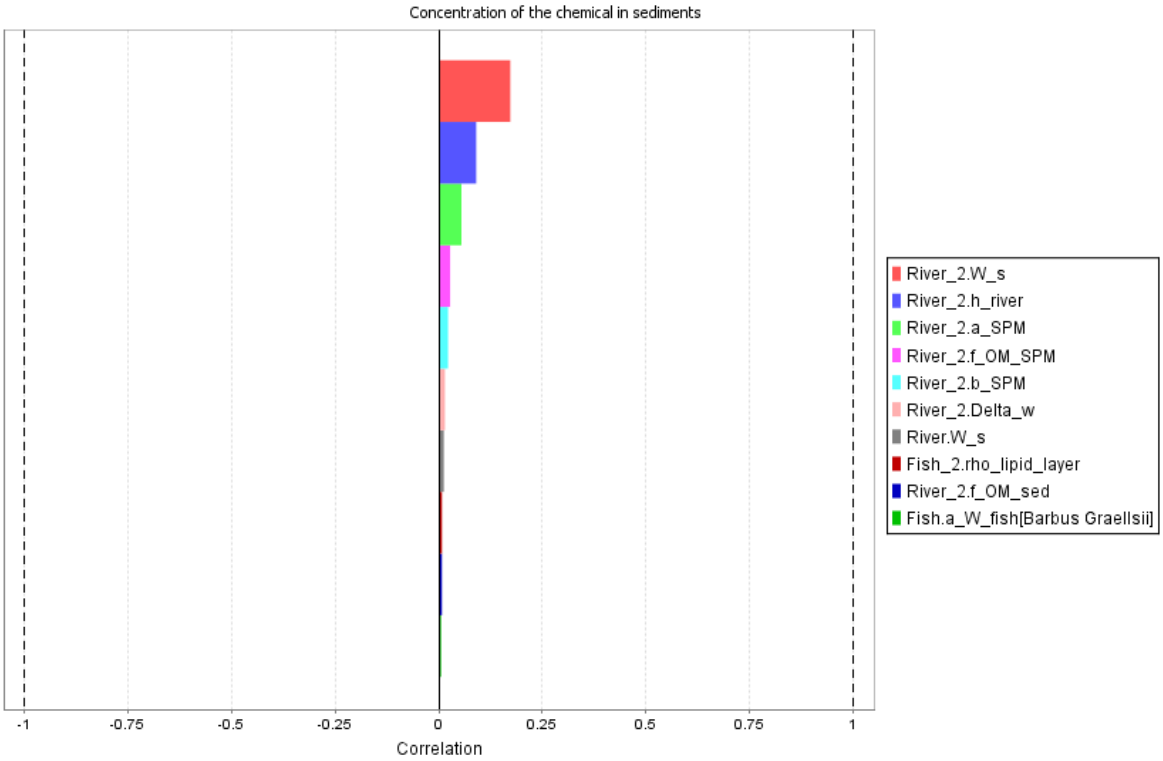

Figure 13: First order sensitivity analysis of modeling parameters-sediments

The same analysis for the water departments shows that the River length is the most important parameters that influence the concentrations of both chemicals (PFOA and PFOS) in river water (as shown in Figure 14).

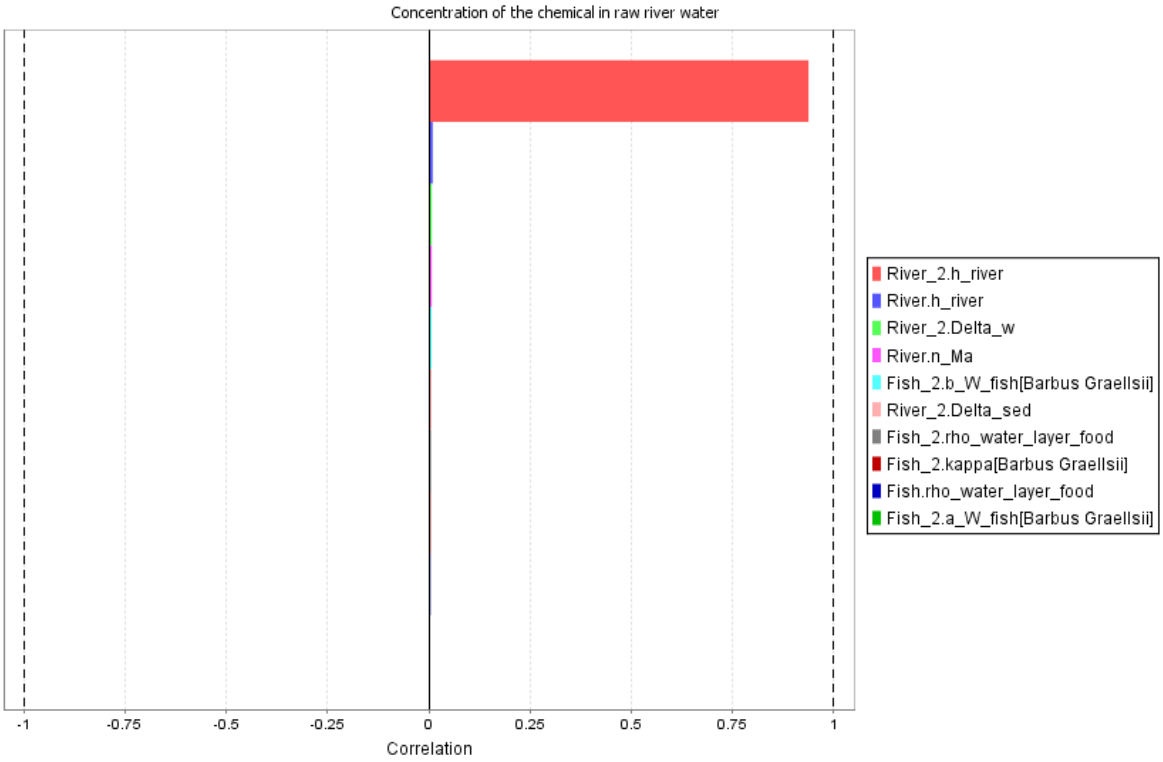

Figure 14: Parameters' sensitivity analysis in the river water

The results obtained from the MERLIN-Expo tool show that predicted concentrations are in the same order of magnitude with the measured results. Therefore, it is possible to use the tool in the exposure assessment of the emerging contaminants such as PFOA and PFOS in the surface water media and in fish. Since the models are usually tested with the neutral chemicals here we have the example of the modeling of acids in the river, sediments and fish compartments. Therefore MERLIN-Expo is a tool that is capable of providing the exposure assessment of acidic compounds. The tool could be used in the higher tiers of the risk assessment process by estimating whether the regulatory thresholds were exceeded (e.g. EQS, PNEC). Since for the PFOS these environmental thresholds in river water, sediments and biota are still not established this case study gives an insight of the fate and occurrence of this pollutant in surface waters which might contribute to the policy making for this contaminant in future.

3 The human related scenario

3.1 Objectives

Perfluorinated compounds (PFC) are a group of fluorinated chemicals with surface-active properties, which have been manufactured for over 50 years. They have been widely used in consumer products. Due to their extensive applications, PFC have been released to the environment, where they persist and may bioaccumulate through the food chain (Houde et al. 2011). In recent years, a number of studies have reported an ubiquitous distribution of PFCs in human tissues (Sturm & Ahrens 2010). Recent investigations have shown that food intake and packaging (Jogsten et al. 2009; Pico et al. 2011; Tittlemier et al. 2007), water (Ericson et al. 2009; Wilhelm et al. 2010; Zhang et al. 2011), house dust, and indoor air (Cornelis et al. 2012; Jogsten et al. 2012; Shoeib et al. 2011) are all potentially significant sources. Among these sources, water consumption has been identified as one of the most important routes of human exposure (Ericson et al. 2009; Ericson et al. 2008b; Post et al. 2009; Thompson et al. 2011). However, dietary intake is probably the main route of exposure to PFCs, including perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) (Domingo 2012; Fromme et al. 2009; Karrman et al. 2009; Noorlander et al. 2011). Among the different foodstuffs, fish seems to contribute the most to the dietary PFC exposure (Berger et al. 2009; Ericson et al. 2008a; Haug et al. 2010; Noorlander et al. 2011).

In this case study, we propose to estimate the external and internal exposure of the local human population in Catalonia. In the following, we present the construction of the exposure scenario, the parameterization of the PBPK model and its evaluation, the coupling of the environmental exposure and the PBPK model to simulate the internal exposure in the population, and the identification of the key determinants of the internal exposure.

3.2 The conceptual model

The purpose of this case study is to estimate the concentrations of PFOA and PFOS in different tissues of individuals living in Catalonia using environmental data. The exposure to PFOA and PFOS was assumed to occur only via drinking water and food.

The first step was to build the conceptual model in MERLIN-Expo. In this study, two modules of the MERLIN-Expo library were used: “Human intake” and “Man” (Figure 15). The “Human intake” module is used to combine the different sources of exposure in order to calculate a total daily intake. The “Man” model is a toxicokinetic model (a physiologically based pharmacokinetic model) used to predict the chemicals concentrations in the human tissues.

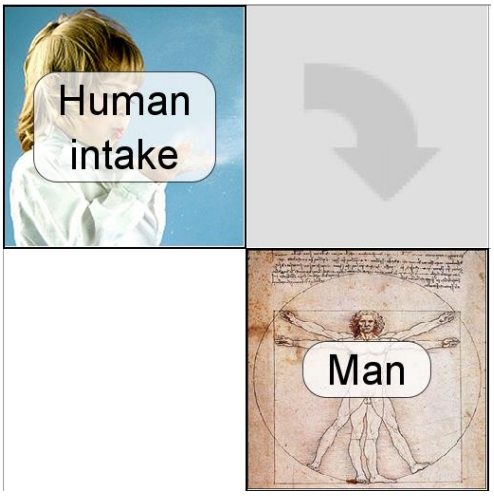

Figure 15: The conceptual model for the human oriented scenario for case study 3

3.3 Environmental human exposure to PFOA and PFOS in Catalonia

Food contamination data were combined with food consumption studies to calculate the daily intake of PFOS and PFOA for the Spanish population as a function of age. Table 1 and Table 2 present the PFOA and PFOS levels in food and drinking water that were used in this study. Several studies have reported concentrations of PFOS and PFOA in the different food groups. We first selected the study of Domingo et al (2012b) but observed that some of the measured concentrations were rather high compared to other studies in Spain, other European countries or Canada. For example, Domigo et al (2012b) measured a concentration in fish (2.6 ng/g fw) that is 40 times higher than the one measured in other studies (Table 3). Several studies were then used.

Table 4 gives the different levels of consumption of food in Spain as a function of age according to the European Food Safety Authority (EFSA 2011). These consumption rates are in good agreement with local data of the ENCAT study (Serra-Majem L et al. 2003).

Table 1: PFOA levels in drinking water and food

Sources of exposure	Levels of PFOA	Reference
Drinking water	0.0024 ng/g	Domingo et al. 2012a
Food		
Meat and meat products	0.026 ng/g fw	Haug et al. 2011
Fish and seafood	0.044 ng/g fw	Haug et al. 2011
Vegetables	0.027 ng/g fw	Ericson et al. 2008a
Tubers	0.0091 ng/g fw	Haug et al. 2011
Fruits	0.036 ng/g fw	Ericson et al. 2008a
Eggs	0.055 ng/g fw	Ericson et al. 2008a
Milk and dairy products	0.005 ng/g fw	Haug et al. 2011
Pulses	0.045 ng/g fw	Ericson et al. 2008a
Oils	0.14 ng/g fw	Domingo et al. 2012b

Table 2: PFOS levels in drinking water and food

Sources of exposure	Levels of PFOS	Reference
Drinking water	0.00108 ng/g	Domingo et al. 2012b
Food		
Meat and meat products	0.034 ng/g fw	Domingo et al. 2012b
Fish and seafood	2.7 ng/g fw	Ericson et al. 2008a
Vegetables	0.1 ng/g fw	Ericson et al. 2008a
Tubers	0.005 ng/g fw	Haug et al. 2011
Fruits	0.005 ng/g fw	Ericson et al. 2008a
Eggs	0.0053 ng/g fw	Haug et al. 2011
Milk and dairy products	0.154 ng/g fw	Ericson et al. 2008a
Pulses	0.0017 ng/g fw	Ericson et al. 2008a
Oils	0.0011 ng/g fw	Domingo et al. 2012b

Table 3: Comparison of the PFOA levels measured in several food groups from different studies

Concentration (ng/g fw)	Ericson et al (2008a) Spain	Domingo et al (2012b) Spain	Domingo et al (2012a) Spain	Haug et al (2010) Norway	Tittlemier et al (2007) Canada	RIVM (2009) Netherlands
Meat and meat products	< 0.225	< 0.3		0.026	< 0.975	0.008
Fish and seafood	< 0.352	2.6	0.074	0.04	< 1.00	0.077
Vegetables	< 0.027	0.37		0.004		0.005
Fruits	< 0.036	< 0.36				0.005
Eggs	< 0.055	< 0.36		0.03		< 0.032
Milk and dairy products	0.08	0.29		0.005		0.001
Cereals	< 0.08	< 0.12				
Pulses	< 0.045	< 0.26				
Tubers		< 0.36		0.0091		
Oils	< 0.247	< 0.14				0.004
Water		0.0032	0.0024	0.0035		

Table 4: Food consumption in Spain (kg/day) reported by EFSA (2011)

Food Consumption in Spain (kg/day)	Toddlers 0 – 3 y	Children 4 – 12 y	Adolescents 13 – 18 y	Adults >18 y
Pulse	0.0873	0.1966	0.2614	0.1831
Vegetables	0.0696	0.0641	0.0915	0.2077
Tubers	0.0431	0.043	0.0508	0.0566
Fruits	0.0794	0.1037	0.1014	0.1602
Meat and meat products	0.0766	0.1125	0.1527	0.1447
Fish and seafood	0.0263	0.0365	0.0441	0.0645
Milk and dairy products	0.5196	0.4873	0.4559	0.3492
Eggs	0	0	0	0.0244
Oils	0.0099	0.0188	0.0259	0.0356
Drinking water	0.3285	0.627	0.915	0.7975

The concentration in the different food groups and the consumption rates were used as input of the human intake module of MERLIN-Expo. Figure 16 and Table 5 present the total quantity ingested of PFOA and PFOS. As an example, the total daily intakes for PFOS and PFOA were predicted respectively at 216 ng/day and 35 ng/day for adults.

We compared the predicted intakes with other studies. We found 11 publications from different countries and different age groups. In general, the daily intake for PFOA and PFOS

vary between 7 and 700 ng/d with an average of 166 ng/d for PFOA and 167.4 ng/d for PFOS (Table 10).

Table 5: Total daily intake in ng/day for PFOA and PFOS in function of the age

Compounds	Toddlers 0 – 3 years	Children 4 – 12 years	Adolescents 13 – 18 years	Adults >18 years
PFOA	17	26	31	35
PFOS	122	146	165	216

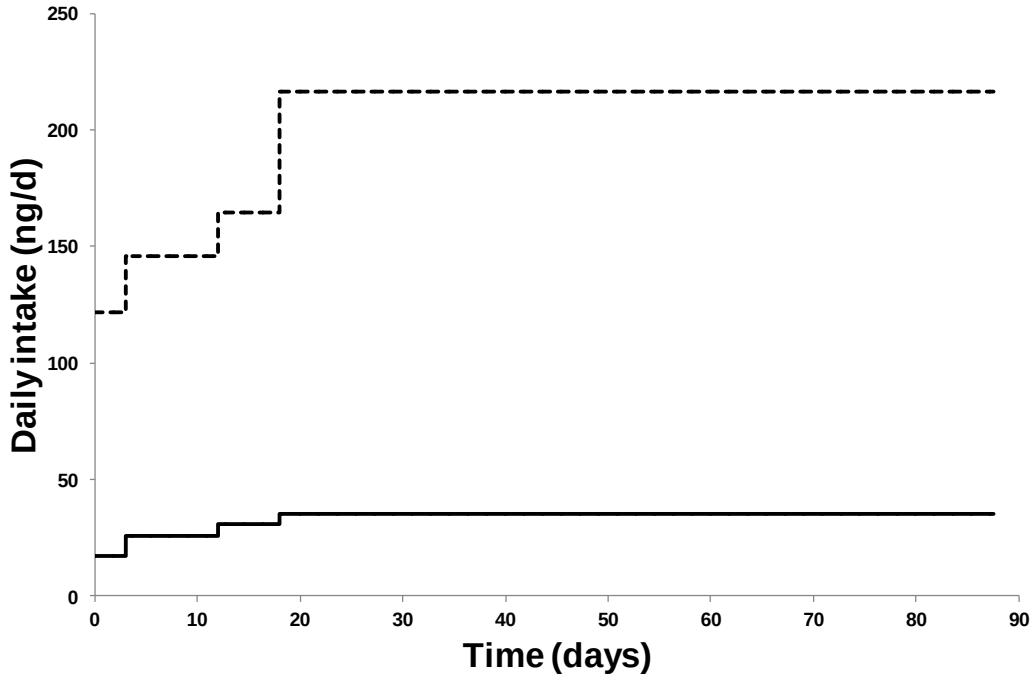

Figure 16: Total amount ingested over time to PFOA (black line) and PFOS (black dotted line)

Table 6: PFOA and PFOS daily intakes from different studies

Country	Year	Reference		PFOA (ng/d)	PFOS (ng/d)
Spain	2009 - 2011	MERLIN-Expo	Toddler	17	122
			Other children	26	146
			Adolescents	31	165
			Adults	35	216
Spain	2009	Ericson, et al. (2012)	Toddler	-	60
			Adult	-	80
Spain	2009	Domingo, et al. (2012a)	Toddler	8.1	-
			Adult	24	-
Spain	2011	Domingo, et al. (2012b)	Children	456	107.52
			Adolescents	313.04	92.4
			Adults	353.5	128.8
			Seniors	357.5	148.85
Germany	2009	Fromme et al. (2009)		269.4	123.4
Japan	2004	Karrman et al. (2009)		50.4	75.6
Canada	2004	Tittlemier et al. (2007)		-	250
Canada	1998	Ostertag et al (2009a)		14 – 28	7 – 14
Canada	2004	Ostertag, et al. (2009a)		7 – 28	56 – 140
Canada	1997-1998	Ostertag, et al. (2009b)		7 - 35	14 - 168
Norway	2008-2009	Haug et al. (2010)		31	18

3.4 Parameterization of the human (PBPK) model

The published PBPK model for PFOS and PFOA in humans developed by Loccisano *et al.* (2011) was selected to parameterize the PBPK model in MERLIN-Expo for the compound-specific parameters. The structure of both models slightly differs for the urinary elimination of PFOS and PFOA. Indeed, Loccisano et al. (2011) developed a PBPK model that integrates the reabsorption of the two perflurinated compounds in kidneys. Even if this process is not modeled in MERLIN-Expo, we applied our model without modifications. Chemical-specific parameters used for the human PFOA and PFOS models are presented in Table 7.

The PBPK model was benchmarked using two published datasets to compare our model predictions to experimental data. This preliminary step of our analysis aimed to assess the reliability of the parameterization of the PBPK model. First we run a scenario for the population of Little Hocking in USA. Because the exposure duration was not known, the model was run until a steady state was reached in blood as proposed by Loccisano et al. (2011). To obtain the steady state, the initial age was set to 20 years old and the model was then run for approximately 30 years. The PFOA daily intake (3.55 ppb) was converted in µg/d and we assumed a consumption of 1 liter of water per day. The intake was then 3.55 µg/d. For PFOS the intake was set to 0.34 µg/d. The model predictions were then compared to measured concentrations in serum of the Little Hocking population (Figure 17). The experimental data are reported in Annex 1. We observed that the model prediction is included in the range of the observations even it is slightly superior to the mean or median of

the measured concentrations. On the same figure, we also observed that 20 years are needed to eliminate PFOA from serum.

Table 7: Parameters of the PBPK model in MERLIN-Expo for PFOS and PFOA in humans (Loccisano et al, 20011)

Parameters	PFOA	PFOS
Quantity ingested rate (in µg/d)		
From 20 to 50 years old	3.55	0.34
From 50 years to 70 years old	0	0
Initial Age	20 years	20 years
Excretion rate per kg of BW (1/min/kg)	2.07^{-7}	6.89^{-7}
Fraction absorbed via ingestion	0.9	0.9
Partition coefficients		
Adipose	0.04	0.14
Adrenal	0.12	0.2
Blood	1	1
Blood_Arterial	1	1
Blood_Venous	1	1
Bones	0.12	0.2
Bones_NP	1	1
Brain	0.12	0.2
Breast	0.12	0.2
Gut	0.05	0.57
Gut_Lumen	1	1
Heart	0.12	0.2
Kidneys	1.05	0.8
Liver	2.2	3.72
Lungs	0.12	0.2
Marrow	0.12	0.2
Muscle	0.12	0.2
Pancreas	0.12	0.2
Sexual_Organs	0.12	0.2
Skin	0.1	0.29
Spleen	0.12	0.2
Stomach	0.12	0.2
Stomach_Lumen	1	1
Thyroid	0.12	0.2
Urinary_Tract	0.12	0.2

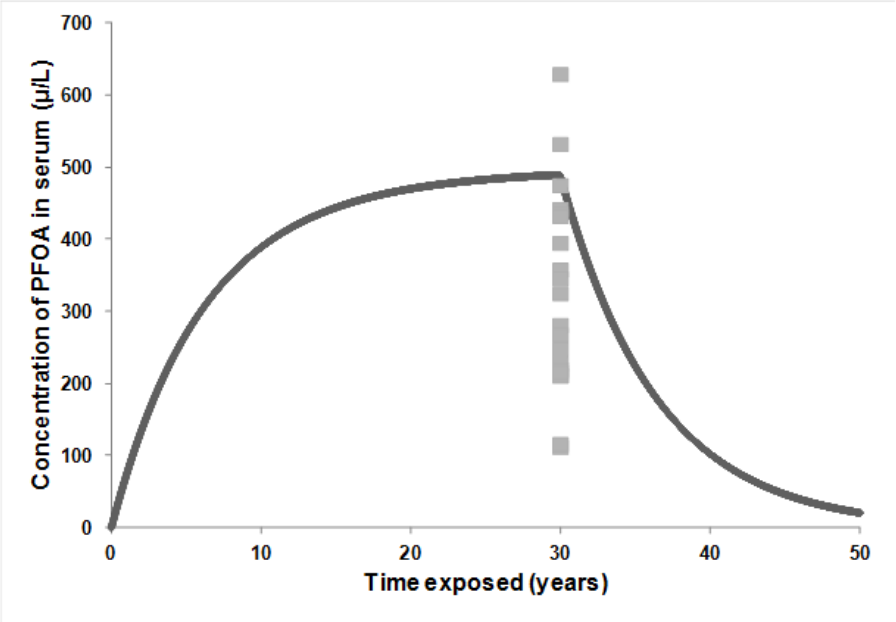

Figure 17: Comparison of model simulations (lines) from MERLIN-Expo (B) with experimental data from Emmett et al. (2006) (squares) and the Little Hocking Water Association website. The Little Hocking population was exposed to drinking water contaminated by PFOA (3.55 ppb). The simulations were run for an exposure period of 30 years.

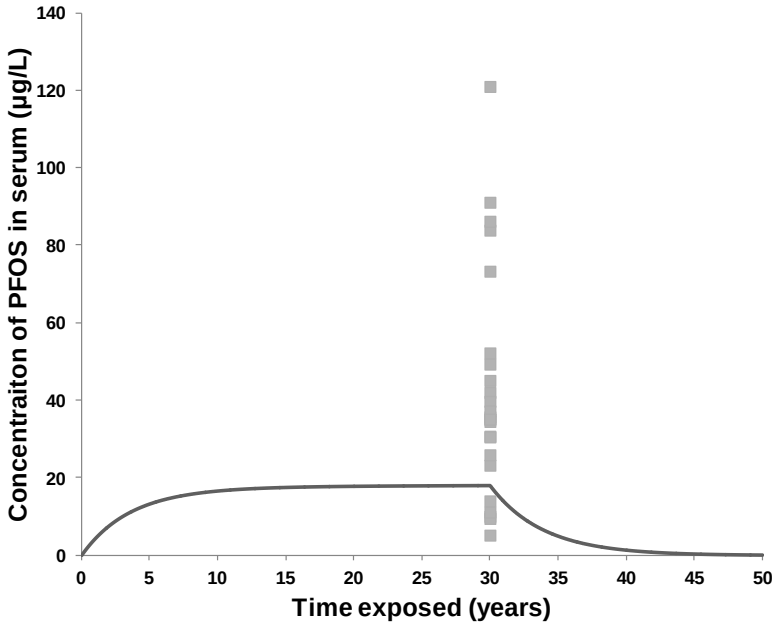

Figure 18 : Simulation of PFOS in blood with MERLIN-Expo (line). Drinking water daily intake was set to 0.34 µg/d for 30 years and no exposure afterwards. Experimental data from the Little Hocking Water Association website in 2005 are represented by grey box.

We slightly modified the values of the tissue:blood partition coefficients proposed by Loccisano et al. (2011) and used the study by Maestri et al. (2006). The values provided by Loccisano et al. (2011) were obtained in laboratory animals and the ones reported by Maestri et al. (2006) were derived using data collected in human autopsies (Table 8). We then chose to use the human data.

Table 8: Values of tissue:blood partition coefficients obtained from human autopsies (Maestri et al. 2006)

Tissues	PFOA	PFOS
Liver	1.03	2.67
Fat	0.47	0.33
Brain	0.17	0.26
Kidneys	1.17	1.26
Lungs	1.27	0.15

3.5 Running the conceptual model in MERLIN-Expo

The human related scenario was run using the human intake module and the PBPK model. We used the data collected in the study of Perez et al. (2013) that measured the PFCs concentrations in tissues obtained from autopsies of 20 individuals of Tarragona (Catalonia, Spain) and the study of Ericson et al. (2007) that measured the PFCs blood concentrations of residents in Catalonia to compare the model predictions to actual human levels in Spain. The comparison between the predictions and data are shown in Figure 19 for PFOS and Figure 20 for PFOA. Data are also reported in Appendix 2 and 3.

The predictions of the blood concentration are in good agreement with the data for both compounds. Concentrations in liver are close to the data for PFOS but are slightly under-estimated for PFOA (by a 2-factor). PFOA is detected in only one subject in kidneys, so no conclusion can be drawn, and for PFOS the predictions are under-estimated by a factor of 3. Concentrations in lungs are clearly under-estimated for both compounds. So the predictions are comprised in a 3-factor, except for lungs which value of the partition coefficient used to parameterize MERLIN-Expo is clearly under-estimated according to the experimental data.

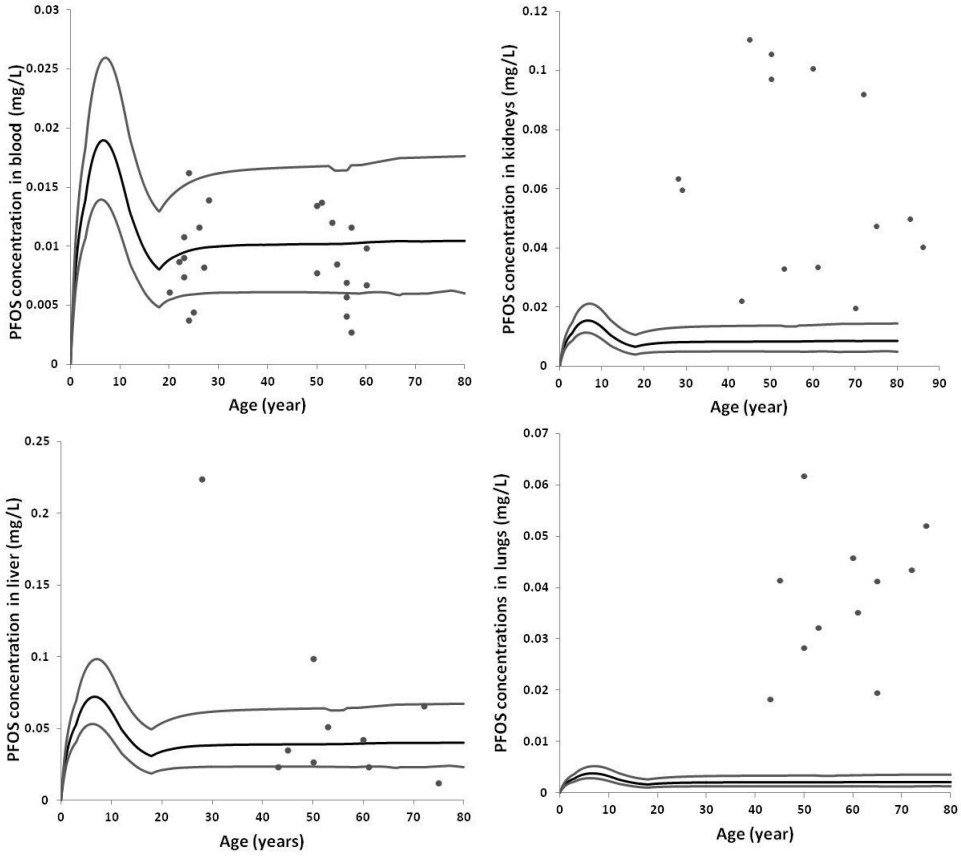

Figure 19. Simulated (lines) and measured (circles) PFOS concentrations in blood, kidneys, liver, and lungs. The two grey lines represent the 95% interval of confidence.

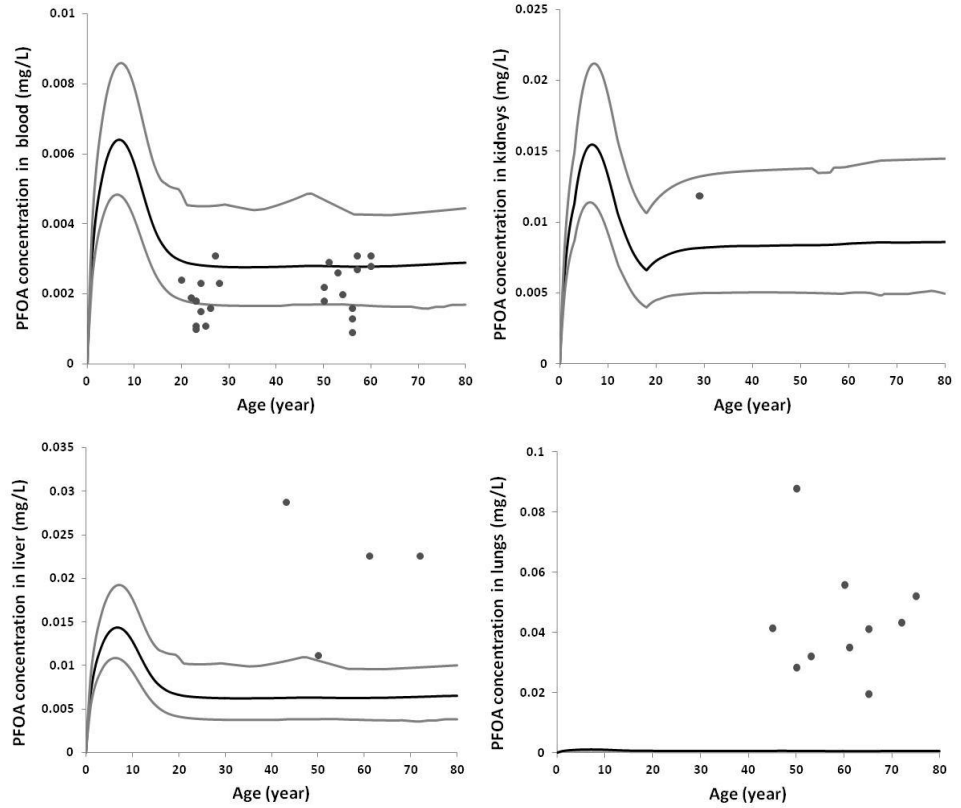

Figure 20. Simulated (lines) and measured (circles) PFOA concentrations in blood, kidneys, liver, and lungs. The two grey lines represent the 95% interval of confidence.

3.6 Sensitivity analyses

Sensitivity analysis (SA) is the study of how the uncertainty in the output of a mathematical model or system (numerical or otherwise) can be apportioned to the different sources of uncertainty in its inputs (Saltelli et al, 2008). The sensitivity of an input parameter depends on its influence on the output. A small change in a sensitive input parameter will cause a large change in the output variable. Similarly, an input parameter is considered non-influential if a high variation produces only a small change in the output variable.

SA can be used to screen the non-influential factors in the model, i.e. identify those factors that can be fixed at any given value in their domains without significantly reducing the output variance (factors fixing setting). This setting is useful for model simplification or when the user has prior beliefs about the importance of some input factors, as it can help in proving or disproving a given model representation. In other cases the objective of SA can be the reduction of the output variance to a lower threshold (variance cutting setting) by simultaneously fixing the smallest number of input factors. This setting could be of use when SA is part of risk assessment study. In the case of factor prioritization, the scope is to identify the most influent factors one by one, while in the variance cutting setting the objective is to reduce the output variance down to a pre-established level by fixing the smallest subset of factors at once.

Finally, the aim of such analyses can be to study which values of the input factors lead to model realizations in a given range of the output space, e.g. above or below an assigned threshold (factors mapping setting). For example, the user wishes to divide the realizations of the Monte Carlo simulations into two groups, e.g. by categorizing them as acceptable or non-acceptable. If SA is carried out according to good practices, it may allow (i) measuring the exposure model properly (e.g. the model is fitted to the observations) and appropriate (e.g. extrapolation of robust model); (ii) identifying critical areas in the input parameter space (e.g. what combination of settings matches the highest risk to detect interactions between input parameters); (iii) establishing research priorities and (iv) simplifying the exposure model. SA is not useful if it cannot be interpreted.

Here we propose a two-step approach to conduct SA for the scenario of human exposure to PFOS and PFOA in Catalonia. The first step was dedicated to reduce the dimensionality of the models. We proposed to use the Morris method as a screening method to identify influential and non-influential parameters. The Morris method can extract assumptions classifying inputs in three categories: (i) input parameters having negligible effects (μ^* and σ have very low values), (ii) influential input parameters, having linear effects and no interaction (μ^* is high and σ is low), (iii) input parameters with nonlinear effects and/or interactions (σ is high regardless of μ^*). This method allows identifying only the relative influence of parameters on the output variables and therefore providing a qualitative ranking of the parameters. For the rest of the analysis, only the most influential parameters will be allowed to vary within their probability distribution functions (PDFs).

The Morris method was applied to the human model whose total number of parameters is 47. All PDFs are normal distributions and are reported in the Appendix 4. The output of interest was the concentration in blood as measured in human biomonitoring studies. Figure 21 presents the results of this analysis. We observed that most of the parameters do not have a high impact on the blood concentrations of PFOS and PFOA. Only parameters having an impact on the blood concentrations (Table 9) were selected for the second step of the analysis.

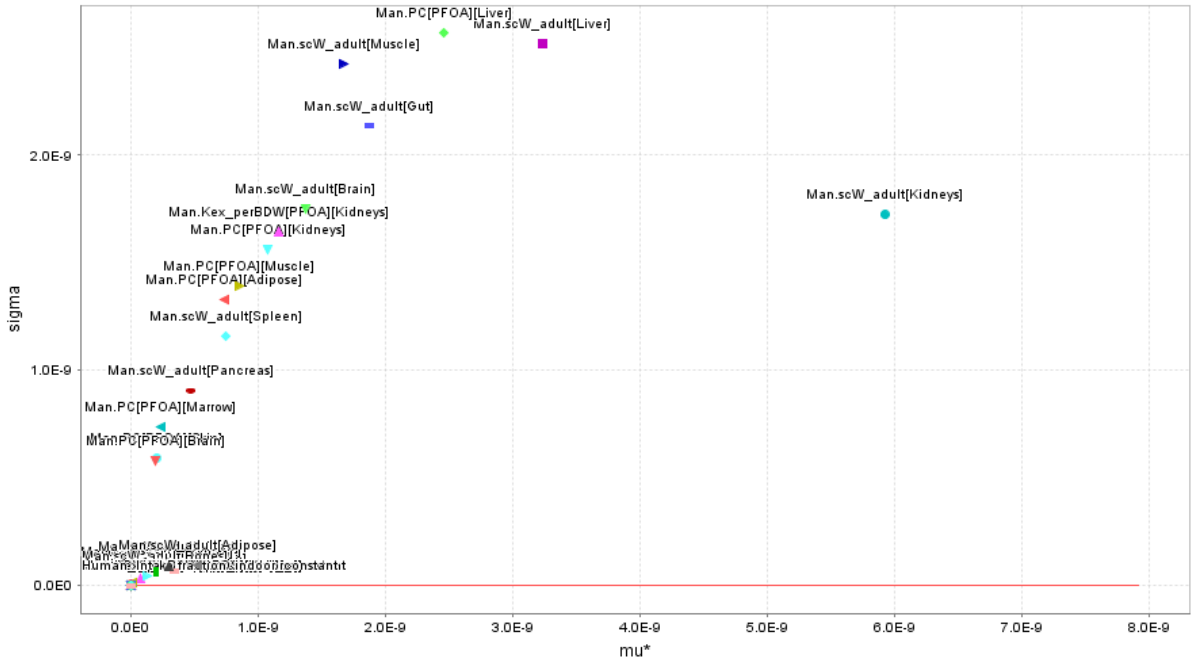

Figure 21 : Results of the Morris method for the PBPK model. The concentration in blood was the selected output

Table 9: List of sensitive PBPK model's parameters for the blood concentration selected by the Morris method

Sensitive parameters	
Partition coefficient kidneys:blood	Partition coefficient marrow:blood
Partition coefficient liver:blood	Partition coefficient muscle:blood
Partition coefficient adipose:blood	Partition coefficient brain:blood
Relative weight of kidneys	Relative weight of liver
Relative weight of muscle	Relative weight of gut
Relative weight of adipose	Relative weight of brain
Relative weight of spleen	Relative weight of pancreas

In the second step, variance-based methods e.g. EFAST and Sobol methods, were applied to quantify the influence of the input parameters on the output of interest. These global SA provide the first order effect of each input parameter on the output variation and also the total order effect. The total order effect is more representative than the first order effect since it takes into account the interactions between all the input parameters. Results for first order effect (between 0 and 1) represent the fraction of model output variance explained by the input variation of a given parameter. To estimate the total-order sensitivity index, TSi, of a given parameter, this method first calculates the summed sensitivity index of the entire complementary set of parameters using their identification frequencies. This includes higher-order, nonlinear interactions between the parameter of interest and the complementary set of parameters. For example, a model with two inputs and one output, one might find that 70% of the output variance is caused by the variance in the first input, 20% by the variance in the second, and 10% due to interactions between the two. These percentages are directly interpreted as measures of sensitivity. Variance-based measures of sensitivity are attractive because they measure sensitivity across the whole input space (i.e. it is a global method), deal with nonlinear responses, and measure the effect of interactions in non-additive systems. The main limitation of these methods is the computational efficiency. Indeed, when

the number of parameters exceeds 20, these methods are difficult to use because of the computational cost.

We then applied the EFAST method on the PBPK model to study the impact of the parameters on the PFOS and PFOA blood concentration. The parameters selected by the Morris method were the only varying parameters. The first and total orders are presented in Figure 22. We observed that the influence of the parameters varied between childhood and adulthood. The liver:blood and kidney:blood partition coefficients are the main parameters impacting the PFOS and PFOA blood concentration. The liver:blood partition coefficients is highly sensitive at birth and his impact decreases in adulthood. On the contrary, the kidney:blood partition coefficient is the most influential parameters in adulthood (orders between 0.7 and 1). We observed only one notable difference between the first and total order of the the adipose:blood partition coefficient for PFOA. These results are in agreement with the knowledge on the toxicokinetic processes that are described in the PBPK model, with a rapid absorption after ingestion and accumulation in the serum, kidney and liver (EPA, 2009).

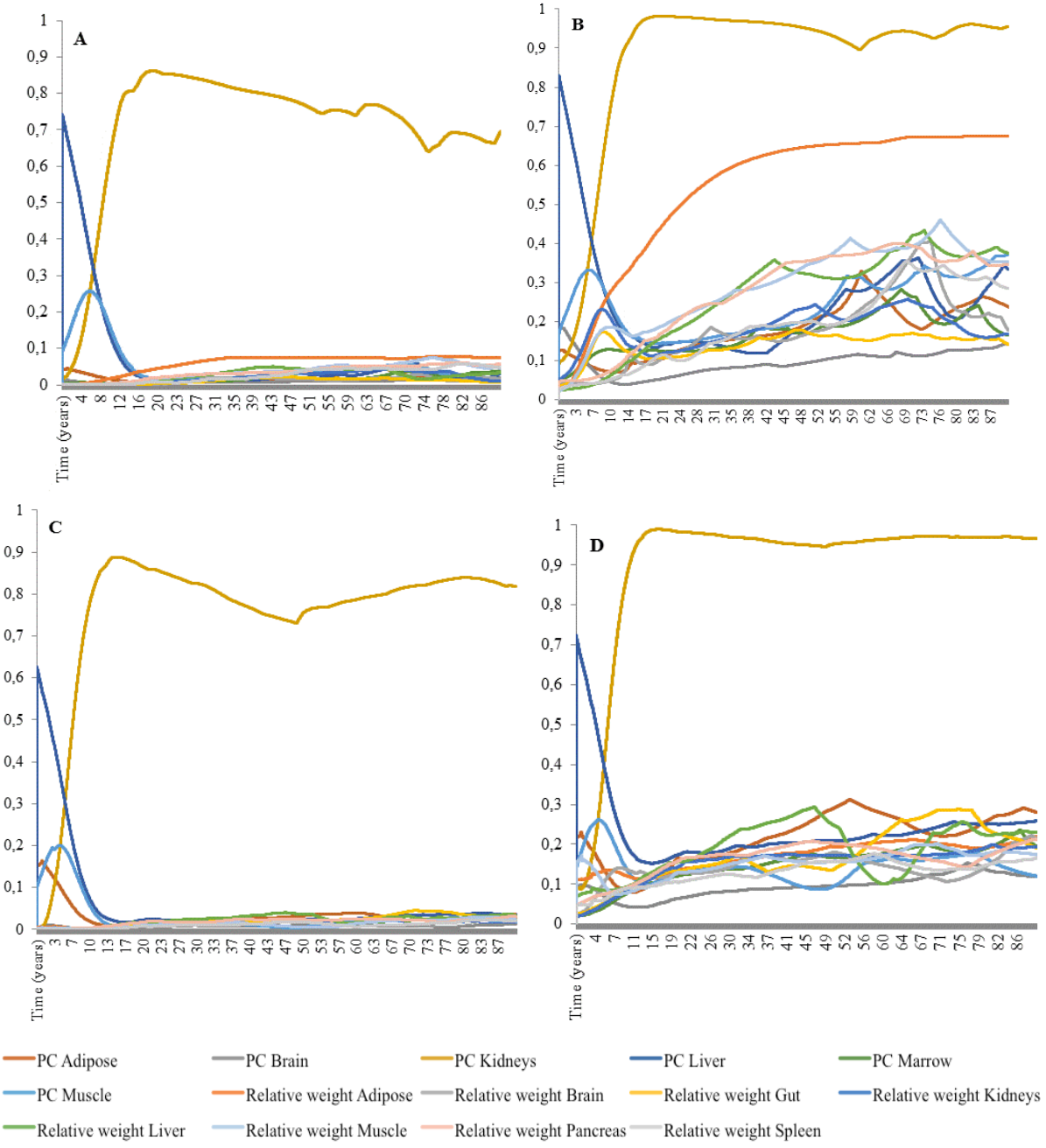

Figure 22: Results for the EFAST method for PFOA first order (A), total order (B) and for PFOS first order (C) and total order (D)

Another sensitivity analysis was performed to identify the main sources of exposure (diet and bodyweight variability) that influence the PFOA and PFOS body burden for each group of age, i.e. toddlers (0 – 3 years), other children (4 – 12 years), adolescents (13 – 18 years) and adults (> 18 years). The PDFs of the parameters related to the food consumption were obtained from EFSA (2011) and are reported in Appendix 5. Results obtained with the EFAST method are presented in Figure 23, Figure 24, Figure 25 and Figure 26.

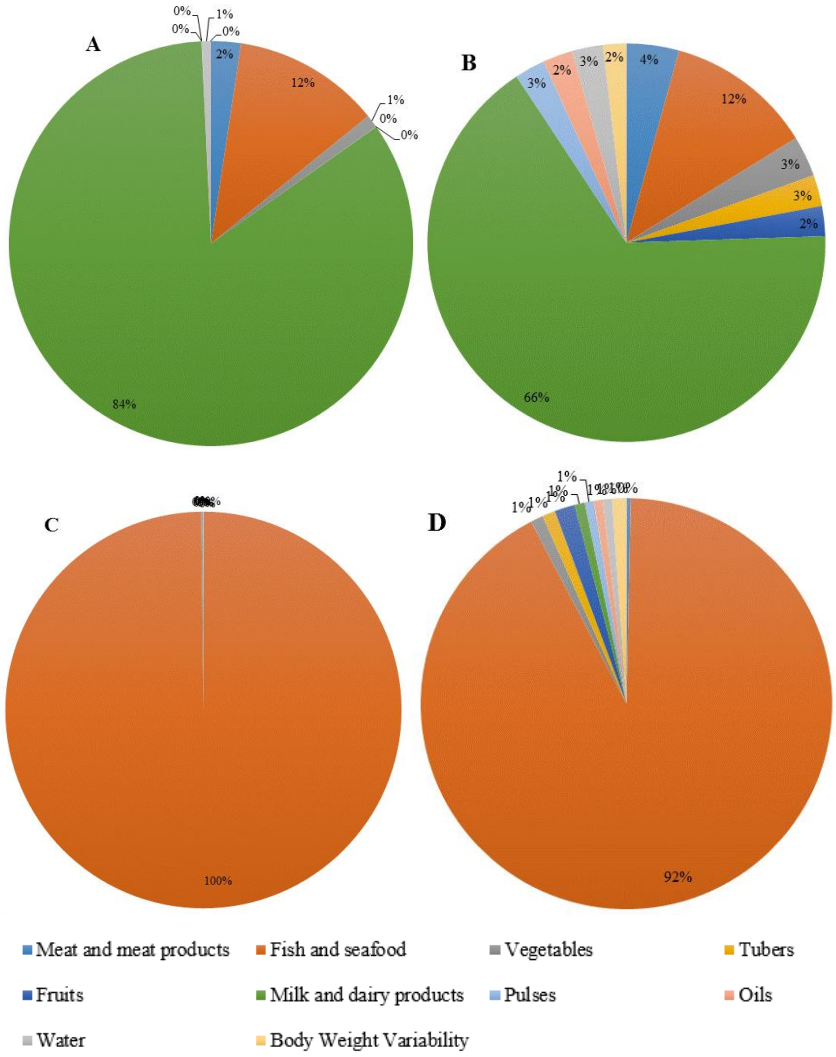

Figure 23: Results for the EFAST method for the toddlers for PFOA first order (A), total order (B) and for PFOS first order (C) and total order (D).

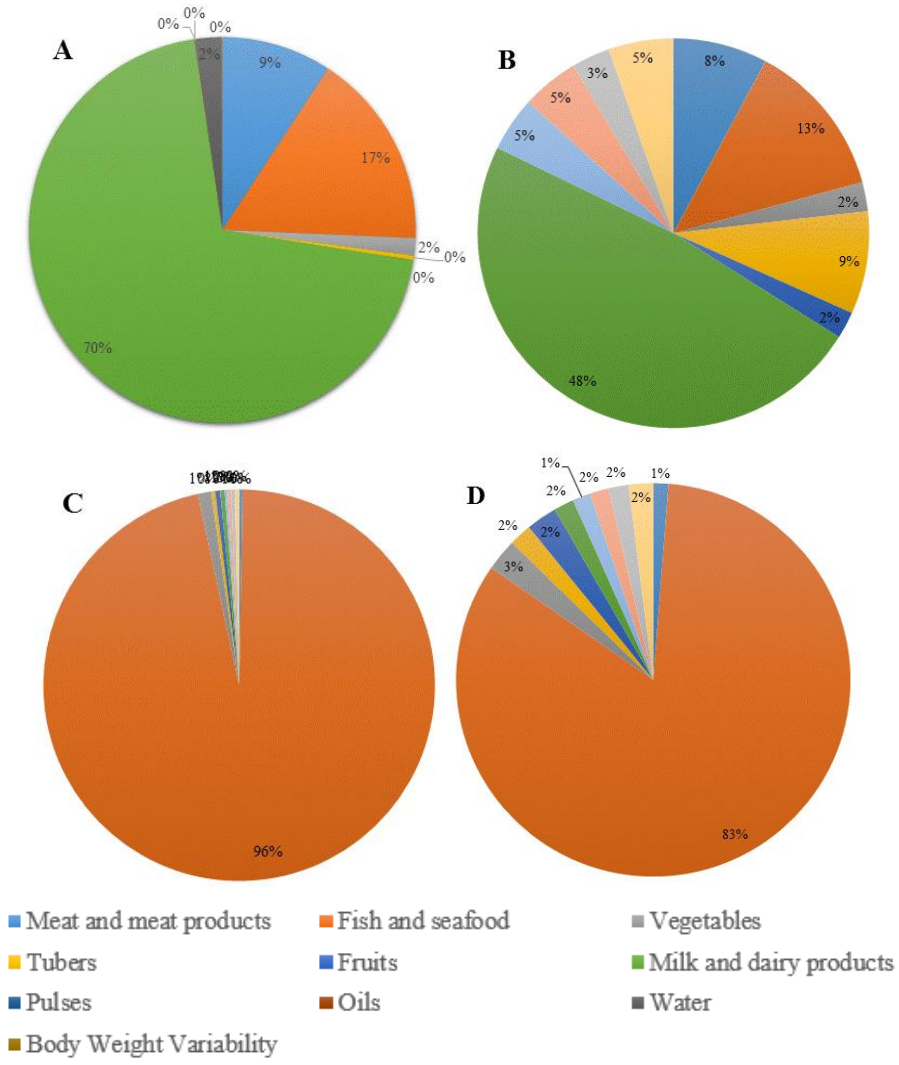

Figure 24: Results for the EFAST method for children (4-12 years) for PFOA first order (A), total order (B) and for PFOS first order (C) and total order (D).

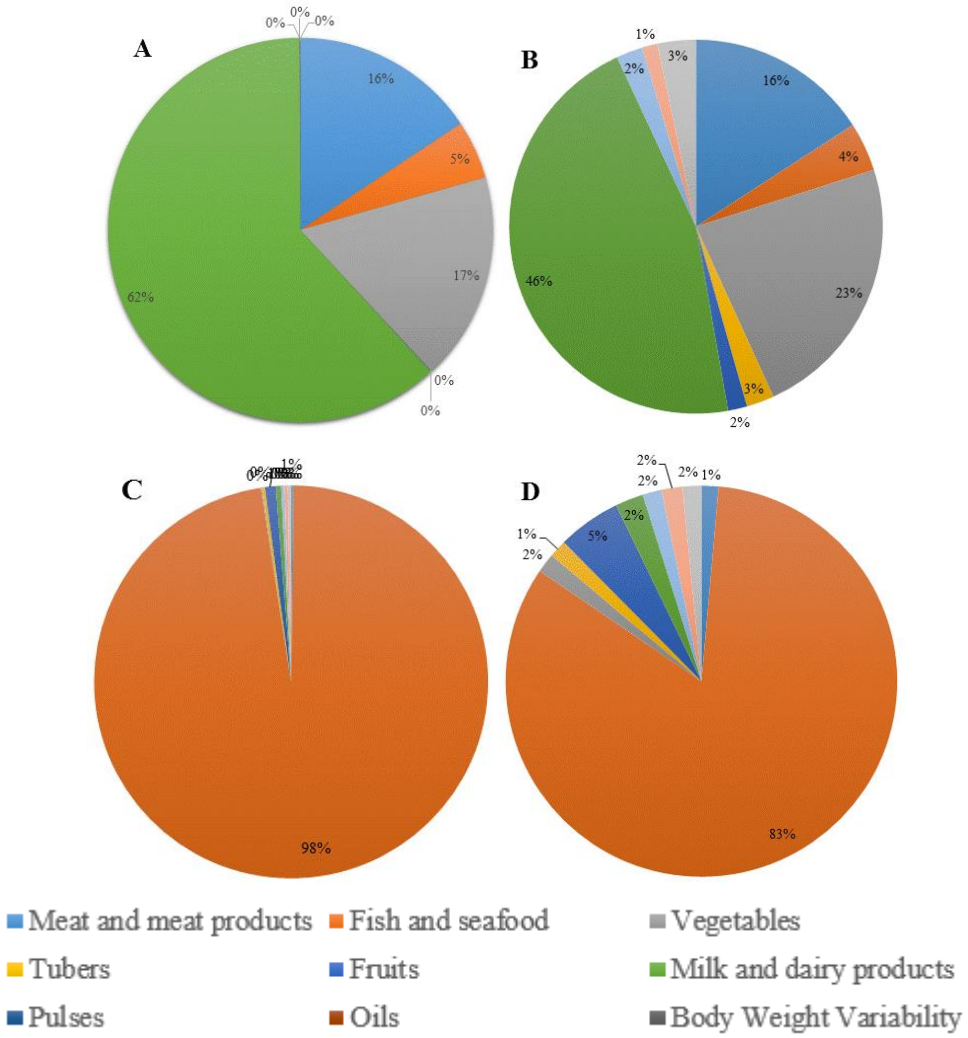

Figure 25: Results for the EFAST method for the adolescents for PFOA first order (A), total order (B) and for PFOS first order (C) and total order (D).

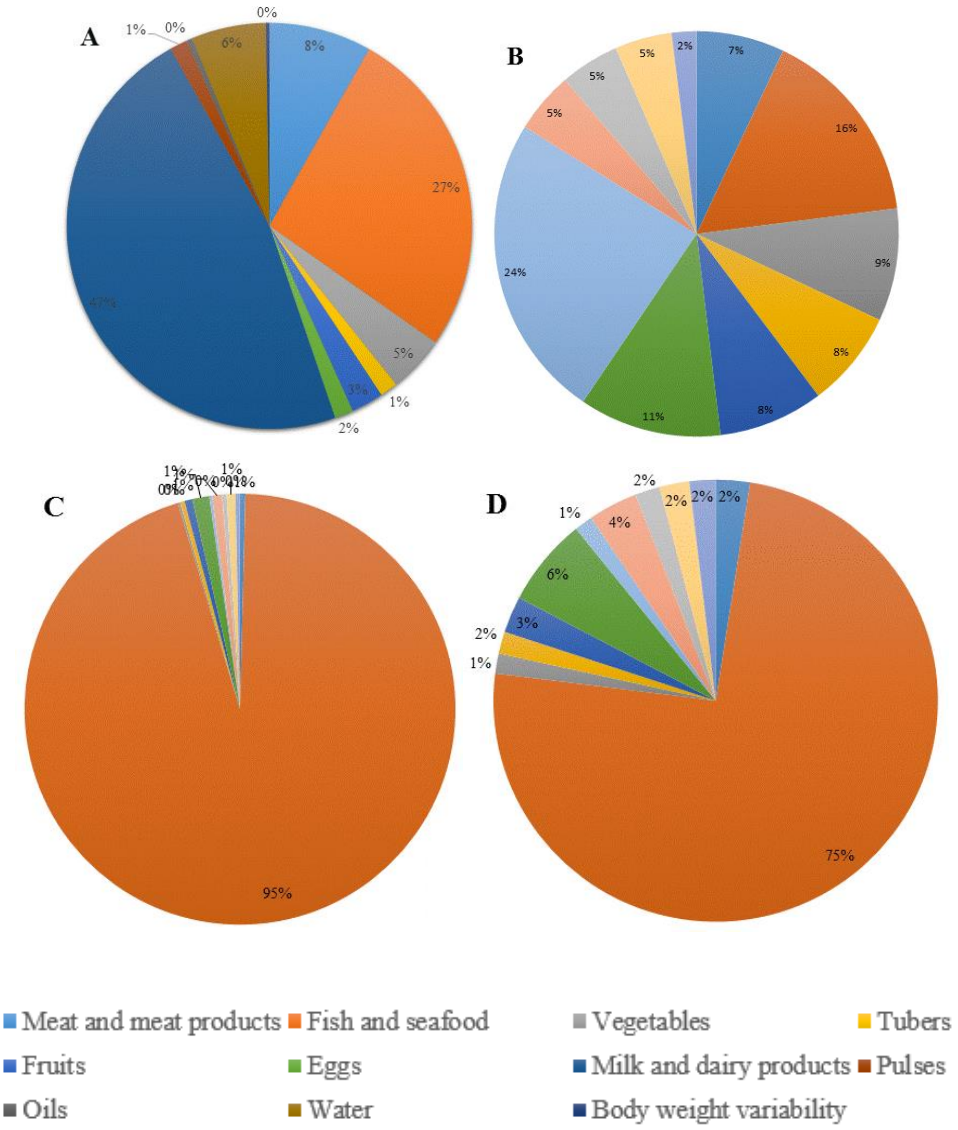

Figure 26: Results for the EFAST method for the adults for PFOA first order (A), total order (B) and for PFOS first order (C) and total order (D).

The results of the SA for the diet and the variability of the bodyweight for PFOA showed that when age increases, various input parameters influence blood concentrations. Indeed during the first years of life, milk is the main source of contamination and then in adults, meat and fish become significant sources. For PFOS the same conclusion can be drawn but to a lesser extent. Indeed fish still represents the main exposure source for adults i.e. 75% taking into account the interactions between parameters.

The conclusions of these sensitivity analyses are in good agreement with the conclusions made by the European Food Safety Authority (EFSA, 2008). EFSA's Scientific Committee concluded that fish was the major source of contamination for PFOS. In addition, fish seems to be a less important source for PFOA.

4 Conclusions

The case study 3 deals with the contamination of the Ebro basin in Spain by perfluorinated compounds (PFOS and PFOA). Two realistic exposure scenarios related to the environment and to the human population were tested to assess the applicability and robustness of MERLIN-Expo.

MERLIN-Expo was used to build a scenario describing the exposure of the human population living in Catalonia via food contamination. Two modules of the model library were used: the intake module to compute the intake from the different food groups, and the human model to predict the concentrations of PFOS and PFOA in the human body. Deterministic and probabilistic simulations were run. All our results showed a good agreement with the experimental data available and with previous knowledge on the main contributors to human exposure.

The models implemented in MERLIN-Expo are generic models that can be applied to a large number of chemical substances. Even if the models are quite flexible and can be adapted to some extent to the specificities of the compounds of interest, some processes cannot be reproduced as the renal reabsorption of the perfluorinated compounds, PFOS and PFOA, in the PBPK model. However, this had no impact on our results since our model provided similar predictions as models developed specifically for perfluorinated compounds, and that a factor less than 3 was observed between the model predictions and the actual experimental data. Such an agreement between predictions and measurement is generally judged acceptable in a purely predictive framework.

Probabilistic and sensitivity analyses were applied to study the impact of uncertainty and variability in parameter values of the different modules on the final model outputs, such as the blood concentration in humans. The probabilistic simulation tools implemented in MERLIN-Expo were used together with the default probability density functions (pre-)defined for model parameters and probability density functions derived from experimental data (e.g., food consumption rates). These analyses produced a mean prediction associated to an interval of confidence for the model outcomes of interest. 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 key input parameters of the exposure, and to assess the relative contribution of the food groups to the human internal contamination. The conclusions of the sensitivity analyses for the human scenario were in good agreement with the conclusions made by the European Food Safety Authority (EFSA, 2008). EFSA's Scientific Committee concluded that fish consumption was the major source of contamination for PFOS in humans.

To conclude, MERLIN-Expo is a flexible tool that was applied easily to environmental and human scenarios and has been proven to provide reliable predictions. The tool could be used in the higher tiers of the risk assessment process by estimating whether the regulatory thresholds were exceeded.

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6 Appendices

Appendix 1: Summary of Little Hocking Water Association, Inc. Member Blood Testing Results May, 2005.

Sex	Age	Years on Water	PFOA (ppb)	PFOS (ppb)
F	46-55	28	281	121
M	46-55	51	395	83.9
F	46-55	55	248	23.2
F	26-35	27	228	34.5
M	<15	3	629	73.3
F	>65	35	442	42
M	36-45	35	220	30.7
F	36-45	9	112	10
M	56-65	34	532	49.3
F	26-35	4	116	9.46
F	26-35	6	211	5.15
M	<15	4	268	35.7
F	<15	3	436	39.7
M	26-35	4	216	35.1
M	36-45	25	325	37.2
F	46-55	33	475	11.1
M	46-55	33	432	14
F	>65	25	358	91.1
M	>65	20	346	52.2
F	36-45	35	240	25.9
M	56-65	16	204	35.1
M	26-35	24	176	86.2
F	16-25	16	488	45.1
M	>65	34	238	30.5

Appendix 2: Measured and simulated concentrations of PFOS in blood, liver and kidneys.

Time (years)	Measured Blood (mg/L)	MCSim (mg/L)	MERLIN (mg/L)
26	0.0116	0.0097	0.0103
25	0.0044	0.0097	0.0103
24	0.0037	0.0097	0.0102
23	0.0074	0.0097	0.0100
27	0.0082	0.0097	0.0104
28	0.0139	0.0097	0.0105
23	0.009	0.0097	0.0100
23	0.0108	0.0097	0.0100
24	0.0162	0.0097	0.0102
22	0.0087	0.0097	0.0099
20	0.0061	0.0097	0.0094
54	0.0085	0.0097	0.0108
56	0.0057	0.0097	0.0108
56	0.0069	0.0097	0.0108
60	0.0098	0.0097	0.0108
57	0.0027	0.0097	0.0108
57	0.0116	0.0097	0.0108
50	0.0134	0.0097	0.0108
56	0.0041	0.0097	0.0108
50	0.0077	0.0097	0.0108
60	0.0067	0.0097	0.0108
51	0.0137	0.0097	0.0108
53	0.012	0.0097	0.0108

Time (years)	Measured Liver (mg/L)	MCSim (mg/L)	MERLIN (mg/L)
86	0.0442	0.0361	0.0409
50	0.0986	0.0361	0.0400
43	0.0231	0.0361	0.0398
61	0.0229	0.0361	0.0403
53	0.0511	0.0361	0.0401
72	0.0655	0.0361	0.0405
28	0.2239	0.0361	0.0389
83	0.2188	0.0361	0.0408
29	0.3786	0.0361	0.0391
75	0.0118	0.0361	0.0406
60	0.0419	0.0361	0.0402
50	0.0266	0.0361	0.0400
45	0.0346	0.0361	0.0399
70	0.4054	0.0361	0.0405

Time (years)	Measured Kidneys (mg/L)	MCSim (mg/L)	MERLIN (mg/L)
86	0.0404	0.0097	0.0088
50	0.0974	0.0097	0.0088
43	0.0221	0.0097	0.0088
61	0.0337	0.0097	0.0088
53	0.033	0.0097	0.0088
72	0.0922	0.0097	0.0088
28	0.0637	0.0097	0.0088
83	0.0501	0.0097	0.0088
29	0.0599	0.0097	0.0088
75	0.0475	0.0097	0.0088
60	0.1009	0.0097	0.0088
50	0.1058	0.0097	0.0088
45	0.1105	0.0097	0.0088
70	0.1679	0.0097	0.0088
70	0.2689	0.0097	0.0088
70	0.0197	0.0097	0.0088

Appendix 3: Measured and simulated concentrations of PFOA in blood, liver and kidneys.

Time (years)	Measured Blood (mg/L)	MCSim (mg/L)	MERLIN (mg/L)
26	0.0016	0.0033	0.0031
25	0.0011	0.0032	0.001
24	0.0015	0.0032	0.0032
23	0.0011	0.0032	0.001
23	0.0018	0.0032	0.0032
27	0.0031	0.0033	0.0008
28	0.0023	0.0033	0.0032
23	0.0018	0.0032	0.001
23	0.001	0.0032	0.0032
24	0.0023	0.0032	0.001
22	0.0019	0.0032	0.0033
20	0.0024	0.0031	0.0012
54	0.002	0.0034	0.0028
56	0.0013	0.0034	0.0011
56	0.0016	0.0034	0.0028
60	0.0031	0.0034	0.001
57	0.0031	0.0034	0.0029
57	0.0027	0.0034	0.001
50	0.0022	0.0034	0.003
56	0.0009	0.0034	0.0009
50	0.0018	0.0034	0.0031

60	0.0028	0.0034	0.0007
51	0.0029	0.0034	0.0033
53	0.0026	0.0034	0.0007

Time (years)	Measured Liver (mg/L)	MCSim (mg/L)	MERLIN (mg/L)
86	0.013	0.0035	0.0084
50	0.0112	0.0035	0.0091
43	0.0288	0.0035	0.0091
61	0.0226	0.0035	0.0086
53	0.0095	0.0035	0.0090
72	0.0226	0.0035	0.0081
29	0.0989	0.0034	0.0091
75	0.004	0.0035	0.0079

Time (years)	Measured Kidneys (mg/L)	MCSim (mg/L)	MERLIN (mg/L)
29	0.0119	0.0048	0.0043

Appendix 4: Probability Density Functions (normal distribution) for the parameters of the human model for PFOS and PFOA

Compound specific parameters

Parameters	PFOA		PFOS	
	Mean	SD	Mean	SD
Tissue:blood partition coefficients				
Adipose	0.04	0.0096	0.14	0.0336
Adrenal	0.12	0.024	0.2	0.04
Blood	1	-	1	-
Blood Arterial	1	-	1	-
Blood Venous	1	-	1	-
Bones	0.12	0.024	0.2	0.04
Bones NP	1	-	1	-
Brain	0.12	0.024	0.2	0.04
Breast	0.12	0.0288	0.2	0.048
Gut	0.05	0.01	0.57	0.114
Gut Lumen	1	-	1	-
Heart	0.12	0.024	0.2	0.04
Kidneys	1.05	0.231	0.8	0.176
Liver	2.2	0.396	3.72	0.6696
Lungs	0.12	0.0324	0.2	0.054
Marrow	0.12	0.024	0.2	0.04
Muscle	0.12	0.0288	0.2	0.048
Pancreas	0.12	0.0168	0.2	0.028
Sexual Organs	0.12	0.0228	0.2	0.038
Skin	0.1	0.024	0.29	0.0696
Spleen	0.12	0.018	0.2	0.03
Stomach	0.12	0.0372	0.2	0.062
Stomach Lumen	1	-	1	-
Thyroid	0.12	0.024	0.2	0.04
Urinary Tract	0.12	0.024	0.2	0.04
Excretion parameters				
Excretion rate per kg of BW (1/min/kg)	2.07E-07	6.89E-08	6.89E-07	2.3426E-07

Physiological parameters

Tissues organ weights	Mean	Standard Deviation
Adipose	0.1986	0.024825
Adrenal	0.0002	0.000025
Blood	0.0767	0.0095875
Blood Arterial	0.0189	0.0024
Blood Venous	0.0569	0.0071

Bones	0.0753	0.0094125
Bones NP	0.0185	0.0023125

Appendix 5: Probability Density Functions for the parameters related to food consumption obtained from EFSA (2011)

Food groups	Toddlers		Other children		Adolescents		Adults	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pulse	0.0873	0.0472	0.1966	0.076	0.2614	0.1105	0.1831	0.0897
Vegetables	0.0696	0.0643	0.0641	0.0579	0.0915	0.0742	0.2077	0.1504
Tubers	0.0431	0.0378	0.043	0.0433	0.0508	0.052	0.0566	0.0588
Fruits	0.0794	0.0753	0.1037	0.0973	0.1014	0.1174	0.1602	0.1474
Meat and meat products	0.0766	0.0459	0.1125	0.062	0.1527	0.0865	0.1447	0.0935
Fish and seafood	0.0263	0.0658	0.0365	0.0363	0.0441	0.0507	0.0645	0.0636
Milk and dairy products	0.5196	0.2752	0.4873	0.1686	0.4559	0.2193	0.3492	0.1966
Eggs	0	0	0	0	0	0	0.0244	0.0268
Oils	0.0099	0.0092	0.0188	0.0098	0.0259	0.0138	0.0356	0.0163
Drinking water	0.3285	0.3234	0.627	0.383	0.915	0.693	0.7975	1.061