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PU	Public	x
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1 Introduction

According to the objectives of Task 4.2 “Verification and benchmarking process”, a validation process has been performed in order to assess the consistency of the mathematical models included in MERLIN-Expo tool.

The benchmarking of the Multimedia models was completed by comparing the results obtained for specified reference scenarios using the MERLIN-Expo tool and EUSES.

For the PBPK model the benchmarking was carried out by simulating the scenarios from studies published in scientific literature and comparing the model predictions to actual experimental data.

This report aims to describe how the benchmarking process was conducted and to provide the conclusions derived from this verification work. A more general comparison of MERLIN-expo versus other exposure tools can be found in Deliverable 2.4.

2 Benchmarking of MERLIN-Expo with EUSES

In order to guarantee the consistency of results provided by the MERLIN-Expo tool, benchmarking of the MERLIN-Expo tool with other exposure models is required. This was carried out by comparing results obtained for a specified reference scenario using the MERLIN-Expo tool and EUSES.

The following models available in EUSES and MERLIN-Expo will be considered for comparison:

- River model
- Soil model
- Atmosphere model
- Fish model

2.1 Introduction to EUSES

EUSES is designed to be a decision-support system for the evaluation of the risks of substances to man and the environment. The system is fully based on the EU Technical Guidance Documents for the risk assessment of new and existing substances and biocides.

In the European Union, the predecessors of the REACH regulation (EC 793/93), CLP regulation (Directive 92/32/EC) and the biocide regulation (Directive 98/8/EC) required the risk assessment for new substances, existing substances and biocides, respectively. The principles for this risk assessment have been laid down supported by a detailed package of Technical Guidance Documents. Against this background the European Union System for the Evaluation of Substances, EUSES, was developed.

Risks to man pertain to consumers, workers and man exposed through the environment. Protection goals in the environment include sewage treatment plant populations of micro-organisms, aquatic, terrestrial and sediment ecosystems and populations of predators. This assessment includes the marine environment. The system can be used to carry out tiered risk assessments of increasing complexity on the basis of increasing data requirements. Virtually all default settings can be changed and all estimated parameter values and intermediate results can be overwritten by measured data.

The exposure assessment in EUSES covers the whole life cycle of substances as well as their fate in all environmental compartments at three spatial scales: the personal scale for consumers and workers, the local scale for man and ecosystems near point sources and the regional scale for man and ecosystems exposed as a result of all releases in a larger region. Both short- and long-term time scales are considered, where appropriate. The following media are considered in the EUSES model: atmosphere, surface water (fresh and marine water), sediment (fresh and marine environment), soil (natural, agricultural and industrial soil) and two terrestrial compartments (natural and agricultural soil). EUSES is a steady state, simulation, deterministic, distributed and analytical model. The exposure assessment aims at ‘reasonable worst case' results by applying unfavorable, but not unrealistic, standard exposure scenarios and, as much as possible, mean, median or typical parameter values.

2.2 Reference scenario

The simulations were performed using the substance **TCDD** (2,3,7,8-tetrachlorodibenzodioxin). TCDD is a polychlorinated dibenzo-p-dioxin, which is usually formed as side product in organic synthesis and burning of organic materials. It is a persistent environmental contaminant usually present in a complex mixture of dioxin-like compounds, and is a carcinogen. TCDD characteristics used in the simulation are listed in Table 1.

Table 1: TCDD characteristics

Parameter	Value	Source
Molecular weight	321.97 g/mol	US EPA
Henry's Law Constant	0.36 Pa m^3/mol	MERLIN-Expo
K_{oc}	380 L/kg	EUSES
Log K_{ow}	1,6	US EPA
K_d-suspended matter	38 L/kg	EUSES
BAF fish	4.57 L/kg wwt	EUSES
BMF fish	1	EUSES
Vapour pressure	1.95 x 10^{-8} Mm Hg	EPISuite
Water Solubility	0.001103 mg/L	EPISuite

The scenario followed is based on an ERC, which is defined as an Environmental Release Category and describes the conditions of use from the environmental perspective. ERCs are developed under REACH and are described in the technical guidance documents (R16 – Environmental Exposure Estimation).

The industrial scenario simulated uses the following assumptions:

- TCDD is produced in a volume of 1000 ton/year
- Release occurs to water, air and soil. Releases to water are assumed to be treated in a municipal sewage treatment plant (STP). By default, a municipal STP is available as a standard RMM for local release from industrial settings. Indirect releases to air via the STP, as a result of water treatment in the STP, are also considered in the industrial setting scenario. Release to soil at the local scale will occur via application of sludge from an STP to agricultural soil and via atmospheric deposition of substances released to air. Direct releases to soil from industrial settings are not assessed at the local scale.
- The fractions released of the used amount to the environmental compartment under consideration are defined in environmental release categories (ERCs). In this scenario, ERC1 – Manufacture of substances will be used. This ERC assumes 5% emission to air (137 kg/d), 6% emission to water (before STP) (164,4 kg/d) and 0% to soil. The number of emission days is set to 365.
- Direct application to soil is not taken into account. For sludge application (originating from the municipal STP treating the wastewater of the industrial plant) to agricultural soil an application rate of 5000 kg/ha dry weight per year is assumed. Sludge application is treated as a single event once a year. The contribution to the overall impact from wet and dry deposition (from the air) is based on the release calculation of a point source. Atmospheric deposition is assumed to be a continuous flux throughout the year.

2.3 Benchmarking

2.3.1 River model

2.3.1.1 Surface water

The River model in MERLIN-Expo dynamically simulates the distribution of organic contaminants and metals in abiotic media (i.e. water, suspended particulate matter and sediments) of river systems. It provides an estimation of the contaminant in raw water or filtered water and the concentration in bottom sediments, which is comparable to the

predicted environmental concentration in water (PEC_{water}) and sediment (PEC_{sed}). The media, inputs, losses and exchanges in the River model are presented in Figure 1.

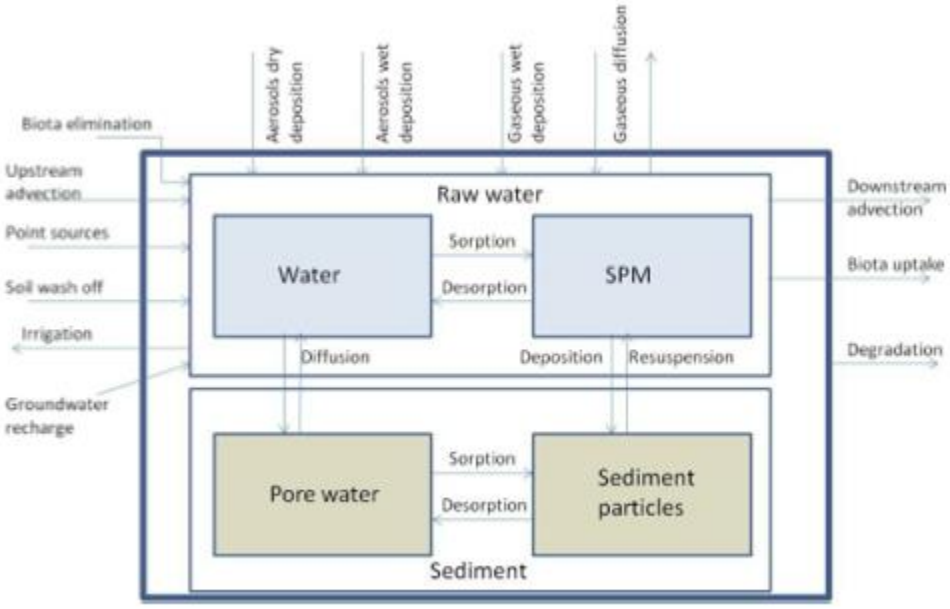


Figure 1: Media considered + loading inputs + losses + exchanges in the river model of MERLIN-Expo (organics)

In EUSES, the calculation of the local PEC_{water} involves several sequential steps. The calculations originate from mass balances (differential equations) similar to those in MERLIN-Expo, however, these were further simplified by assuming steady-state conditions. It includes the calculation of the discharge concentration of a STP to a water body, dilution effects and removal from the aqueous medium by adsorption to suspended matter. The fate processes in the surface water are presented in Figure 2.

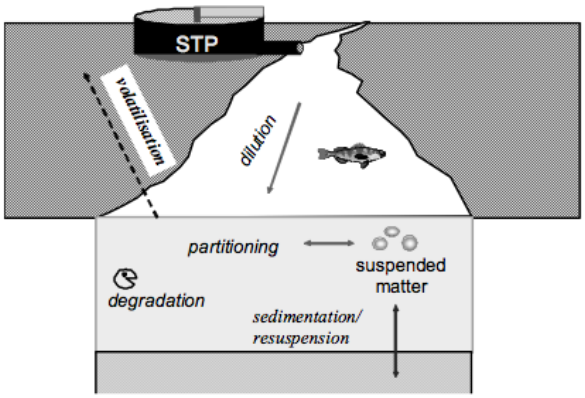


Figure 2: Fate processes in the surface water (EUSES)

The calculation of the contaminant in the water phase is more complex in MERLIN-Expo compared to EUSES, therefore in order to obtain a similar model, the following processes were disabled in MERLIN-Expo to achieve similar conditions as in EUSES:

Output processes:

- Irrigation
- Diffusion to gaseous atmosphere
- Deposition to sediment
- Degradation

- Deposition of particles into the sediment

Input processes:

- Upstream river input
- Elimination from fish
- Diffusion from air
- Atmospheric Deposition
- Recharge from groundwater
- Wash-off
- Erosion from sediment

Further assumptions and input parameters:

- 6% of the 1000 tonnes manufactured/year is released to water = 164,4 kg/day. This quantity is directed to an STP. The number of inhabitants feeding this STP is 10000 and the effluent discharge rate of this STP is 20000l/day. The fate of contaminants in the STP is process using the model SimpleTreat which is incorporated in EUSES. The following distribution of the emission is used:
  - Fraction of emission directed to air by STP: 0.326%
  - Fraction of emission directed to water by STP: 13,8%
  - Fraction of emission directed to sludge by STP: 4.52%

  This results in a concentration of the contaminant of 11.3 mg/L in the STP effluent.

Taking the flow rate of 20000l/day into account, this results in 22,6 kg/d. This STP output can be used as a time series input in MERLIN-Expo (D point source) because STP cannot be simulated in the current MERLIN-Expo version. This concentration remains constant in time, hence the daily input into the river is constant.

- A dilution factor of 10 is taken into account for rivers. The flow rate used in EUSES and implemented in MERLIN-Expo is 0.21 m^3/s.

The predicted environmental concentration in surface water (dissolved concentration) is presented in Table 2.

Table 2: Predicted environmental concentration in surface water (PEC_{water}) calculated using EUSES and MERLIN-Expo

	PEC_{water}
EUSES	1.13 mg/L
MERLIN-Expo	1.24 mg/L

CONCLUSION

The obtained values indicate that the REACH scenario can be fairly well reconstructed in MERLIN-Expo by switching off certain processes and modifying the values of certain parameters to be in accordance with the REACH requirements. It can be concluded that EUSES is a highly simplified version of the river model in the MERLIN-Expo tool.

2.3.1.2 Sediment

Processes taking place in the MERLIN-Expo tool are:

- Input:
  - Deposition from suspended particle matter
  - Sorption from the pore water
- Output:
  - Re-suspension from the sediment
  - Desorption from the sediment

In EUSES, the concentration in freshly deposited sediment is taken as the PEC for sediment. Processes considered in EUSES are partitioning between the dissolved water concentration and the sediment. The partitioning is however modelled using the partition coefficient of suspended matter (instead of sediment) because it is assumed the freshly deposited sediment is more similar to suspended matter characteristics (e.g. organic content) than to sediment. PEC_{sed} is calculated as follows:

$$PEC_{sed} = \frac{K_{susp-water}}{\rho_{susp}} \cdot PEC_{water} \cdot 1000$$

$K_{susp\text{-}water}$: suspended matter-water portioning coefficient

RHO_{susp}: bulk density of suspended matter (kg/m^3)

Hence, comparison between the two tools is not straightforward for sediment as the available fate processes do not correspond: there is no partitioning available between sediment and water in MERLIN-Expo (through the suspended matter partition coefficient), but deposition and resuspension of suspended matter as well as diffusion between water and sediment porewater.

CONCLUSION

Sediment processes in MERLIN-Expo do not correspond with the processes described in EUSES, which makes benchmarking between the two tools for the sediment model not straightforward.

2.3.2 Fish model

The fish model in MERLIN-Expo includes two media that correspond to two input/output pathways for chemical accumulation in fish, i.e. the fish respiratory system and the fish gastro intestinal tract (GIT) system. The media and processes considered are represented in Figure 3.

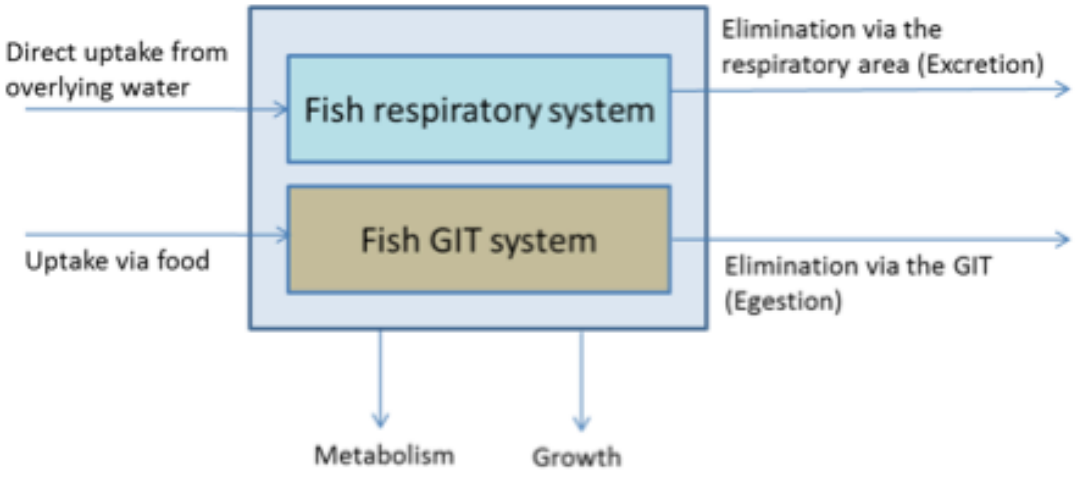


Figure 3: Media considered + loading inputs + losses in the fish model (for organic substances)

In EUSES, the concentration in fish is a result of uptake from the aqueous phase and intake of contaminated food (aquatic organisms). $PEC_{oral,\ predator}$ is calculated from the bioconcentration factor (BCF) and biomagnification factor (BMF).

$$PEC_{oral,predator} = PEC_{water} \times BCF_{fish} \times BMF$$

The BCF used in EUSES is similar to the BCF used in MERLIN-Expo.

Comparing the two models, the following processes are not taken into account or are not similar in EUSES:

- Input:
  - Direct uptake in water is also present in EUSES, but in MERLIN-Expo this occurs via membrane diffusion via the respiratory area and is determined by the respiratory uptake rate constant. This constant is determined by the water-layer diffusion resistance for uptake of chemicals, the lipid-layer permeation resistance, the K_{ow}, the fish weight at maturity and the allometric rate exponent.
  - The direct uptake via food is in EUSES only characterized by the BMF, which is defined as the relative concentration in a predatory animal compared to the concentration in its prey.

- Output:
  - Elimination from fish via the respiratory area
  - Elimination from fish via elimination into egested faeces
  - Via metabolic transformation
  - Via growth of fish mass

Benchmarking of EUSES with MERLIN-Expo for the fish model is not straightforward because:

- Simplified approach in EUSES only taking bioconcentration and biomagnification into account
- Dynamic approach in MERLIN-Expo compared to the steady-state approach of EUSES
- Disabling the excretion and elimination processes from the fish results in an error which makes simulation impossible (because a mass-balance model like MERLIN-Expo must by nature include both inputs and outputs in a given compartment).

This result in the following concentrations in wet fish calculated in EUSES and MERLIN-Expo (Table 3).

Table 3: Predicted environmental concentration in fish ($PEC_{oral,predator}$) calculated using EUSES and MERLIN-Expo

$PEC_{oral,predator}$	
EUSES	5.16 mg/kg wwt
MERLIN-Expo	46100 mg/kg wwt*

*Steady-state concentration of the chemical in fish caught for human food, reached after 365 days

CONCLUSION

The behavior of the chemical in the fish is much more elaborated in MERLIN-Expo compared to EUSES. This results in a different outcome of the fish concentration, which is used for human consumption. The concentration in fish calculated by MERLIN-Expo depends also on

the fish species and on its diet. Hence, it is concluded that MERLIN-Expo can not easily simulate the more simple processes described in EUSES.

2.3.3 Soil model

The soil model includes the following media: pore water and soil particles, which are duplicated in several soil layers with a constant height. The media and processes considered are represented in Figure 4.

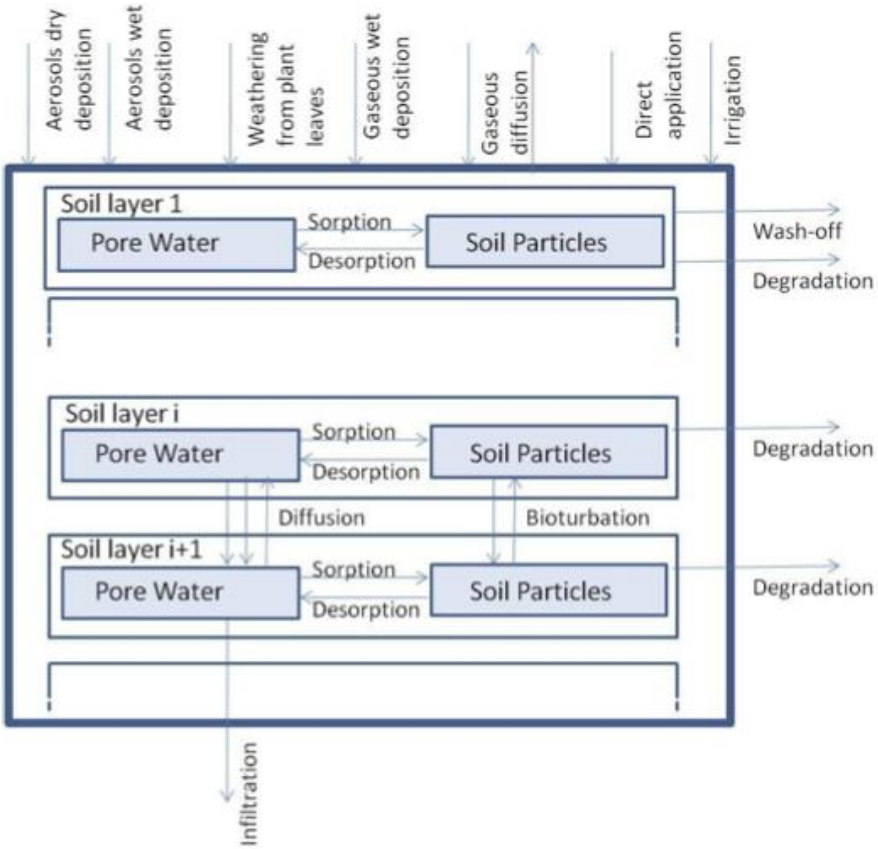


Figure 4: Media considered + loading inputs + losses in the soil model (for organic compounds)

In EUSES, differentiation in soil layers is not taken into account; only one soil layer is available. Direct application of substances is not taken into account. Input into the soil is based on sludge application and on atmospheric deposition. For the benchmarking of EUSES vs. MERLIN-Expo the concentration in agricultural soil is calculated. Sludge application on agricultural soil is by default 5000 kg/ha dry weight per year. Sludge application is treated as a single event once a year. Atmospheric deposition is assumed to be a continuous flux throughout the year.

The output processes considered in EUSES are diffusion (volatilization to air), degradation and advection (leaching to deeper soil layers) (Figure 5).

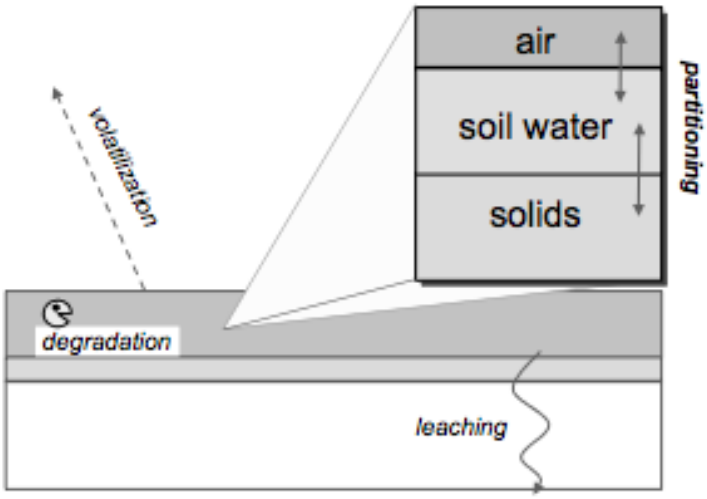


Figure 5: Processes considered in EUSES

The soil model present in EUSES is a fairly simplified model as this is more appropriate for a generic risk assessment at EU-level compared to the extensive numerical soil and groundwater models available (mainly for pesticides). The concentration in soil in EUSES is described by following simple differential equation:

$$\frac{dC_{soil}}{dt} = -k \times C_{soil} + D_{air}$$

D_{air}: aerial deposition flux per kg of soil (mg/kg/d)

t: time (d)

k: first order rate constant for removal from top soil (d)

Accumulation of the substance may occur when sludge is applied over consecutive years. This is illustrated in Figure 6. As a realistic worst-case assumption for exposure, it is assumed in EUSES that sludge application takes place for 10 consecutive years. The soil concentration used in risk assessment is the integrated concentration in soil over a period of 180 days after 10 years of sludge application (Figure 7).

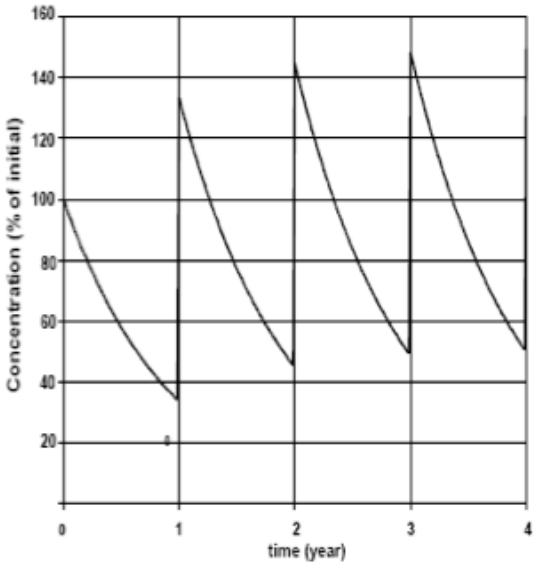


Figure 6: Accumulation in soil due to several years of sludge application

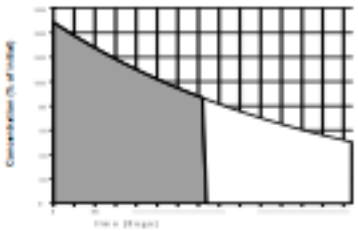


Figure 7: Soil concentration after 10 years

Processes which are present in MERLIN-Expo and which are not taken into account in EUSES are the following:

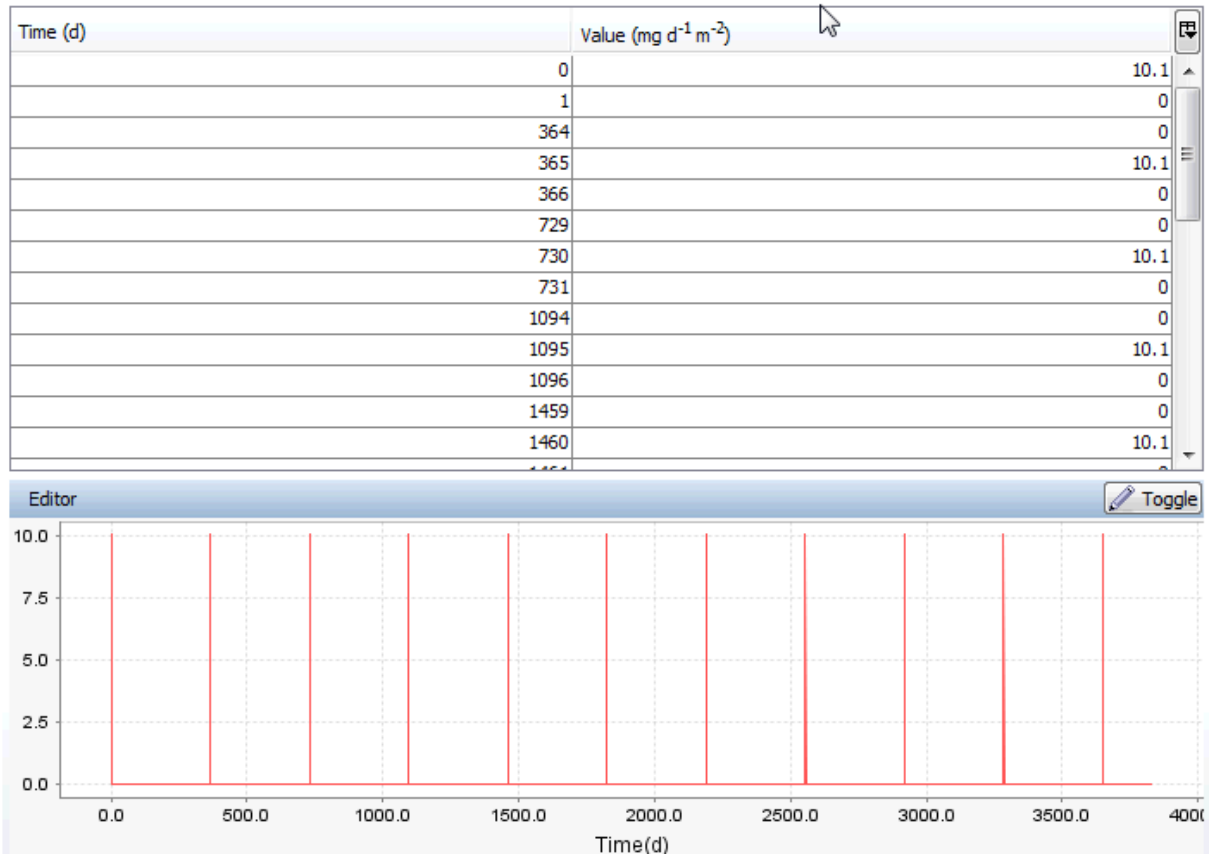
- Input:
  - Wet deposition (No differentiation is made between dry and wet deposition in EUSES, therefore, deposition calculated in EUSES was used as a direct input in MERLIN-Expo in dry deposition (2,59 mg/m^2/d))
  - Diffusion from atmospheric gas to gas present in soil pores
  - Input from plant leaves
  - Irrigation
- Output
  - Wash-off

Rate of sludge application (used as input for the direct application on topsoil per day and per surface unit) was calculated as follows:

Rate of sludge application = sludge application x contaminant concentration in sludge (input from EUSES)

= 0.5 kg/m^2/y x 7,36 x 10^3 mg/kg = 10.1 mg/m^2/d

This value was used as a time series input value in MERLIN-Expo:



The soil concentrations of the last 180 simulated days from MERLIN-Expo were averaged (this was done manually), which resulted in 9517.4 mg TCDD in the soil, which corresponds to 11.20 mg/kg dw.

A comparison of the results obtained with EUSES and MERLIN-Expo are presented in Table 4.

Table 4: Predicted environmental concentration in soil (PEC_{soil}) calculated using EUSES and MERLIN-Expo

	$PEC_{oral,predator}$
EUSES	72 mg/kg wwt
MERLIN-Expo	11.2 mg/kg wwt

CONCLUSION

The results obtained with the two tools are in the same order of magnitude, thus it is feasible to obtain similar results with both exposure models. A more thorough detailing and comparison of all intermediate calculations steps and parameters may further refine the discrepancy. Nonetheless, we need to take the following limitations into account:

- Deposition in EUSES combines wet and dry deposition, while this is separate in MERLIN-Expo, allowing to simulate wet deposition according to actual meteorological conditions (rainfall)
- REACH requires sludge concentrations, which can not be modeled in MERLIN-Expo, thus the input from another model is required.
- PEC_{soil} is calculated as the concentration after 10 years sludge application and in the 10^{th} year averaged over 180 days, averaging over the last simulated 180 days is not

possible in MERLIN-Expo and needs to be done manually outside the tool. However, the criteria chosen by EUSES for defining the soil contamination (why only the 180 last days of the 10th year) remains disputable because it totally hides the dynamics of chemicals in soils over the complete simulation timeframe. The same criteria could be implemented in MERLIN-Expo if needed, but the relevance of such a criteria remains subject to debate.

2.3.4 Atmosphere model

The atmosphere model in MERLIN-Expo is based on the following processes:

- Input:
  - Diffusion to air
  - Wind in (flux of contaminants into the atmosphere box from the surrounding region)
- Output:
  - Diffusion from air
  - Dry deposition of aerosol
  - Wet deposition of aerosol
  - Wet deposition of gas
  - Wind out (flux of contaminants from the atmosphere box to the surrounding region)

The air compartment in EUSES receives its input from direct emission to air, and volatilisation from the sewage treatment plant. These processes could be combined and could be entered into MERLIN-Expo as input in 'Wind in'. Diffusion to air can be disabled, as this is not taken into account in EUSES.

The local air concentration does not take output processes into consideration. Deposition from the air is calculated as an input into other compartments, but is not withdrawn from C_{air}.

As specific information on scale, emission sources, weather conditions, etc. is normally not available for a lot of chemicals, a standardised exposure assessment is carried out in EUSES making a number of explicit assumptions and using a number of fixed default parameters.

CONCLUSIONS/LIMITATIONS:

- Input into the air model can not be directly coupled to other models (STP volatilization not available in MERLIN-Expo)
- The air model available in EUSES is an empirical model. The atmosphere model available in MERLIN-Expo is more advanced.

2.4 Conclusion on benchmarking of MERLIN-Expo with EUSES

The following conclusions are made on the benchmarking of MERLIN-Expo in the simulation of a EUSES scenario:

- Framework related specifics like in the EUSES tool recommended under REACH (Registration, Evaluation, Authorisation and restriction of CHemicals) and BPR (Biocidal Product Regulation) are not yet available in MERLIN-Expo (e.g. presence of an STP, sludge application,…). In case an EUSES-like scenario is to be implemented in MERLIN-expo (for the purposes of REACH or BPR), this could be solved in the short term by making these calculations outside MERLIN-Expo and in the long term by implementing these specifics (e.g. an STP model, such as Simpletreat) in the MERLIN-Expo tool. On the other hand, MERLIN-Expo contains the latest 20 years scientific developments on state-of-the-art multimedia modelling and has many more functionalities compared to EUSES (see SWOT deliverable for more information on strengths for MERLIN-Expo).
- Benchmarking MERLIN-Expo and EUSES models was possible for the aquatic and terrestrial compartment. In those cases, EUSES can be considered as a simplified version of MERLIN-expo (by disabling a number of fate processes). However, benchmarking is not always straightforward for other compartments due to different model approaches in the calculation as well as differences due to dynamic versus steady-state approach. Overall, MERLIN-Expo is more mechanistic and more complete. This way, MERLIN-Expo can be seen as a more advanced scientific tool compared to EUSES.

3 The human model

The human model implemented in MERLIN-Expo is a physiologically based pharmacokinetic (PBPK) model. Different software or tools have been developed and some of them are freely available. However there is currently no consensus among stakeholders, risk assessors or researchers on a tool. Therefore we chose to benchmark our model using published studies in the scientific literature and to compare the model predictions to actual experimental data. Several scenarios were tested with different exposure times (i.e. chronic and sub-acute), age classes (i.e. adults and children) and chemical compounds, 2,3,7,8-tetrachlorodibenzo-p-dioxin, two perfluorinated compounds, and lead. For lead, a reference model (IEUBK) is available and was then used for the benchmarking.

3.1 Life-time exposure to a persistent compound

3.1.1 Chemical

Polychlorinated dibenzo-p-dioxin (PCDDs) (also called dioxins), including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), are persistent environmental contaminants. The main current sources of PCDDs in the environment are combustion processes, such as waste incineration, and metal smelting and refining. Among this chemical class, TCDD is one of the most toxic compounds and is classified as a human carcinogen by IARC.

Food is the major source for human exposure to dioxins. Because the persistence of TCDD in the human body (or the half-life) is of the order of many years, continuous exposures from contaminated food might lead in the long run to extremely high body burdens.

3.1.2 Reference study

We used the study of Maruyama et al. (2003) to benchmark our model for TCDD. In their study, Maruyama et al. (2003) applied a PBPK model developed for dioxins to predict the concentrations in several tissues and compared the predictions to experimental data. The data measured concentrations in blood, fat, liver and richly perfused tissues of Japanese men whose ages range between 20 and 60 years. This data was obtained from reports by Environment Agency (2000) and by Iida *et al*. (1999).

The TCDD exposure route was assumed to be solely food ingestion. The daily intake was calculated using concentration data in Japanese food obtained in 1998 (Toyoda et al., 1999) and was set to 12.8 pg/day.

3.1.3 Parameterization of the PBPK model in MERLIN-Expo

Although chemicals absorbed from gut lumen enter the liver first, ingested TCDD were set to enter the blood flow directly in this model, assuming that dioxin passes liver fast enough to avoid accumulation or first pass effects such as metabolic elimination. We then used the option “Ingestion via the liver” in MERLIN-Expo. The absorption rate was obtained by Mclachlan (1993)

Only one elimination route was considered in the liver via biliary excretion, since urinary excretion of dioxins can be neglected. The excretion rate was set to the value provided by Milbrath et al. (2009).

Tissue-blood partition coefficients of liver, kidney, fat, muscle and richly perfused tissue were calculated using dioxin concentration data in human tissues (Iida et al., 1999), or determined based on structural information of the chemicals (Parham et al., 1997).

The parameters values are reported in Table 5.

Table 5 : Values of the PBPK model parameters used the different tests for benchmarking

Parameters	TCDD	Lead	PFOA	PFOS
Absorption rate				
Oral	0.97	0.11	0.9	0.9
Inhalation	-	0.05	-	-
Metabolism and excretion (min/kg)				
Liver	4.257×10^{-7}	1.85×10^{-6}		
Kidneys		4.347×10^{-6}	2.07×10^{-7}	6.89×10^{-7}
Partition coefficients				
Adipose	247	20	0.04	0.14
Adrenal	9.8	100	0.12	0.2
Blood	1	1	1	1
Blood_Arterial	1	1	1	1
Blood_Venous	1	1	1	1
Bones	9.8	1 000	0.12	0.2
Bones_NP	1	1	1	1
Brain	4.1	100	0.12	0.2
Breast	17	20	0.12	0.2
Gut	9.8	100	0.05	0.57
Gut_Lumen	1	1	1	1
Heart	9.8	100	0.12	0.2
Kidneys	3.1	100	1.05	0.8
Liver	9.8	100	2.2	3.72
Lungs	4.1	100	0.12	0.2
Marrow	1	100	0.12	0.2
Muscle	17	20	0.12	0.2
Pancreas	9.8	100	0.12	0.2
Sexual_Organs	9.8	100	0.12	0.2
Skin	2.5	20	0.1	0.29
Spleen	9.8	100	0.12	0.2
Stomach	9.8	100	0.12	0.2
Stomach_Lumen	1	1	1	1
Thyroid	9.8	100	0.12	0.2
Urinary_Tract	9.8	100	0.12	0.2
Partitioning in blood				
BIND (mg/L)	-	2.7	-	-
KBIND (mg/L)	-	0.0075	-	-

3.1.4 Results

The predicted concentrations in blood, liver, fat and richly perfused tissues obtained using MERLIN-Expo are presented in Figure 8. The average prediction is represented together with its interval of confidence (IC) at 90%. The 90% confidence interval encompassed the majority of the data points for all organs. The toxicokinetic profiles in the various organs were quite similar.

Because the concentrations are quite stable over the time period considered, we also calculated the average concentration for the 4 organs and compared the predictions with the measurements (**Table** 6). We observed that the predictions provided by MERLIN-Expo were quite close to the data, and also outperformed the model by Maruyama et al. (2003). Indeed the predictions obtained by MERLIN-Expo are closer to the actual measurement data in four tissues than the predictions obtained by the other model (Table 6).

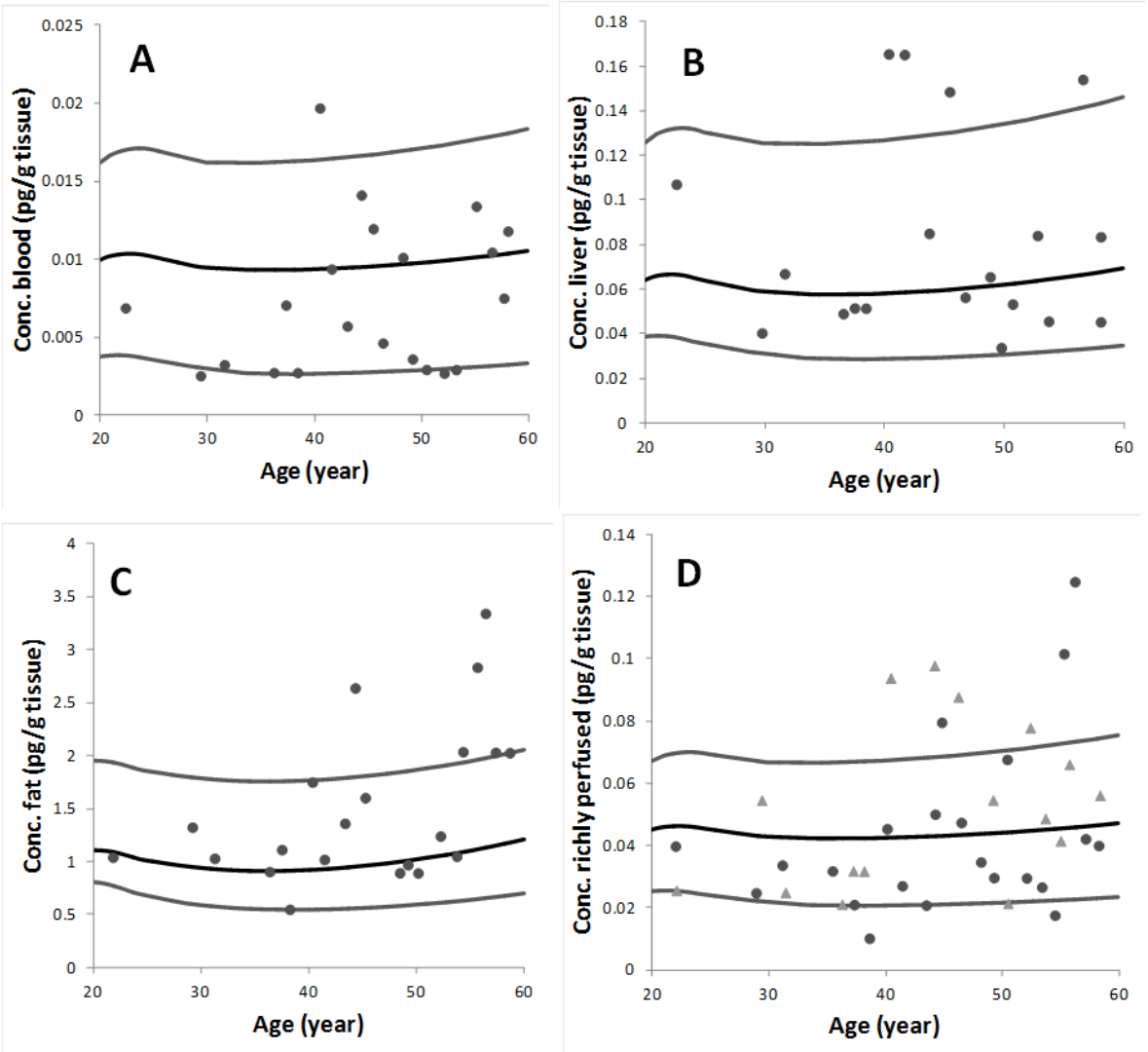


Figure 8 : Simulated (lines) and measured (circles and triangles) TCDD concentrations in blood (A), liver (B), fat (C) and richly perfused tissue (D). The two grey lines are represented the 90% IC. Measured concentrations represented by circles were obtained from the report by Environment Agency (2000) and the ones represented by triangles were obtained from Iida et al. (1999).

Table 6 : Predicted and measured average concentrations of TCDD in blood, liver, fat and richly perfused tissue by MERLIN-Expo and a published model (Maruyama et al., 2003). The mean is given with the standard deviation (SD) for the data, and the predictions of MERLIN-Expo with the 90% interval of confidence (IC).

Tissue	Concentrations (pg/g tissue)	
Blood	Maruyama *et al.* (2003)	0.048
	Measured (± SD)	0.007 ± 0.005
	MERLIN-Expo (90% IC)	0.009 [0.003; 0.017]
Liver	Maruyama *et al.* (2003)	0.112
	Measured (± SD)	0.082 ± 0.045
	MERLIN-Expo (90% IC)	0.062 [0.031; 0.131]
Fat	Maruyama *et al.* (2003)	6.31
	Measured (± SD)	1.51 ± 0.735
	MERLIN-Expo (90% IC)	1.07 [0.61; 1.84]
Richly perfused tissues	Maruyama *et al.* (2003)	0.197
	Measured (± SD)	0.052 ± 0.026
	MERLIN-Expo (90% IC)	0.044 [0.022; 0.069]

3.2 Exposure to semi-persistent compounds during adulthood

3.2.1 Chemicals

Perfluorinated compounds (PFCs) 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, PFCs have been released to the environment and bioaccumulate through the food chain. Recently, a number of studies have reported internal exposures to PFCs in human tissues. We focused on two compounds, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the most extensively investigated PFCs.

Although the relative importance of the routes of human exposure to these compounds is not quite established yet, recent investigations have shown that food intake and packaging, water, and house dust and indoor air are all potentially significant sources (Domingo et al., 2012a; Shoeib et al., 2011, Haug et al., 2011). Among these sources, water consumption and food (specially, fish) have been identified the most important routes of human exposure to PFCs.

3.2.2 Reference studies

We used two datasets to benchmark the PBPK implemented in MERLIN-Expo on the two perfluorinated compounds of interest, PFOS and PFOA. The first study reported the measured concentrations of PFOA in blood serum for residents from Little Hocking, Ohio (USA). The data were obtained from the website of the Hocking Water Association (LHWA) and from the article by Emmet et al. (2006). The exposure of the population was assumed to occur only via drinking water and the daily intake was set to 3.55 ppb.

The second study was conducted by Ericson et al. (2007) and Perez et al. (2013). They measured the PFOS and PFOA concentrations in blood and several tissues (liver, kidneys and lungs) of people living in Catalonia (Spain). Food was identified as a major source of human exposure but contribution from drinking water was not negligible (Ericson et al., 2009). To estimate the daily intake for PFOA we used three studies in Spain (Domingo et al., 2012a, 2012b; Ericson, et al., 2008) and age specific food consumption in Spain from EFSA

database in exposure assessment (EFSA, 2011). The total daily intakes for PFOS and PFOA were set respectively to 204.84 ng/day and 33.45 ng/day.

3.2.3 Parameterization of the PBPK model in MERLIN-Expo

We used the values provided by Loccisano et al. (2011) to parameterize the PBPK model in MERLIN-Expo for the compound-specific parameters (Table 5).

3.2.4 Results

First, we ran the scenario for the population of Little Hocking. Because the exposure duration was not known the model was run until a steady state was reached in blood. The model predictions were then compared to the data (Figure 9). 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 blood after that type of exposure.

Then we ran the second scenario for the population in Catalonia. The model predictions are represented together with the data on Figure 10 for PFOA and on Figure 11 for PFOS. 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 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 where value of the partition coefficient used to parameterize MERLIN-Expo is clearly under-estimated. However this value is affected by high uncertainty and was not studied in the model used to parameterize MERLIN-Expo. The predictions could certainly be improved by conducting additional analyses.

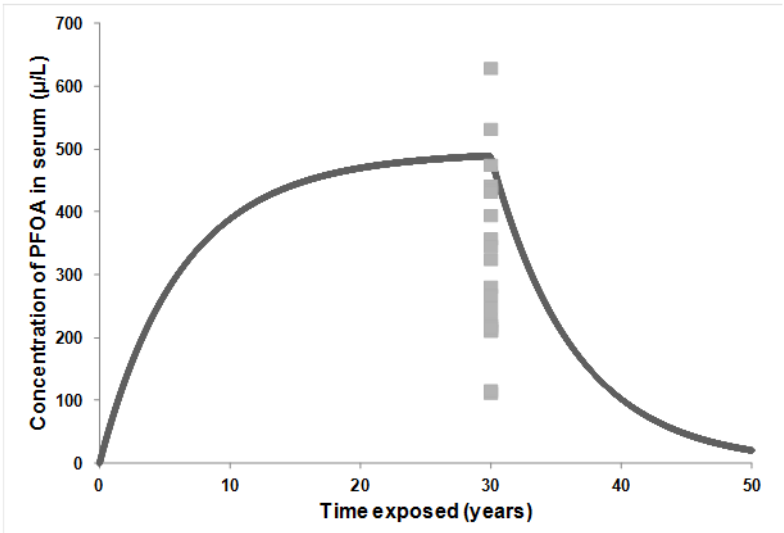


Figure 9 : Comparison of model simulations (lines) from MERLIN-Expo (B) with experimental data from Emmett et al. (2006) (squares) and the Little Hocking Water Association website. The Little Hocking population was exposed to drinking water contaminated with PFOA (3.55 ppb). The simulations were run for an exposure period of 30 years.

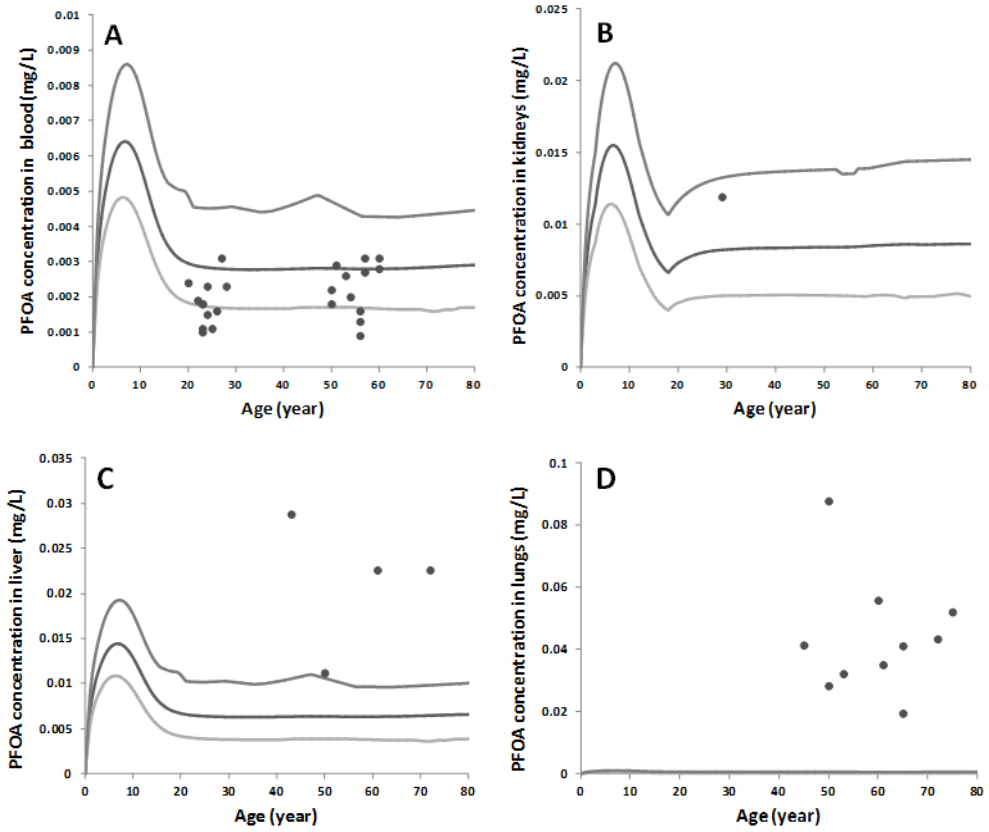


Figure 10 : Simulated (lines) and measured (circles) PFOA concentrations in blood (A), kidneys (B), liver (C), and lungs (D).The two grey lines represent the 90% interval of confidence.

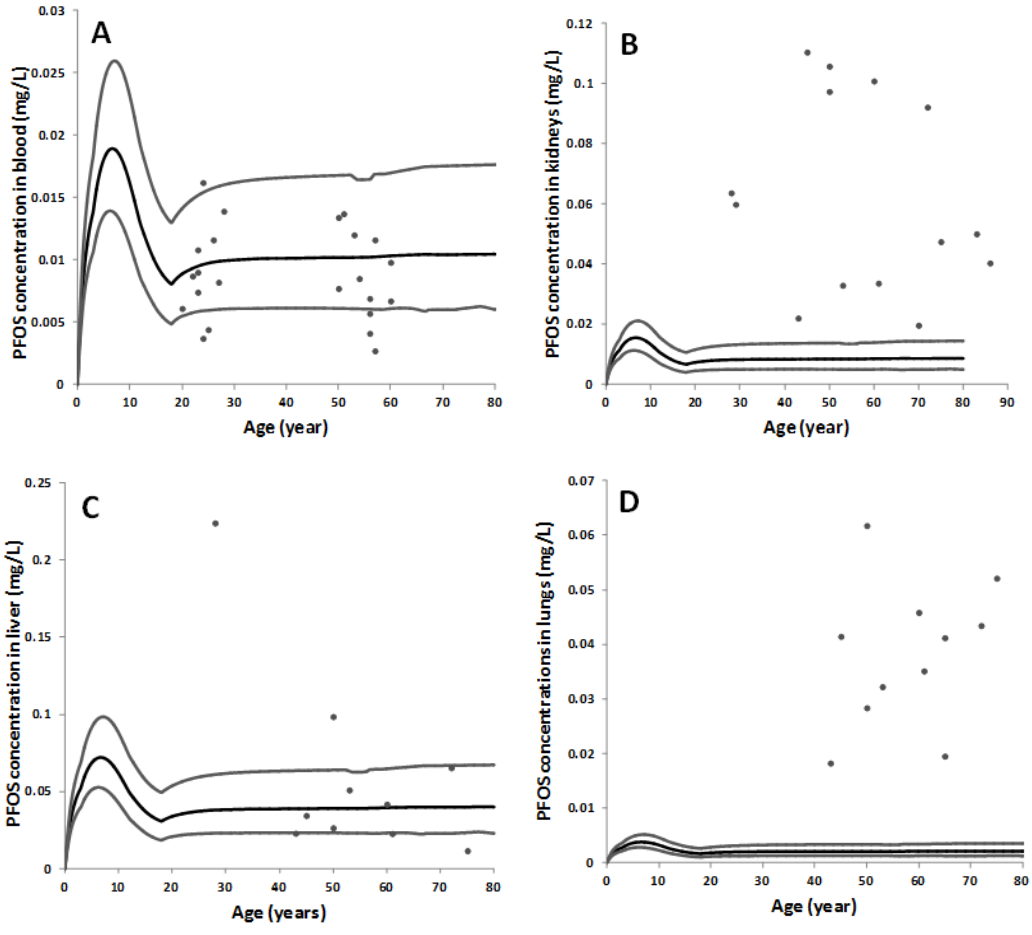


Figure 11 : Simulated (lines) and measured (circles) PFOS concentrations in blood (A), kidneys (B), liver (C), and lungs (D).The two grey lines represent the 90% interval of confidence..

3.3 Adult and children exposure to a metal

3.3.1 Chemicals

Lead is a metal that can be found in all parts of our environment: air, soil, water, and indoor environment. Lead compounds have been used in a wide variety of products, including lead-based paints, ceramics, pipes and plumbing materials, solders, gasoline, batteries, ammunition, and cosmetics. Long-term exposure to lead can cause toxicity effects and particularly in children. The principal interest adverse effect is neurotoxicity. The lead blood level is a widely used biomarker to estimate the individual body burden.

3.3.2 Reference software and studies

Because lead causes health issues especially for children, several models and software have been developed. For instance, the US Environmental Protection Agency developed the Integrated Exposure Uptake Biokinetic (IEUBK) model that became a reference software for children exposure to lead. To provide direct comparisons between IEUBK and MERLIN-Expo, the IEUBK daily lead intakes were used as input to the MERLIN-Expo model (Table 7).

We also tested MERLIN-Expo for adult exposure and used experimental data from (Azar et al., 1975). In their study, Azar et al. (1975) assessed the relationship between exposure to inorganic lead in the atmosphere and indices of lead absorption such as blood lead levels. The air lead exposure of 30 male subjects in five locations in the United States was measured with personal air samplers for twenty-four hours a day for two to four weeks. During this time period, blood samples were obtained for analyses. The demographic characteristics of the study are shown in Table 8. The five sites were selected to represent a wide range of air lead exposures.

Table 7: The daily intake of lead for children from 6 months to 7 years old in µg/day

Intake µg/day	Age (years)						
	0.5-1	**1-2**	**2-3**	**3-4**	**4-5**	**5-6**	**6-7**
Air	0.07	0.11	0.19	0.21	0.21	0.29	0.29
Diet	5.53	5.78	6.49	6.24	6.01	6.34	7.00
Drinking water	0.80	2.00	2.08	2.12	2.20	2.32	2.36
Soil	7.65	12.15	12.15	12.15	9.00	8.10	7.65
Dust	9.35	14.85	14.85	14.85	11.00	9.90	9.35
Total intake	**23.40**	**34.89**	**35.76**	**35.57**	**28.42**	**26.95**	**26.65**

Table 8 : Demographic characteristics of the study conducted by Azar et al. (1975), and the predicted and measured blood levels.

Region	Occupation	Air lead levels (microgram/m^3)	Observed mean blood levels (microgram/dL)	MERLIN-Expo estimated blood levels (microgram/dL)
Philadelphia	Cab driver	2.62	22.4	19.43
Starke, FL		0.81	16.4	16.99
Barksdale, WI		1.01	13.8	17.27
Los Angeles	Cab driver	6.10	24.6	23.81
Los Angeles	Office worker	3.06	19.9	20.01

3.3.3 Parameterization of the PBPK model in MERLIN-Expo

Parameters values used for the MERLIN-Expo model are shown in Table 5 and were taken from the article by Sharma et al. (2005). For lead, we used the option “binding with erythrocytes”.

3.3.4 Results

Figure 12 represents the predictions for the 2 models tested (MERLIN-Expo and IEUBK) for children exposure between 1 and 7 years old. The results show a good agreement between the predictions of MERLIN-Expo and IEUBK model (differences range between 5% and 30%).

Figure 13 presents the predictions for the second scenario. The predictions of the blood lead levels in adults using MERLIN-Expo are very close to the measured concentrations. Table 8 also presents the numerical values for the predictions and measurements and highlights the good correspondence between them (differences range between 1% and 20%).

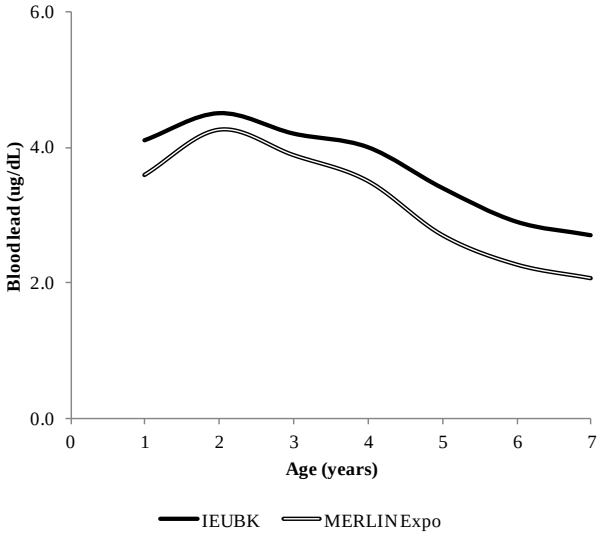


Figure 12 : Predicted blood lead levels in children obtained by the IEUBK model and MERLIN-Expo

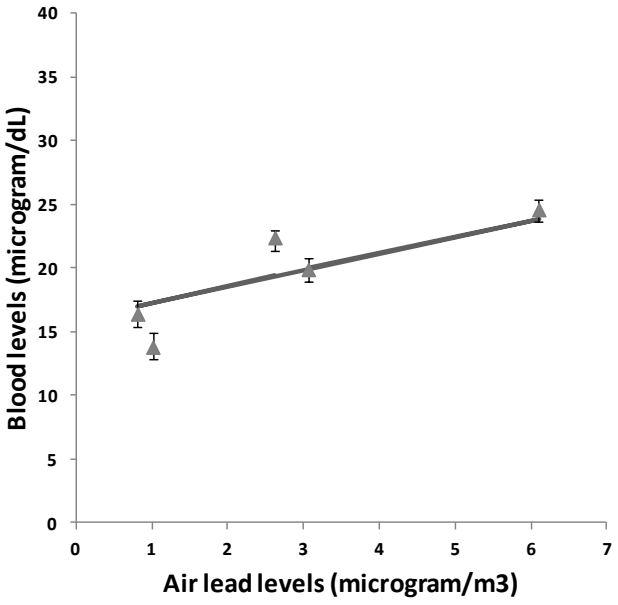


Figure 13 : Blood levels measured in (Azar et al., 1975) and predictions using with MERLIN-Expo.

3.4 Conclusion on the benchmarking of the PBPK model

We used three different test studies to benchmark the PBPK model implemented in MERLIN-Expo. Our results show a good predictability of our model compared to experimental data and published or reference models. It should be noted that all the models tested were usually developed for a specific chemical or chemical family, and that MERLIN-Expo is intended to be a generic model to be applied for numerous contaminants. The discrepancies observed with the data or other models are in the range of what is acceptable in toxicokinetic modeling as the knowledge of the behavior of the compounds is affected by uncertainty (for example, in parameter's values).

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