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Studying physiologically based pharmacokinetic modeling reliability in human exposure assessment. A case study of drinking water contamination by perfluoroalkyl substances in northern Italy

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ABBREVIATIONS

ADME = absorption, distribution, metabolism and excretion

AOP = Adverse Outcome Pathway

ARPAE = Environmental Protection Agency in the Emilia-Romagna region

ARPAV = Environmental Protection Agency in the Veneto region

BMI = Body Mass Index

CNR = National Research Council

GAC = Granular Activated Carbon

HBM = Human Bio-monitoring

ISS = Istituto superiore di sanità (= Italian National Institute of Health)

PBPK = physiologically based pharmacokinetic

PFAS = in this work it is used to indicate PFOA and PFOS only

PFASs = perfluoroalkyl substances

PFHxS = perfluorohexane sulfonic acid

PFNA = Perfluorononanoic acid

PFOA = Perfluorooctanoic acid

PFOS = Perfluorooctanesulfonic acid

WHO = World Health Organization

ABSTRACT

Introduction. A huge drinking water contamination due to perfluoroalkyl substances (PFASs) occurred in the Veneto Region (northern Italy). The contamination started likely in the late Sixties and high PFASs concentrations are still present in rivers and groundwater. More than 130 000 people have been exposed to PFASs for many years. Very high PFOA serum concentrations and quite low PFOS serum concentrations were observed in the study population in the context of a Human Bio-monitoring (HBM) study.

Methods. Three well-known physiologically based pharmacokinetic (PBPK) models (Loccisano et al., 2011; Thompson et al., 2010 and Bartell, 2017) were tested and one of them (Loccisano et al., 2011) was modified to improve the accuracy of the result. In the testing procedure, predicted PFOA and PFOS serum concentrations were compared (at individual and at aggregate level) with observed data collected in the HBM study. The adjustment of the human PBPK model proposed by Loccisano consisted of a model optimization obtained modifying some input parameters and equation terms in the original model code.

Results. All the tested models underestimated the observed PFOA serum concentrations and overestimated the observed PFOS serum concentrations. However, the predicted and the observed values were of the same order of magnitude for all the investigated municipalities, both for men and women. The results were very similar between the tested models and a significant improvement was obtained with the modified model.

Conclusions. The suggested analysis for this case study (and similar) was the use of the modified model at aggregate level, in order to obtain the most accurate results without spending too much time in simulation settings. However, using the Bartell model, satisfying results can be obtained, especially for low PFOS serum concentrations and when the end of the exposure is not distant in time.

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INTRODUCTION

A huge drinking water contamination due to perfluoroalkyl substances (PFASs) occurred in the Veneto Region (northern Italy). The contamination started likely in the late Sixties and high PFASs concentrations are still present in rivers and groundwater. More than 130 000 people have been exposed to PFASs for many years.

Even if PFASs represent an emerging environmental issue and have been claimed to pose concerns for the environment and human health, the epidemiology and toxicology of these chemicals is still limited and their toxicokinetics and underlying mechanisms poorly understood. Due to the presence of strong carbon-fluoride bonds, PFASs are generally stable to metabolic transformation and to environmental degradation. They have been suggested to bioaccumulate into the liver of living organisms and for some of them, such as the perfluorooctanoic acid (PFOA), the absorption, distribution, metabolism and excretion (ADME) processes seem to be supported by transporter families, which may facilitate the gastrointestinal absorption, the uptake by the tissues, and the excretion and reabsorption via the kidney. Understanding these processes, as well as identifying the main routes of exposures, would be essential to define the real risk for human population and set up scientific-based approaches to risk management. Moreover, finding an accurate and reliable health risk assessment framework to investigate the risk of a population exposed to pollutants in the environment is one of the main issue in the Risk Assessment science nowadays (EPA, 2019). At the same time, the use of a fast method giving a hint of the entity of the risk for the target population is of equal importance for Local Authorities and environmental protection agencies to design and carry out timely, efficient and cost-effective health interventions and site remediations.

Following the purpose to produce evidences to be used by decision makers about the risk posed by PFASs for the Veneto population, Veneto region set up an international call for projects on two separate aspects of this issue: epidemiological aspects and mechanisms. The environmental protection agency of the Emilia-Romagna region (ARPAE), together with the environmental protection agency of the Veneto region (ARPAV), London School of Hygiene and Tropical Medicine (LSHTM) and with the participation of the University of Modena and Reggio Emilia (UNIMORE), presented a project proposal on the second issue, that was accepted and financed by the CORIS consortium. The project, called "Pharmacokinetics Modeling for Pfas Exposure and related Risk" (PAMPER) started in 2018 and will end in December, 2021.

The project main purpose is to test and adjust physiologically based pharmacokinetic (PBPK) models developed for PFASs and highlight the toxicological behaviour and risk for human health associated to PFASs exposure.

The project has the general ambition to create a system to support the collection and processing of information for a complete and comprehensive picture of the fate of the PFASs present in the territories of the Veneto region in order to support the regional policies of public health. However, the project can be considered also as a prototype framework, exportable to other situations and areas, and usable in other actual or presumed contamination of substances released in environmental matrices. The understanding of the final destiny of PFASs in humans and the timing of excretion after cessation of exposure are information that will be helpful in defining the measures to reduce concentrations in the environment to useful indications on time windows of possible damage in humans, due to acute and chronic exposures. The project will increase knowledge about the mode and mechanism of action of PFASs, which is currently unknown, and the level of dose or concentration related to possible adverse effect, supporting the scientific-based process of risk assessment to human. Indeed, the integration of the data on the PFASs toxicological profile, their effective concentrations, toxicokinetics and fate in human body will provide

the essential information to the decision makers about the risk of PFASs and the scientific evidence to refine the process of risk management.

This approach integrated three main phases. Phase 1 focused on the exposure assessment of the population, including the development of a geographical approach to reconstruct historical exposures. Phase 2 included use, validation and modification of PBPK models. Phase 3 saw the description of the chemical-agnostic toxicodynamics based on the AOP (Adverse Outcome Pathway) approach. This integrated model will allow the quantitative prediction of in vivo response to PFASs exposure, and the complete description of the pathway leading to the AOP. In fact the use of the PBPK model to develop a model-based contextualisation of in vitro toxicity data aims to understand the mechanisms underlying PFASs toxicity, identify their mode of action, predict the risk for the exposed populations, when tissue and organ critical concentrations are reached, and to identify thresholds of effect.

The main work carried out in the first two phases of the PAMPER project is described in the present thesis. More specifically, the work consisted first in developing an effective exposure assessment strategy, and subsequently in testing several PBPK models (Loccisano et al. 2011; Thompson et al., 2010; Bartell, 2017) predicting PFOA and PFOS (PFAS) concentrations in organs and tissues of the human body. In the model testing procedure predicted PFAS serum concentrations were compared with the observed ones, derived by a blood sampling campaign conducted in the context of a Human Bio-monitoring (HBM) study that involved the exposed population in the study area. Finally, the code of one of the tested models (Loccisano et al. 2011) was adjusted setting the input parameters and adding some terms in the differential equations in order to obtain a new model that improved the accuracy of the prediction (i.e. lower deviation from the observed data).

The first purpose of this work was to provide indications and suggestions to understand which is the best PBPK model to adopt in case of a widespread

contamination of PFAS like the one occurred in the Veneto Region. Following this purpose, the PFAS serum concentrations predicted using a complex multi-compartment PBPK model were compared with those from more “user friendly” one-compartment PBPK models to provide information on the most appropriate model for a specific case-study.

The development of an efficient procedure to assess the environmental exposure and the PFASs internal dose also pursued the aim to create a reliable model that in the next future could replace expensive, invasive and time consuming HBM campaigns, or at least, provide an efficient support to public health programs and plans.

Many analysis were developed to assess the efficiency of the testing procedure, the importance of some exposure variables and the uncertainty associated to predicted values. Attention was paid to the gender influence on the toxicokinetics profile by comparing the concentration values in men and women, to take into account the possible role of menstruation, cord-blood transfer and breastfeeding in PFASs excreting pathways. Moreover, we analyzed the predicted effect of the exposure to the internal dose throughout time, studying the memory effect, i.e. the elapsed time from the exposure to human body burden.

INTRODUCTION ON PFASs

Per- and polyfluoroalkyl substances (PFASs) are a class of industrial chemicals that consist of a fully-fluorinated carbon chain with a non-fluorinated functional group at the end. The huge strength of the carbon-fluorine bond (due to the high electronegativity of the fluorine atom) and the molecular structure give, in particular to the long chain PFASs (> 6 carbon atoms) properties like a strong thermal and chemical stability, a low volatility and a high hydrophobicity. This class of compounds is therefore widely produced and used for a variety of industrial applications all over the world since the late 1940's (Barry et al., 2013; Steenland et al., 2010a; Steenland et al., 2010b). In fact, many companies manufactured fabrics, carpets, clothes, paper coatings, insecticides, paints, cosmetics, cookware, food packaging and fire-fighting foams (Sarigiannis et al., 2017; Domingo et al., 2012a; Wong et al., 2014), adding PFASs in their products precisely for their chemical-stabilising, flame-retardant, heat-resistant, water-resistant, oil-repellent, and surfactant-like properties (OECD, 2018).

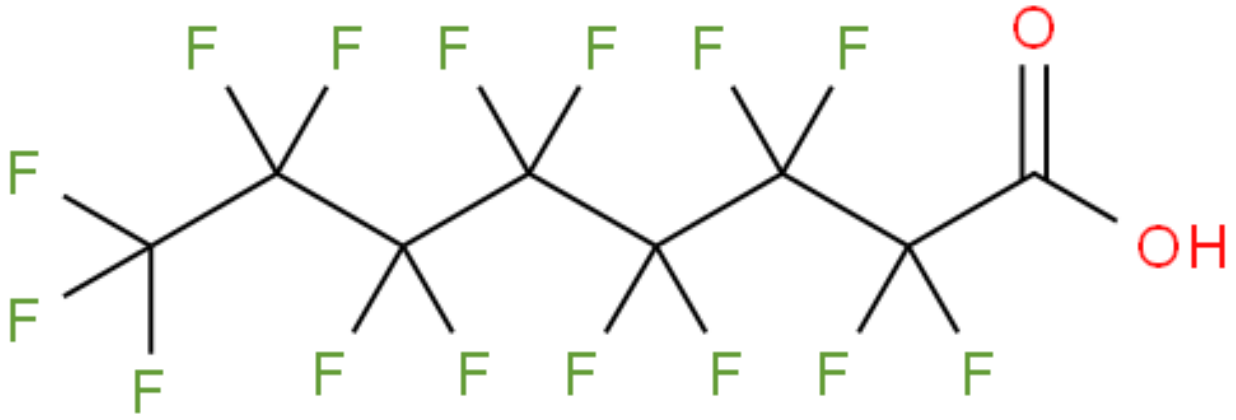


Figure 1Chemical structure of perfluorooctanoic acid (PFOA). (Sorokin et al., 2019).

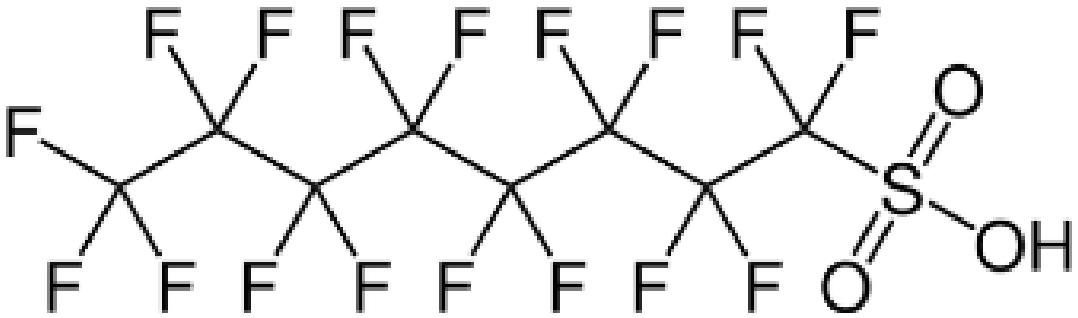


Figure 2 Chemical structure of perfluorooctane sulfonate (PFOS). (Tahziz et al., 2020).

Unfortunately, because of these same properties, PFASs have also a great mobility and a long persistence in the environment, since they can be carried by air and water for long distances. The result is a bioaccumulation and bio-magnification in living organisms. Moreover, the presence of PFASs in many products of daily use increases the entity of the background exposure for the general population in industrialized countries. This is the reason why nowadays, they can be found in traces in human blood and animals worldwide, despite the ban occurred in the last decades in many countries (Gomis, 2017).

The general population is directly exposed to PFAS through diet, drinking water, house dust, indoor air, and dermal contact. Previous studies have indicated that, especially at a certain age (e.g. toddlers) also indirect exposure can contribute quite significantly to human exposure to PFAS (Gebbink et al., 2015). Indirect exposure to PFAS may also occur in the body following uptake and biotransformation of PFAS precursors. Besides the exposure pathways that adults experience, children are additionally exposed to PFAS during the parental period via transplacental transfer and during infancy via breastfeeding (Poothong et al., 2020; Rappazzo et al., 2017).

Associations between concentrations of PFAS in human body and a range of health outcomes have been observed in epidemiological studies including low birth weight (Apelberg et al., 2007a; Washino et al., 2009), testicular cancer (Barry et al., 2013), metabolic alteration, immunotoxicity, thyroid cancer, high cholesterol (ATSDR,

2018; EFSA, 2018). The potential toxicities of PFAS, in particular perfluorooctane sulfonate (PFOS), have also been examined via high-throughput screening in vitro studies with a broad dose range (1 nM to 100 µM), and PFOS exposure has been associated with several toxicity endpoints, including PPAR/PXR/RAR receptors, neurotoxicity, aquatic toxicity, immunotoxicity, endocrine disruption, and activation of cytochrome P450s (U.S. EPA, 2016).

INTRODUCTION ON PBPK MODELS

Due to the concern increase for the health risk associated to long-chain PFAS exposure over the last few years, the challenge is now to find out a valid method to assess human PFAS uptake and effective dose in organs and tissues. Physiologically based pharmacokinetic (PBPK) models are a promising tool in providing an answer to this challenge. PBPK modelling employs a compartmental structure that incorporates anatomic and physiologic characteristics of the body and its tissues to map chemical movement. PBPK models is based on the rates of pharmacokinetics (PK), describing the processes of chemical absorption, distribution, metabolism, and excretion (ADME) and takes into account the dynamic interactions that exist between the physiological, physiochemical, and biochemical properties of the compound and the biological system (Paini et al., 2019; WHO, 2010). PBPK models can assess implications of variability and uncertainty for a predicted outcome of interest in a population), can assimilate biomonitoring data (Pletz et al., 2020) to estimate the individual exposome (Sarigiannis et al., 2019b) and can also assess the aggregate exposure for environmental chemicals via multiple sources and routes of exposure (Kenyon et al., 2016).

PBPK models requires in input all the parameters necessary to assess the external exposure, the intake and the toxycokinetics of the chemical in human body. Therefore environmental measurements, life-style information and human toxicokinetic and toxicodynamic data for substances are needed.

The main advantage of PBPK models compared to biomonitoring studies is the avoidance of invasive procedures in predicting the effective dose in the target organs. So, in the next future, these models, once they are properly validated, will likely be able to save time and efforts avoiding waste of energies and resources in long human samples measurements campaigns. That’s a reason why it’s so important to test PBPK models predictions with observed data.

Modeling PFASs pathway in the human body can occur using two different kind of PBPK models: generic or compound specific. “Generic models are toxicokinetic models that account for the main human anatomy (i.e. all major tissues and body compartments) and physiology (i.e. all major processes related to absorption, distribution, metabolism and elimination), independently of the compound” (Sarigiannis, et al. 2019b) but they provide for the compound-specific parameterization option. So, they are created to be used for many chemicals and are very flexible.

“In contrast, compound specific PBTK models focus on the compartments and the processes that are essential to capture the toxicokinetic properties of the specific compound(s)” (Sarigiannis, et al. 2019b) at best. So, they are developed for one specific chemical only.

In our opinion compound specific models developed for PFAS should be more reliable in predicting the inner dose, since they take into account compound specific mechanisms. In the case of PFASs, the renal resorption, responsible for the PFASs high half-lives in humans, is the principal mechanism that today is considered in compound specific models only.

So, even if good results were obtained for many chemicals using generic PBPK models (Sarigiannis, et al. 2019b; Vaccari et al., 2020), they can overlook important compound characteristics, that often produce an important effect on the compound ADME processes. That is even more true when pollutants show an uncommon toxicodynamics and toxicokinetics, like PFASs. On the contrary, compound specific models are created with all the necessary compartments to model pollutant pathway at best, taking into account the specific toxicodynamic and toxicokinetic properties of the chemical.

The pharmacokinetic and pharmacodynamic characteristics of PFAS have been studied in animals (Lau et al., 2007). PFAS are well absorbed in oral intake, not metabolized and poorly eliminated (Cui et al., 2009; Hundley et al., 2006). The

main tissues of distribution are plasma and liver, being the concentration in liver several times higher than concentration in plasma.

Recently, a number of PBPK models have been developed for PFAS. However, most of them are limited to the study of PFOS and PFOA. These include a human model for PFOA and PFOS (Fàbrega et al. 2014, 2016; Loccisano et al. 2011; Worley et al. 2017b), models for PFOA and PFOS in monkeys (Loccisano et al. 2011), models for PFOA and PFOS in rats (Harris et al., 2008; Loccisano et al. 2012a, 2012b; Tan et al. 2008; Worley et al., 2015), and a model for PFOA in mice (Rodriguez et al. 2009). Models of PFOA and PFOS kinetics during gestation and lactation in rats and mice also have been reported (Loccisano et al. 2012a, 2012b; Rodriguez et al. 2009). In addition, there are few studies on other PFAS. Gomis (Gomis, et al. 2017) proposed an interesting population-based model to assess PFOA, PFOS and PFHxS concentrations in human serum. Given the toxicokinetic differences between compounds, the PBPK models implemented for PFOA and PFOS may not be appropriate for other compounds.

PBPK MODELLING DEVELOPED FOR PFASS: A COMPREHENSIVE REVIEW

A comprehensive review of PBPK models available for PFAS is provided in the present study, to highlight their advantages and deficits.

Moreover, the analysis of the literature on PBPK models implemented for PFASs was necessary to collect many values for several parameters required as input in the PBPK models in order to use them in the optimization of an existing model. So, the final purpose of the review was providing data to optimize a model that can predict more accurate PFAS serum concentrations for the subjects of the Veneto region respect to the other models tested.

The comprehensive review of the literature (last update: 17/09/2020) was performed by searching Web of science, Scopus and PubMed for studies on published PBPK models for PFAS. Search terms included an extensive list of perfluorinated compounds and PBPK models. Searches were performed by joining two parenthetical terms with an AND operator. The first term was comprised of PFAS terms linked with OR operators, and the second with PBPK modeling terms linked with OR operators. Duplicates were removed from results.

All the PBPK models developed to simulate the ADME of PFAS in animals or human found with this research were collected and analyzed.

PFAS Search Terms		Pubmed	Web of Science	Scopus
Perfluorinated	Physiologically based pharmacokinetic	((((((((((Perfluorinated) OR (perfluorooctane sulfonate))	TOPIC: (Perfluorinated) *OR* TOPIC: (perfluorooctane	(perfluorinated OR perfluorooctane sulfonate OR

perfluorooctane sulfonate	Physiologically based toxicokinetic	OR (perfluorooctanoate)) OR (Polyfluoroalkyl*)) OR (Perfluorinated*)) OR (Perfluorooctanoic acid)) OR (perfluorooctane sulfonic acid)) OR (perfluorinated acid)) OR (fluorocarbons)) OR (Perfluorinated alkyl substances)) OR (fluorinated organic compounds)) OR (PFAS)) AND (((((((Physiologically based pharmacokinetic) OR	sulfonate) *OR* TOPIC: (perfluorooctanoate) *OR* TOPIC: (Polyfluoroalkyl*) *OR* TOPIC: (Perfluorinated*) *OR* TOPIC: (Perfluorooctanoic acid) *OR* TOPIC: (perfluorooctane sulfonic acid) *OR* TOPIC: (perfluorinated acid) *OR* TOPIC: (fluorocarbons) *OR* TOPIC: (Perfluorinated alkyl substances) *OR* TOPIC: (fluorinated organic compounds) *OR* TOPIC: (PFAS) AND TOPIC: (Physiologically based pharmacokinetic) *OR* TOPIC: (P	perfluorooctanoate OR polyfluoroalkyl* OR perfluorinated* OR perfluorooctanoic acid OR perfluorooctane sulfonic acid OR perfluorinated acid OR fluorocarbons OR perfluorinated alkyl substances OR fluorinated organic compounds OR pfas) AND (physiologically based pharmacokinetic OR physiologically based
Perfluorooctanoate	Physiologically based biokinetic			
Polyfluoroalkyl*	PBTK			
Perfluorinated*	PBBK			
Perfluorooctanoic acid	Physiologically based kinetic			
perfluorooctane sulfonic acid	PBK			
perfluorinated acid				
fluorocarbons				
Perfluorinated alkyl substances				
fluorinated organic compounds				
PFAS				

		(Physiologically based toxicokinetic)) OR (Physiologically based biokinetic)) OR (PBTK)) OR (PBBK)) OR (Physiologically based kinetic)) OR (PBK)): 76 results	hysiologically based toxicokinetic) *OR* TOPIC: (Physiologically based biokinetic) *OR* TOPIC: (PBTK) *OR* TOPIC: (PBBK) *OR* TOPIC: (PBK) *OR* TOPIC: (Physiologically based kinetic): 35 results	toxicokinetic OR physiologically based biokinetic OR pbtk OR pbbk OR physiologically based kinetic OR pbk): 16 RESULTS

Table 1 Search terms used for comprehensive review.

The prisma diagram is reported in the figure below:

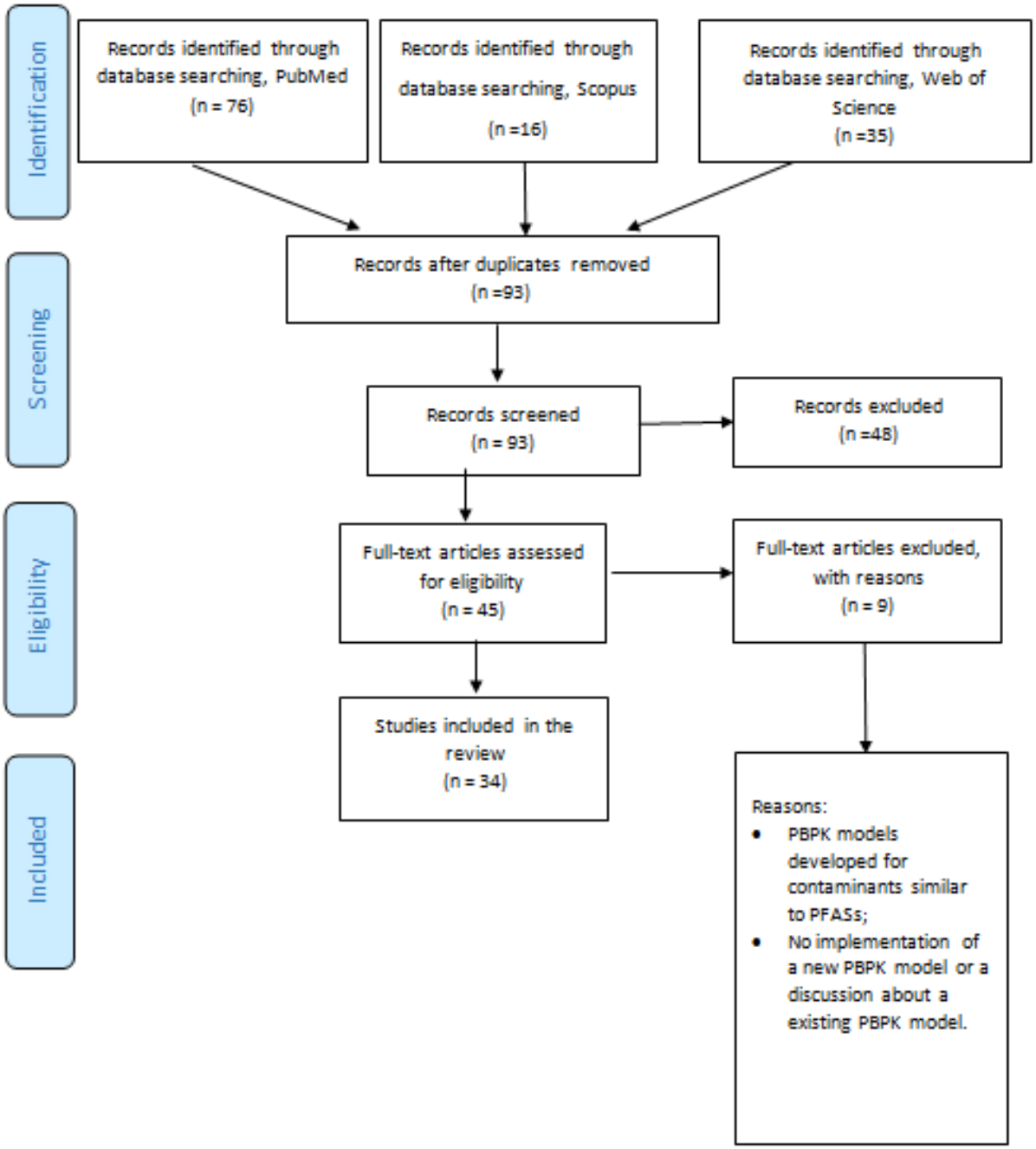


Figure 3Prisma diagram.

From the analysis of the literature collected as the result of the comprehensive review a complex multi-compartment PBPK model (Loccisano et al., 2011) was chosen for the testing procedure. In the next section that model and the similar models found in the context of the comprehensive review and useful for the present study, are briefly described.

THE "ORIGINAL LOCCISANO" MODEL (LOCCISANO ET AL., 2011) AND SIMILAR MODELS

The model that was deeply studied and modified to obtain a better prediction of the PFAS serum concentrations for the subjects living in the Veneto region was the model proposed by Loccisano (Loccisano et al., 2011), called in the present study the "original Loccisano" model to distinguish between the model developed by Loccisano and the same model with some changes and adjustments.

The model was chosen for its completeness in describing the ADME of PFAS in human body and its accuracy in predicting observed data (Loccisano et al., 2011; Fabrega et al., 2014). Moreover the model code was reported in detail by the authors as supplementary material to the article (Loccisano et al., 2011) and this fact provided the possibility to easily modify it to adapt the test the model at best and to adapt it to the subjects studied.

The original Loccisano model was based on the model developed by Andersen (Andersen et al., 2006), that was one of the first PBPK models implemented specifically for PFOS and PFOA. The model, who described the elimination of PFOS and PFOA in monkey, included three compartments (central compartment, tissue and filtrate) and included two key features of the ADME characteristics of PFOS and PFOA. The first feature was the PFAS elimination mainly through urine, following a resorption mechanism, i.e. when the chemicals are in urine they are resorbed back to plasma following a saturable process. This kinetic is analogous to the Michaelis-Menten kinetics. The second feature of the PBPK model developed by Andersen was the binding of PFOS and PFOA to plasma albumin. Consequently, only a fraction of the total amount of PFASs present in urine was available to be distributed to other tissues. Afterwards, the PBPK model was extended to rats and humans, and the pharmacokinetics and pharmacodynamics properties of PFOS and PFOA were studied more deeply (Loccisano et al., 2012a; Lou et al., 2009; Tan et al., 2008).

Rat Models developed by Loccisano (Loccisano et al., 2012a; 2012b):

Loccisano (Loccisano et al., 2012a) developed a model for simulating the kinetics of PFOA and PFOS in male and female rats. The model was based, in part, on a multi-compartmental model developed by Tan (Tan et al. 2008; Andersen et al. 2006). The female rat model (Loccisano et al., 2012a) was subsequently extended to include gestation and lactation (Loccisano et al., 2012b). Complete lists of parameters, parameter values and the bases for parameter values, other than the evaluations of model predictions in comparison to observations are described in Loccisano articles (Loccisano et al. 2012a; 2012b).

The basic (i.e., adult non pregnant rat) model includes compartments representing plasma (including a bound and free fraction), kidney and renal glomerular filtrate, liver, and a lumped compartment representing all other tissues. Two storage compartments are included in the model: one receives perfluoroalkyl from the gastrointestinal tract (unabsorbed) and liver (bile) and the other receives perfluoroalkyl from the glomerular filtrate. The storage compartments were included in the model to simulate time delays between elimination from plasma and appearance of perfluoroalkyl in feces or urine. Absorption from the gastrointestinal tract is simulated as the balance between first-order absorption and fecal excretion of unabsorbed chemical. Absorbed PFOA and PFOS are assumed to be delivered to the liver where saturable binding of PFOS (but not PFOA) to liver proteins occurs. Saturable binding of PFOS in liver was included to simulate the relatively long retention times of PFOS in liver that have been observed in rats. Exchanges between PFOA or PFOS in liver (free fraction), kidney, and other tissues with the free pool in plasma are assumed to be flow-limited (governed by blood flow) with equilibrium determined by the tissue:blood partition coefficient. PFOA and PFOS in plasma are simulated as instantaneous distributions into free and bound fractions. Extensive binding of PFOA and PFOS to plasma proteins has been demonstrated in various animal species including rats (see Table 2). For PFOA, the free fraction is assigned a constant of 4.5% in females and 0.6% in males. These values were

optimized to fit observed kinetics of PFOA in plasma and urine of rats following intravenous and oral exposures (Loccisano et al. 2012a). Adequate fit to observed PFOS plasma kinetics following single doses of PFOS required introducing a time-dependence in binding of PFOS to protein (Loccisano et al. 2012a; Tan et al. 2008). The free fraction for PFOS in plasma decreases from an initial value (after dosing) of 2.2% to a minimum of 0.1% with a t1/2 for the change of approximately 14 hours in a 0.25-kg rat (k = 0.035 hours-1/kg-0.25). The relatively short t1/2 for the change limits the effects of the time-dependent plasma kinetics over the first 1–2 days of dosing (including peak concentrations) and has no effect on longer-term kinetics or steady state. Although the time-dependence of the free fraction in plasma was needed to simulate short-term plasma PFOS kinetics in rats, the physiological mechanism for a dependence of plasma binding on the time following dosing (i.e., not on concentration of PFOS in plasma or some other dose surrogate) has not been established. Elimination of absorbed chemical occurs by biliary excretion and urinary excretion. Transfer from liver to feces (representing excretion following biliary transfer) is represented as a first-order process acting on the free fraction in liver. Excretion in urine is simulated as the balance between transfer from the free fraction to the glomerular filtrate and renal tubular reabsorption, which removes PFOA and PFOS from the glomerular filtrate and returns it to kidney tissue. Renal tubular reabsorption is simulated as a capacity-limited process with parameters Tm (μg/(hour*kg body weight)), representing the maximum rate of transport, and Kt (μg/L), representing affinity for the transporter (the concentration in the glomerular filtrate at which reabsorptive transport rate is half of maximum). This representation of renal tubular reabsorption is used to simulate observed sex differences in elimination of PFOA from plasma, which have been attributed to higher reabsorptive capacity in male rats. Values for the maximum and affinity parameters for PFOA result in higher reabsorptive clearances from the glomerular filtrate (Tm/Kt=4.1) in male rats compared to female rats (Tm/Kt=0.045), and correspondingly lower urinary clearance of PFOA from plasma in male rats.

Reabsorption parameters for PFOS are the same in both sexes and result in reabsorptive clearances that are approximately twice that of PFOA in female rats (Tm/Kt=7.2).

The basic rat model was extended to simulate gestation with inclusion of additional compartments representing adipose and mammary tissue in the dam, placenta, and fetus (Loccisano et al. 2012b). Transfer of PFOA and PFOS to the fetus is simulated as a flow-limited transfer to the placenta, with first-order exchange between the placenta and the free fraction in fetal plasma. The free fraction in fetal plasma is simulated with as a constant fraction for PFOA and PFOS (i.e., no dependence on time as in the adult). Within the fetus, PFOA in the free fraction of plasma exchanges with a single lumped compartment representing the fetal body, which exchanges with PFOA in amniotic fluid. The fetal PFOS model subdivides fetal tissue into brain, liver, and a lumped compartment for other tissues, all of which undergo flow-limited exchanges with the free fraction of PFOS in fetal plasma. Binding of PFOA and PFOS in fetal liver is assumed to be negligible. Differences in the structure of the fetal models for PFOA and PFOS reflect the differences in the availability of data of for estimating parameter values for the various compartments (e.g., perfluoroalkyl concentrations in amniotic fluid, liver).

The lactation model extends the dam portion of the gestational model to include milk and pup (Loccisano et al. 2012b). Transfer of PFOA to milk occurs through the mammary gland with flow-limited exchange between plasma and mammary tissue and diffusion into milk from mammary tissue. The model also includes transfer from the pup to the dam, which occurs during maternal stimulation of the neonatal pup to induce elimination and during pup grooming. Data on PFOS in mammary tissue of rodents were not available to establish parameters for a mammary tissue compartment; therefore, the mammary tissue compartment was left out of the PFOS model, and transfer of PFOS to milk is simulated as diffusion directly from plasma. The pup model includes compartments representing the free fraction in plasma, liver, kidney, glomerular filtrate, and a lumped compartment representing all other

pup tissues. This structure is essentially identical to the non-pregnant rat model (Loccisano et al. 2012a) with a few differences. Absorption from the gastrointestinal tract is assumed to be complete in pups, and binding in pup liver is assumed to be negligible in pups. There are no storage compartments for biliary or glomerular filtrate perfluoroalkyl in the pup model. Sex differences in renal tubular reabsorption of PFOA are assumed to develop in response to sexual maturation and, therefore, are not present during lactation (i.e., parameter values are allometrically scaled to pup body weight from the male rat values). Reabsorptive transport parameters for PFOS are allometrically scaled from the lactating dam. The liver/plasma partition coefficient for PFOS in the pups was set lower than that in the dam, based on observations in rats. All other parameters for PFOA and PFOS in the pup were the same or allometrically scaled from values for the dam.

Optimization of parameter values and evaluations of the rat models are described in Loccisano et al. (2012a, 2012b). Data sets utilized in developing and evaluating the nonpregnant rat models included single-dose intravenous and gavage studies and short-term feeding studies (Johnson et al., 1979; Kemper et al., 2003; Kudo et al. 2007; Perkins et al. 2004).

Applications for Dosimetry Extrapolation and Risk Assessment based on the rat model developed by Loccisano (Loccisano et al., 2012a,; 2012b):

The wealth of data on pharmacokinetics of PFOA and PFOS in rats allowed an extensive evaluation of the rat models for predicting plasma urinary and liver PFOA and PFOS following single intravenous or single and repeated oral dosing. Inclusion of renal tubular reabsorption parameters in the model provided accurate simulations of sex differences in elimination rates of PFOA from plasma and excretion in urine, and differences in rates of elimination of PFOA and PFOS. The gestation model successfully predicted fetal plasma and liver PFOA and PFOS at the end (or near the end) of pregnancy. Consistent with observations, the model predicts higher fetal plasma concentrations and lower fetal liver concentrations of PFOS compared to maternal, and lower internal exposure (plasma concentrations) to PFOA in the fetus

compared to maternal (fetal liver data were not available for PFOA). The lactation model successfully predicted PFOA and PFOS in pup plasma following dosing of the dam. Predicted plasma concentrations of PFOA in nursing pups were approximately 10–50% lower than maternal concentrations, whereas maternal and pup concentrations of PFOS were similar. The model could be used to estimate liver doses and corresponding plasma profiles resulting from single or repeated dosing of adult male or female rats, and maternal-fetal and maternal-pup transfer of PFOA and PFOS. The rat model was evaluated with data from a 14-week oral dosing study and has not been tested for longer exposures. Harris and Barton (Harris et al., 2008) developed a PBPK model for PFOS in the rat and found that time adjustments that increased renal clearance and decreased the liver-plasma partition coefficient as a function of time and dose improved predictions of plasma and liver PFOS in adult rats exposed for a period of 105 weeks. Although the Harris and Barton (Harris et al.,2008) model is very different from the Loccisano et al. (2012a) model, these results suggest the possibility that clearance of PFOS may be age- and/or dose-dependent in rats. This may reflect age- or dose-related changes in kidney function, including tubular reabsorption or secretion of PFOS.

THE ORIGINAL LOCCISANO MODEL (LOCCISANO ET AL., 2011, 2013): MONKEY AND HUMAN MODELS

The original Loccisano model (Loccisano et al., 2011) was developed for simulating the kinetics of PFOA and PFOS in monkeys and humans. The human model described in (Loccisano et al., 2011) was subsequently extended to include simulations of pregnancy and lactation (Loccisano et al. 2013). The monkey model was based, in part, on a multi-compartmental model developed by Tan et al. (2008; Andersen et al. 2006) for simulating the kinetics of plasma and urinary PFOA in monkeys. The structures of the monkey and human models are very similar to the structure of the rat model (Loccisano et al. 2012a), with inclusion of compartments representing fat and skin, and absence of a storage compartment for biliary transfer. Complete lists of parameters and parameter values and the bases for parameter

values and evaluations of model predictions in comparison to observations are reported in Loccisano et al. (2011).

Parameters in the monkey and human models differ in several ways from the rat model. The free fraction in plasma is represented as a constant for both PFOA and PFOS; time-dependency for PFOS in the rat model is absent in the monkey and human models. The parameters for renal tubular reabsorption of PFOA and PFOS are the same for males and females. This is consistent with the absence of evidence for a sex difference in elimination kinetics in monkeys (Butenhoff et al. 2002, 2004a; Seacat et al. 2002).

Values for the affinity constant (Kt) and maximum (Tm) for tubular reabsorption were optimized to plasma concentration kinetics in monkeys. The value for Kt in monkeys was used in the human model. The value for Tm for PFOA in humans was set to yield a plasma elimination t1/2 of 2.3 or 3.8 years. The latter two values derive from estimates of the serum t1/2 in populations exposed to PFOA in drinking water (2.3 years; Bartell et al. 2010) or in retired fluorochemical workers (3.8 years; Olsen et al. 2007). The value for Tm for PFOS in humans was set to yield a plasma elimination t1/2 of 5.4 years, based on observations in retired fluorochemical workers (Olsen et al. 2007). Binding of PFOA and PFOS in the liver was assumed to be negligible in monkeys and humans. Tissue-plasma partition coefficients used in both models were derived from observations in rodents and were the same in the monkey and human models.

The model was applied in a case study of human individuals living in Little Hocking (Ohio, USA) and Arnsberg (Germany), and exposed to relatively high concentrations of PFOS and PFOA through consumption of drinking water. The result was a PBPK model reasonably capable to estimate the concentration of PFOS and PFOA in the human body. The model was firstly developed to study the pregnant and lactating concentrations of PFOS and PFOA in rats (Loccisano et al.,

2012b) and after that, the model was scaled to humans to assess the pregnancy and lactational concentrations of PFOS and PFOA (Loccisano et al., 2013).

Optimization of parameter values and evaluation of the monkey and human models are described in Loccisano et al. (2011). Data sets utilized in developing and evaluating the monkey model included single-dose intravenous and oral studies and repeated-dose oral studies conducted in Cynomolgus monkeys (Butenhoff et al., 2004c; Noker et al., 2003; Seacat et al., 2002). Data used in evaluating the human model consisted of serum measurements in people who experienced environmental exposures (Emmett et al., 2006a; Hölzer et al., 2008; Steenland et al., 2009), adult Red Cross donors (Olsen et al. 2003b, 2008), and retired fluorochemical workers (Olsen et al., 2007). In general, PFOA and PFOS intakes and exposure durations were not known with certainty in these populations and, as a result, these data do not yield confident evaluations of the ability of the human model to predict intake-plasma level relationships. Follow-up monitoring after a cessation or decrease in exposure can provide data that allow evaluation of the ability of the model to accurately simulate elimination kinetics. Predicted declines in serum PFOA concentrations encompassed observed group mean declines when the Tm for renal tubular reabsorption was set to yield an elimination t1/2 of 2.3 or 3.8 years. Group mean declines in serum PFOS predicted reasonably well for some populations, but not all populations, when the Tm for renal tubular reabsorption was optimized to yield an elimination t1/2 of 5.4 years.

The human pregnancy model includes additional compartments representing the free fractions in plasma, amniotic fluid, and a lumped compartment for fetal tissue (Loccisano et al. 2013). The same conceptual approach was used in rat pregnancy model (Loccisano et al. 2012b). Rate constants for placental transfer were initially those from the rat model, adjusted to yield predicated maternal/fetal plasma ratios that agreed with observed maternal/fetal ratios in cord blood (Apelberg et al. 2007b; Fei et al. 2007; Midasch et al. 2007; Washino et al. 2009). Transfers from amniotic fluid to fetus were the same as those used in the rat model, as there were no data on

which to base estimates for humans. The lactation model included additional compartments for mammary milk and a lumped compartment representing the infant. Transfer of PFOA to milk is simulated as flow-limited exchange between plasma and milk, governed by mammary tissue blood flow and a milk/plasma partition coefficient. This structure obviated the need to simulate mammary tissue kinetics, for which there were no data in humans. The milk/plasma partition coefficient was calibrated to yield predictions of observed milk/plasma ratios (Fromme et al., 2010; Kärrman et al., 2007). Transfer from maternal milk to infants is the product of the milk concentration and milk production rate (assumed to be equal to sucking rate). The pregnancy model was evaluated by comparing predicted maternal/fetal plasma ratios for PFOA and PFOS with observations from various human monitoring studies (Fei et al. 2007; Fromme et al., 2010; Hanssen et al., 2010; Inoue et al. 2004; Kim et al. 2011; Midasch et al. 2007; Monroy et al. 2008; Tittlemier et al. 2004). The lactation model was evaluated by comparing predicted maternal plasma/milk ratios for PFOA and PFOS with observations from various human monitoring studies (Fromme et al. 2009; Kärrman et al. 2007). In general, most model predictions were within plus or minus 2-fold of observations.

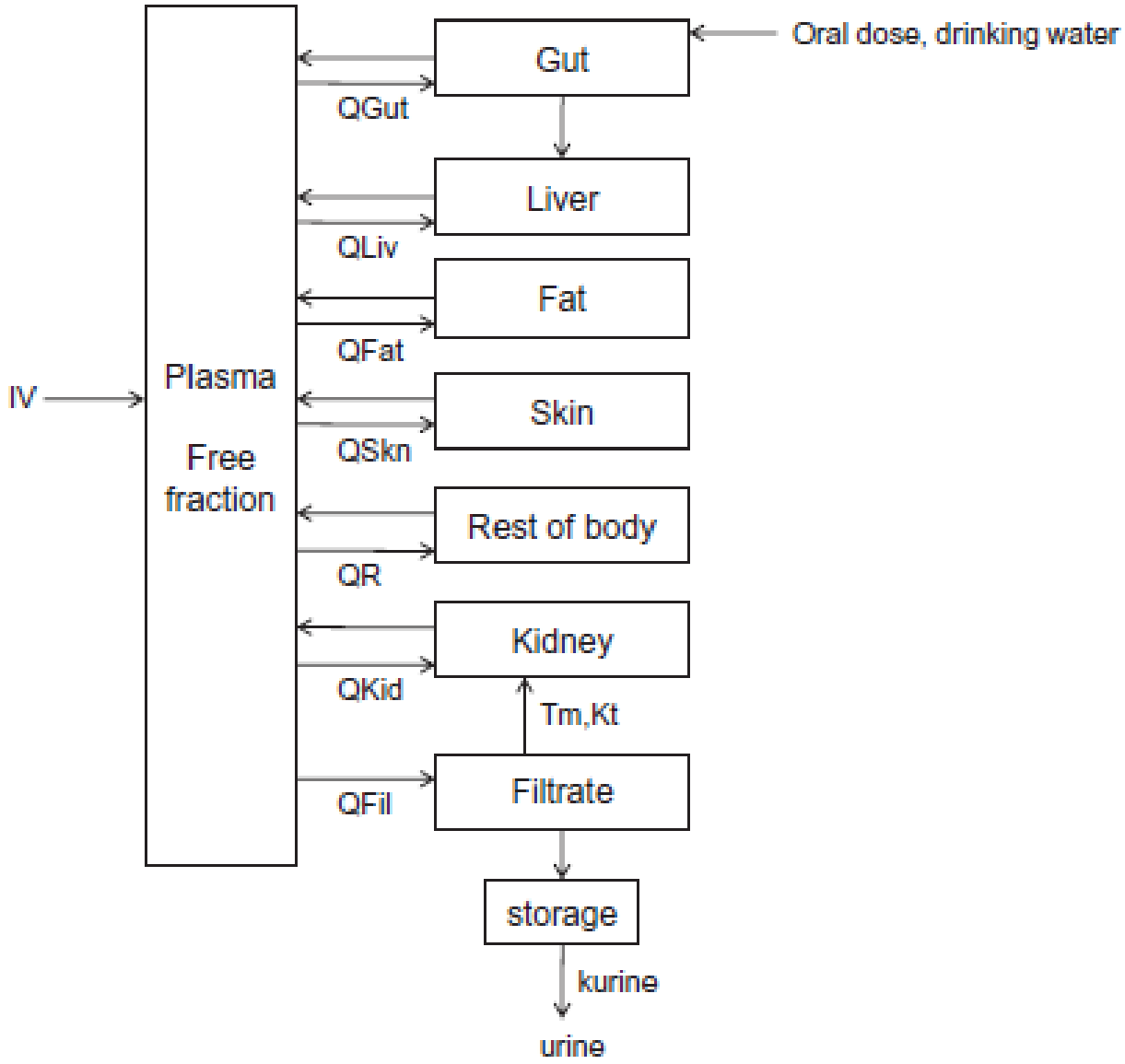


Figure 4 Structure of PBPK model developed by Loccisano (Loccisano et al. 2011) for PFOA and PFOS in monkeys and humans. "Chemical is taken up into the plasma (iv) or into the gut (oral). From the gut, chemical is transported to the liver by the portal blood. Only the free fraction of chemical in plasma is assumed to be available for partitioning into tissues. Chemical is eliminated through the filtrate compartment to storage into urine. While in the filtrate compartment, chemical can be reabsorbed back into the plasma through a saturable process with a transporter maximum Tm and affinity constant Kt. The Qs indicate blood flows into and out of tissues. Qfil is not a blood flow; it is a clearance(L/h) from the plasma to the filtrate compartment" (Loccisano et al. 2011).

Applications for Dosimetry Extrapolation and Risk Assessment based on the original Loccisano model.

The model predicts plasma concentrations and tissue levels of PFOA and PFOS following intravenous or oral dosing. A skin compartment is included in the model, which may serve for simulating absorption and distribution following deposition

onto the skin surface; however, the dermal absorption model was not evaluated in the original Loccisano model (Loccisano et al., 2011). The human model was calibrated to predict t1/2 values estimated for human populations (e.g., 2.3 or 3.8 years for PFOA, 5.4 years for PFOS). As a result, comparisons made between observed and predicted serum concentrations evaluate whether or not the populations actually exhibit the t1/2 to which the model was calibrated, and not the validity of the model to predict the internal distribution of PFOA or PFOS. It is not currently possible to assess with confidence whether the human model can accurately predict doses to liver or any other tissues. Fábrega (Fábrega et al., 2014) applied the human model to estimate plasma concentrations and tissue levels of PFOA and PFOS in human autopsy samples. Exposure inputs to the model were intakes of PFOA and PFOS estimated from public water supply concentrations in the local area where the subjects had resided (Catalonia, Spain) and concentrations in local market basket foods (Domingo et al. 2012a, 2012b). The human model predicted levels of PFOA in plasma and liver that were approximately 10- and 5-fold higher, respectively, than observed. Predicted plasma levels of PFOS were approximately 2-fold higher than observed and predicted levels of PFOS in kidney were approximately 25% of observed. Fábrega et al. (2014) explored alternative values for tissue/plasma partition coefficients, determined from human autopsy tissues (Maestri et al. 2006). The adjusted partition coefficients improved predictions of observed tissue PFOA and PFOS levels. Although the model could be applied to predicting plasma concentrations of PFOA and PFOS or intakes associated with specific plasma concentrations (e.g., oral MRLs), it is not clear what advantages the model offers over simpler empirical or compartmental models similarly calibrated to predict the serum t1/2. The monkey model has been more thoroughly evaluated for predicting plasma and urinary kinetics of PFOA and PFOS. This was possible because of the availability of more extensive experimental data on plasma and urine PFOA and PFOS following intravenous and oral (single and repeated) dosing in male and female monkeys. Nevertheless, data on internal

distribution were not available to allow evaluation of how well the monkey model predicts doses to the liver or other tissues. Predictions of plasma PFOA and PFOS concentrations from the monkey (and human) model were highly sensitive to values assigned to the maximum rate for tubular reabsorption (Tm) and other parameters that govern urinary elimination of PFOA and PFOS (e.g., free fraction in plasma and glomerular filtration rate; Loccisano et al. 2011). Optimization of the monkey models relied heavily on adjusting these same parameters and, for the human model, the target plasma elimination t1/2 was achieved solely by adjusting Tm. Thus, despite the complexity of the models, their potential to accurately predict plasma elimination kinetics and, therefore, steady-state plasma concentrations and associated oral intakes, depends largely on how well they predict plasma clearance. If plasma clearance and the free-fraction in plasma can be reliably predicted empirically for the animal species of interest, then far simpler compartmental models can be used for dosimetry extrapolation of steady-state free plasma concentrations.

FÀBREGA ET AL. (2014, 2016) HUMAN MODEL

Fàbrega et al. (2014, 2016) modified the Loccisano et al. (2011, 2013) human models for PFOA and PFOS with inclusion of brain and lung compartments and removal of the skin compartment. Tissue-plasma partition coefficients were re-estimated using data from human cadavers (Maestri et al. 2006) in place of estimates based on rat data (Loccisano et al. 2011). The major differences in the partition coefficients for PFOA were lower values for liver in humans (1.03) compared to rats (2.20), higher values for fat in humans (0.47) compared to rats (0.04), and inclusion of partition coefficients for brain (0.17) and lung (1.27). For PFOS, the major differences in the partition coefficients were lower values for liver in humans (2.67) compared to rats (3.72) and higher values for fat in humans (0.33) compared to rats (0.14). Values for parameters that control urinary excretion (Tm and Km for reabsorptive transport from glomerular filtrate to kidney tissue) were recalibrated based on plasma concentration data (Ericson et al. 2007). Fàbrega et al.

(2014) compared predictions to observed concentrations of PFOA and PFOS in cadaver samples (from Tarragona County, Spain) for constant intakes of 0.11 µg/day for PFOA or 0.13µg/day for PFOS. Better agreement with observations was achieved with partition coefficients based on cadaver data. Fàbrega et al. (2016) performed a quantitative uncertainty analysis of predictions of tissue PFOA and PFOS concentrations by assigning lognormal probability distributions to renal transport parameters, the unbound fraction in plasma, and intake. Probability distributions for PFOA and PFOS intakes were based on data from Domingo et al. (2012a, 2012b). Distributions for biokinetic parameters were established to achieve a coefficient of variation of 0.3 (Brochot et al. 2007; Sweeney et al. 2001). Observations of tissue PFOA and PFOS were within uncertainty bounds on predictions.

Animal-to-Human Extrapolations

Interspecies differences in the toxicokinetics of perfluoroalkyls and possible differences in the mechanisms of toxicity have been found. The elimination rate for PFOA in female rats is approximately 45 times faster than in male rat, 150 times faster than in Cynomolgus monkeys, and approximately 5,000–9,000 times faster than in humans (Bartell et al. 2010; Butenhoff et al. 2004c; Kemper et al., 2003; Olsen et al. 2007). Elimination of PFOS in male rats is approximately 3 times faster than in Cynomolgus monkeys and approximately 40 times faster than in humans (Chang et al. 2012; De Silva et al. 2009; Olsen et al. 2007; Seacat et al. 2002). These large differences in elimination rates imply that similar external PFOA or PFOS dosages in rats, monkeys, or humans would be expected to result in substantially different steady-state internal doses (i.e., body burdens, serum concentrations) of these compounds in each species. In addition, exposure durations required to achieve steady state would be expected to be much longer in humans than in monkeys or rats. Assuming a terminal elimination t1/2 of 1,400 days for PFOA in humans (Olsen et al. 2007), a constant rate of intake for 17 years would be required to achieve the 95% of steady state. Steady state (i.e., 95%) would be

achieved in approximately 110 days in monkeys (t1/2=25 days, Butenhoff et al. 2004c), 30 days in male rats (t1/2=7 days; Kemper et al., 2003), and 1 day in female rats (t1/2=0.2 days; Kemper et al., 2003). Using an internal dose metric such as serum perfluoroalkyl concentration and PBPK models that can account for these differences in elimination rates can decrease the uncertainty in extrapolating from animals to humans. Many perfluoroalkyl-induced effects in rats and mice are mediated through the PPARα and it is generally agreed that humans and nonhuman primates are refractory, or at least less responsive than rodents, to PPARα-mediated effects (Corton et al. 2014; Klaunig et al. 2003; Maloney and Waxman, 1999). While studies in mice have identified specific effects that require PPARα activation, for example, postnatal viability (Abbott et al. 2007) and some immunological effects (Yang et al. 2002b), other effects such as hepatomegaly and antigen-specific antibody response (DeWitt et al. 2016) were reported to be PPARα-independent (Yang et al. 2002b). Therefore, further studies are needed to expand the knowledge regarding PPARα-dependent and -independent effects that would allow selection of an appropriate animal model for perfluoroalkyls toxicity.

COLLECTED INFORMATION AND PARAMETERS

Many information about the PBPK models and many values for PFAS parameterization (useful for our model optimization) were collected in the context of the comprehensive review, as shown in the following table:

General information	**Physiological parameters**	**Partition coefficients (Tissue/Serum), PFOA/PFOS:**	**Target parameters collected:**	**Structure and mathematical representation**	**Computer implementation**	**Parameterisation**	**Model evaluation of predictive ability**

Study ID: (Study Authors-Article title)	Body weight (BW) [kg]	Tissues: Liver-Fat-Kidney-Gut-brain-lung-Skin-rest of body)	Kurine [/h/kg$^{-0.25}$]	What is the complexity in terms of number of compartments?	What software (package) is used for the implementation?	Are physiological parameter values derived from experiments, literature, or predicted?	Has the model been validated against external data (i.e., not those used for calibration)?
Basis of the PBPK model	Cardiac output, QCC (L/h/kg$^{0.75}$)-blood flows (fraction of cardiac output): fat (QFatC)-Liver (QLiverC)-Kidney (QKC)-Brain (QBrainC)-lungs (QLungsC)-Gut (QGC)-Skin (QSkinC)-Filtrate (QFilC)-Gonads (QGonadC)-rest of body (QRestC)-Richly perfused (QRC) - Slowly perfused (QSC)		Kt [mg/L]	Are steady-state or differential calculations used?		Are physicochemical parameter values derived from experiments, literature, or predicted?	Are the validation data adequately reported?
Purpose of the model	Hematocrit (Htc)		T_{mc} [mg/h/kg	Are the mass		Are biochemi	Is a statistica

			$^{0.75}$]-	balance (ADME-) equations given?		cal parameter values derived from experiments, literature, or predicted?	l analysis performed?
Species/strain	Tissue volume (fraction of body weight) [L/kg BW]: ((Fat (VFC)-Liver (VLiverC)-Plasma (VPlasC)-Brain (VBrainC)-lungs VLungC) – Gut (VGC) - Kidney (VK)-Skin (VSkinC)-Filtrate (VFilC) - rest of body (VRestC) -- Gonads (VGonadC)-Richly perfused (VRC)-Slowly perfused (VSC)		Free: free fraction in plasma (unitless)				Is a sensitivity analysis performed?
Sex-Age						Has the model been	Is a visual inspectio

						calibrated with a dataset?	n of the adequacy of the model predictions possible (e.g., via concentration-time plots?)
Compound -Route of administration/exposure -Dose metric selected						Are the calibration data adequately reported (citation of an article, written)?	
Number of compartments-type of compartments							

Table 3 main information collected in the context of the comprehensive review.

Reference	Chemical studied	PBPK model purpose	species

Choi et al., 2020	PFPeA	Filling the knowledge gap on shortchain PFASs, perfluoropentanoic acid (PFPeA), in terms of its PK properties using non-linear mixed-effect modeling and to explore gender differences in rats. The study aims to develop a PK model to simultaneously analyze all kinetic data to better understand the disposition of PFPeA. Furthermore, gender differences regarding PKs of PFPeA were explored using non-linear mixed-effect (NLME) PK model. In addition, a bio-analytical method for PFPeA in plasma, urine, feces and nine organs or tissues was developed and validated.	Sprague–Dawley rat
Fujii et al., 2015	PFCAs (from C6 to C14)	Investigating the toxicokinetics of PFCAs with six to fourteen carbon atoms (C6 to C14) in mice	mouse
Dzierlenga et al., 2020	PFOS, PFOA	Exposure to PFOA and PFOS has been associated with the occurrence of thyroid disease in some epidemiologic studies. The hypothesis is that in a specific epidemiologic study the association of clinical thyroid disease with serum concentration of PFOA and PFOS was due to reverse causality. Thyroid hormone affects glomerular filtration, which in turn affects excretion of PFOA and PFOS. The researchers evaluated this by linking a model of thyroid disease status over the lifetime to a physiologically based pharmacokinetic model of PFOA and PFOS.	human
Dzierlenga et al., 2020	PFOS, PFOA	testing the hypothesis that thyroid effects on PFAS excretion could account for some or all of the association reported between subclinical thyroid disease and serum PFAS concentration in cross-sectional data on non-pregnant adults	

Ruark et al., 2017	PFOS, PFOA	The association between increased serum concentrations of PFOS and PFOA and early menopause may be explained by the fact that women who underwent menopause no longer excrete PFAS through menstruation. The objective of the study was to assess how much of the epidemiologic association between PFAS and altered timing of menopause might be explained by reverse causality.	human
Ngueta et al., 2017	PFOS, PFOA	evaluation of whether the differential use of oral contraceptives in women with and without endometriosis could contribute to the association between serum PFAS levels and endometriosis	human
Chou et al., 2019	PFOS	developing an open-source physiologically based pharmacokinetic (PBPK) model accounting for species-specific toxicokinetic parameters of PFOS	mouse
Chou et al., 2019	PFOS	developing an open-source physiologically based pharmacokinetic (PBPK) model accounting for species-specific toxicokinetic parameters of PFOS	Sprague Dawley rat
Chou et al., 2019	PFOS	developing an open-source physiologically based pharmacokinetic (PBPK) model accounting for species-specific toxicokinetic parameters of PFOS	monkey
Chou et al., 2019	PFOS	developing an open-source physiologically based pharmacokinetic (PBPK) model accounting for species-specific toxicokinetic parameters of PFOS	human
Worley et al., 2017	PFOA	applying physiologically based pharmacokinetic (PBPK) modeling and Monte Carlo analysis to evaluate the impact of historic non-drinking water PFOA exposure on serum PFOA concentrations	human

Worley et al., 2015	PFOA	Developing a PBPKmodel for PFOA in male and female rats to explore the role of organic anion transporters (Oat1, Oat3, and Oatp1a1) in sex-specific renal reabsorption and excretion of PFOA.	rat
Wu et al., 2015	PFOS, PFOA	assessing how much of the observed association between PFAS and delayed menarche might be caused by the pharmacokinetic correlates of puberty	human
Sakolish et al., 2020	PFOA	modeling reabsorption kinetics and making predictions of overall in vivo renal clearance	human
Vidal et al., 2019	PFOS	elucidating PFOS kinetics in adult rainbow trout	rainbow trout (Oncorhynchus mykiss)
Vidal et al., 2020	PFOS	studying changes in physiological functions and PFAA ADME at different temperatures	rainbow trout (Oncorhynchus mykiss)
Brochot et al., 2019	PFOS, PFOA	providing new indicators of foetal exposure for a spanish birth cohort. First, a pregnancy and lactation PBPK model was calibrated in a population framework to provide quantitative estimates for the PFOA and PFOS placental transfers in humans. The PBPK model was then used to back-calculate the time-varying daily intakes of the INMA mothers corrected for their individual history from a spot maternal concentration. Finally, the foetal exposure was simulated in target organs over pregnancy using the PBPK model and the estimated maternal intakes.	human

Rovira et al., 2019	PFOS, PFOA	establishing the prenatal exposure of the fetus/child and adjusting exposure assessment vs. biomonitoring results	human
Kim et al., 2019		Established based on in vivo study in male and female rats. The predicted rat plasma and urine concentrations simulated and fitted were in good agreement with the observed values.	Sprague–Dawley rat
Kim et al., 2019		Established based on in vivo study in male and female rats. The predicted rat plasma and urine concentrations simulated and fitted were in good agreement with the observed values.	human
Kim et al., 2018	PFHxS	developing and evaluating a PBPK model for PFHxS in male and female rats, and apply this to a human health risk assessment	Sprague–Dawley rat
Kim et al., 2018	PFHxS	developing and evaluating a PBPK model for PFHxS in male and female rats, and apply this to a human health risk assessment	human (derived from: Sprague–Dawley rat)
Khazaee et al., 2018	PFOA	simulating PFOA fate in zebrafish following waterborne exposure	zebrafish
Cheng et al., 2017	PFOA	estimating the toxicokinetics and tissue distribution of PFOA in male rats	rat
van Asselt, et al., 2013	PFOS	describing the uptake of PFOS from contaminated feed by cows and its subsequent elimination through the cows' milk	cow

Sonne C, et al., 2009	PFOS	conducting a risk quotient (RQ) evaluation to more quantitatively evaluate the effect risk on reproduction (embryotoxicity and teratogenicity) using a PBPK model.	polar bear
Loccisano, et al., 2011	PFOS, PFOA	Evaluation and prediction of pharmacokinetics of PFOA and PFOS in the monkey	cynomolgus monkey
Loccisano et al., 2011	PFOS, PFOA	Evaluation and prediction of pharmacokinetics of PFOA and PFOS in humans	human
Loccisano, et al., 2012b	PFOS, PFOA	helping define a relationship between external dose, internal tissue concentrations, and observed adverse effects, and to understand how physiological changes that occur during gestation and lactation affect tissue distribution of PFAAs in the mother, fetus, and neonate	rat
Loccisano, et al., 2012a	PFOS, PFOA	describing the pharmacokinetics of PFOA and PFOS for adult rats	rat
Loccisano, et al., 2013	PFOS, PFOA	understanding how the physiological changes associated with development affect pharmacokinetics of these compounds in the mother, fetus, and infant	human fetus, infant, pregnant and lactating women
Verner, et al., 2015	PFOS, PFOA	simulating PFAS concentrations in maternal and cord plasma to assess how much of the PFAS–birth weight association observed in epidemiologic studies might be attributable to glomerular filtration rate (GFR)	human

Fàbrega et al., 2014	PFOS, PFOA	testing an existing PBPK model for their predictability of PFOS and PFOA in a new case-study and to adapt it to estimate the PFAS content in human tissue compartments	human
Fabrega, et al., 2016	PFOS, PFOA	The parametric uncertainty associated with PBPK models developed for perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) (Fàbrega et al., 2014) were analyzed and the different validation approaches were discussed for a case-study in Tarragona County (NE of Spain)	human
Fabrega, et al., 2015	PFBS, PFHxS, PFOS, PFDS, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnD, PFTeDA	developing a physiologically based pharmacokinetic model to assess the concentration of perfluoroalkyl substances in human tissues, based on an existing model previously validated for perfluorooctane sulfonic acid and perfluorooctanoic acid (Fàbrega et al., 2014)	human
Sharma et al., 2017	PFOS	generating the time course of PFOS concentration in brain	human

Table 4 description of investigated PFASs, PBPK model purpose and species studied in the articles found in the context of the comprehensive review.

		PFOA			PFOS		
Reference	**species**	**Kt [mg/L]**	**T_{mc} $_{[mg/h/kg^{0.75}]}$**	**Free (unitless)**	**Kt [mg/L]**	**T_{mc} $_{[mg/h/kg^{0.75}]}$**	**Free (unitless)**
Choi et al., 2020	Sprague–Dawley rat						

Fujii et al., 2015	mouse						
Dzierlenga et al., 2020	human	0.055	4.8	0.02	0.023	3.27	0.025
Dzierlenga et al., 2020	human	0.055	4.8	0.02	0.023	3.27	0.025
Wu et al., 2015	human	mean:0.055; lower-upper bounds: not available; distribution: not available	mean:4.8; lower-upper bounds: 1.67-11.0; distribution: Log normal	mean: 0.02; lower-upper bounds: 0.007-0.046; distribution: Log normal	mean: 0.023; lower-upper bounds: not available; distribution: not available	mean: 3.27; lower-upper bounds: 1.1-7.5; distribution: Log normal	mean: 0.025; lower-upper bounds: 0.0087-0.058; distribution: Log normal
Ruark et al., 2017	human	mean:0.055; lower-upper bounds: not available; distribution: not available	mean:4.8; lower-upper bounds: 1.77-13.0; distribution: Log normal	mean: 0.02; lower-upper bounds: 0.007-0.054; distribution: Log normal	mean: 0.023; lower-upper bounds: not available; distribution: not available	mean: 3.27; lower-upper bounds: 1.2-8.9; distribution: Log normal	mean: 0.025; lower-upper bounds: 0.009-0.068; distribution: Log normal
Ngueta et al., 2017	human						
Chou et al., 2019	mouse				basolateral: 27.2, apical: 52.3		0.02 (male)

Chou et al., 2019	Sprague Dawley rat				basolateral: 27.2, apical: 278		0.09 (male)
Chou et al., 2019	monkey				basolateral: 20.1, apical: 45.2		0.016 (male)
Chou et al., 2019	human				basolateral: 20.1, apical: 64.4		0.014 (male)
Worley et al., 2017	human	apical transporters 0.0775; basolateral transporters 0.0201		0.02			
Worley et al., 2015	rat			0.09 (male)			
Sakolish et al., 2020	human						
Vidal et al., 2019	rainbow trout (Oncorhynchus mykiss)						prior distribution: Normal(0.025, 50); after calibration: average value ± standard deviation:0.032 ± 0.006, 95% credibility intervals: 0.028-0.045]
Vidal et al., 2020	rainbow trout (Oncorhynchus mykiss)						prior distribution: Normal(0.025, 50); after calibration: average value ± standard

							deviation:0.032 ± 0.006, 95% credibility intervals: 0.028-0.045]
Brochot et al., 2019	human			0.02			0.025
Rovira et al., 2019	human						
Kim et al., 2019	Extrapolated to a human PBPK model based on human physiological parameter. Sprague–Dawley rat						
Kim et al., 2019	human						
Kim et al., 2018	Sprague–Dawley rat						
Kim et al., 2018	human (derived from: Sprague–Dawley rat)						
Khazaee et al., 2018	zebrafish						
Cheng et al.,	rat						

2017							
van Asselt, et al., 2013	cow						
Sonne C, et al., 2009	polar bear						
Loccisano, et al., 2011	cynomolgus monkey	0.055	0.15	0.02	0.023	1.3	0.025
Loccisano et al., 2011	human	0.055	6 (half life = 2.3 y) and 10 (half life = 3.8 y)	0.02	0.023	3.5	0.025
Loccisano, et al., 2012b	rat	67	3	dam: 0.045, pup: 0.006	0.0167	0.12	0.022
Loccisano, et al., 2012a	rat	67	3 (F), 270(M)	0.045 (F), 0.006 (M)	0.0167	0.12	0.022, free fraction adjustment constant: 0.94, rate constant for free fraction variation: 0.035 [/h/kg$^{-0.25}$]
Loccisano, et al., 2013	human fetus, infant, pregnant and lactating women	0.055	10	0.02	0.023	3.5	0.025
Verner, et al., 2015	human	0.055	10	0.02	0.023	3.5	0.025
Fàbreg	human	0.116	147	0.03	0.0176	86.0	0.03

a et al., 2014		[µg/L]	[µg/h], 3.60		[µg/L]	[µg/h], 2.08	
Fabrega, et al., 2016	human	0.116 [min: 1.12E-4, max: 3.0E-02] [µg/L]	6 [min: 1.46, max: 20.9] [µg/h]	0.03	0.018 [min: 3.30E-7, max: 5.0E-02] [µg/L]	3.50 [min: 0.617, max: 17.2] [µg/h]	0.03
Fabrega, et al., 2015	human	0.116 [µg/L]	T_m = 147.4 [µg/h]	0.03	0.018 [µg/L]	T_m = 86.0 [µg/h]	0.03
Sonne C, et al., 2009	polar bear				0.023	7.0 (nmole/h/kg$^{0.75}$)	0.025

Table 5values for several parameters found in the comprehensive review. Kt = resorption affinity, Tmc = maximum resorption rate, Free = free fraction of chemical in plasma.

OTHER PBPK MODELS STUDIED

THE SHIN MODEL (SHIN ET AL., 2011B)

In addition to the models found in the context of the comprehensive review, other well-known pharmacokinetic models created to predict PFAS serum concentrations in humans where found in the literature and analysed. Among these models, one of the most promising is surely the model proposed by Shin (Shin et al., 2011b). That single-compartment ADME model was developed in the context of the C8 Health Project to estimate the PFOA serum concentration in people exposed to DuPont Washington Works facilities.

The strength of that model is surely the low time consumption in comparison to the more complex multi-compartment models. Moreover, the model allows to take into account a time varying and cumulative exposure (that is the most common situation) not relying on the unrealistic condition of steady state, nor on the numerous assumptions present in the multi-compartments PBPK models about different tissue concentration. For all the reasons mentioned above, in the next future also this model will be validated with the data collected and used in the present study.

The model was based on individual residential histories, maps of public water supply networks and on the results of an environmental fate and transport model used to predict PFOA concentrations in water and in the atmosphere (Shin et al., 2011b).

The ADME model was created assuming that serum PFOA concentrations in exposed subjects were contributed from both the emissions by the Washington Works Plant (in water and atmosphere) and background exposures (deriving from the use of consumer products such as non-stick cooking material, food packaging and carpeting). The background serum concentration was fixed equal to 0 µg/L in 1950 and was assumed to vary linearly. Three average serum concentration data in

humans were collected to build the background serum concentration curve over time, corresponding to the periods: 1950, 1999-2000 and 2003-2004. Then, the following ADME model was created to estimate both the PFOA serum concentrations (derived from both the contributions: background and plant) for each year assuming piecewise-constant exposure rate and first order excretion:

$$C_t = C_{t,ww} + C_{t,bc}$$

$$C_{t,bc} = \beta_1 \cdot (t - 1950), if\ t < 1999$$

$$C_{t,bc} = C_{2000,bc} + \beta_2 \cdot (t - 1999), if\ 1999 \leq t \leq 2004$$

$$M_{t,ww} = M_{t-1,ww} \cdot e^{-k} + (1 - e^{-k}) \cdot (I_t/k), if\ 1999 \leq t \leq 2004$$

$$C_{t,ww} = M_{t,ww} + V$$

Where:

C_t = serum PFOA concentration contributed from background concentration and the Washington Works emissions for year t, [µg/L];

$C_{t,bc}$ = background serum PFOA concentration for year t, [µg/L];

$C_{t,ww}$ = serum PFOA concentration due to the emissions from the Washington Works for year t, [µg/L];

β_1 = is equal to 0.11, [(µg/L)/year];

β_2 = is equal to -0.33, [(µg/L)/year];

$M_{t,ww}$ = serum PFOA mass from the Washington Works emissions for year t, [µg];

I_t = total mass of PFOA ingested for year t, [(µg/year];

V = age- and sex-specific volume of distribution , obtained multiplying the recommended volume of distribution per weight: 0.181 L/kg for males and 0.198 L/kg for females (Butenhoff et al., 2004) by self-reported body weight, [L];

K = excretion rate coefficient for PFOA [1/year];

Serum concentrations for newborns was assumed equal to 78.5% of their mother's predicted value to consider the perinatal exposure transplacentally or via breast-feeding.

The model proposed by Shin et al. will be surely tested in the next future using the data collected in this study.

MERLIN AND INTEGRA "FULL-CHAIN" SOFTWARE

Two other promising tools, previously studied and analysed also by this research group (Vaccari et al., 2020), that incorporate a generic PBPK model (i.e. not developed specifically for PFASs) for humans were not tested in the present work. These tools are MERLIN-Expo software (https://merlin-expo.eu/. Ciffroy et al., 2016, Fierens et al., 2016) and INTEGRA platform (http://www.integra-lri.eu/. Sarigiannis et al., 2014; Sarigiannis et al., 2016).

The MERLIN-Expo software is an open-source software developed within the 4FUN Project (funded by the EU 7th Framework Programme) created for the Environmental Exposure Assessment and the Human Exposure Assessment. It models complex environmental phenomena and pollution pathways from the sources to their deposition and accumulation in human organs and tissues. The generic human PBTK model implemented in MERLIN was tested for several

pollutants (e.g. lead, anthracene and fluoranthene; Vaccari et al., 2020) but not for PFASs. The generic human PBTK model developed in INTEGRA platform (Sarigiannis et al., 2017) was designed to cover major ADME processes occurring in the human body at different life stages, to be easily applicable to a broad variety of chemicals after compound specific parameterisation (Sarigiannis et al., 2019b).

The reason why MERLIN software was not tested in the present study is that PFASs substances are not included in the list of contaminants that can be modelled with this tool. So, no essential parameters or other information about PFASs substances are provided from the software database and not an accurate description of the ADME processes (i.e. renal resorption) is provided for these chemicals. INTEGRA seems a promising tool to predict PFAS in the human tissues but the maximum simulation time was equal to 1000 hours, so a comparison with the measured data from the HBM study and the predicted values from the other PBPK models would not have been possible.

PFASs contamination in the Veneto Region

The contamination plume

A huge PFASs contamination occurred in the Veneto Region (located in the northeast of Italy) and involved many municipalities of the provinces of Vicenza in particular, but also of the provinces of Padova, Verona and Rovigo. The contamination started in Trissino (Vicenza) likely at the end of the year 1966, when a fluorochemical factory, today under trial, started to discharge liquid and solid waste containing PFASs into the near river (called Poscola river) and underground (ARPAV, 2017; ARPAV, Mazzola et al., 2018; Veneto region, 2019).

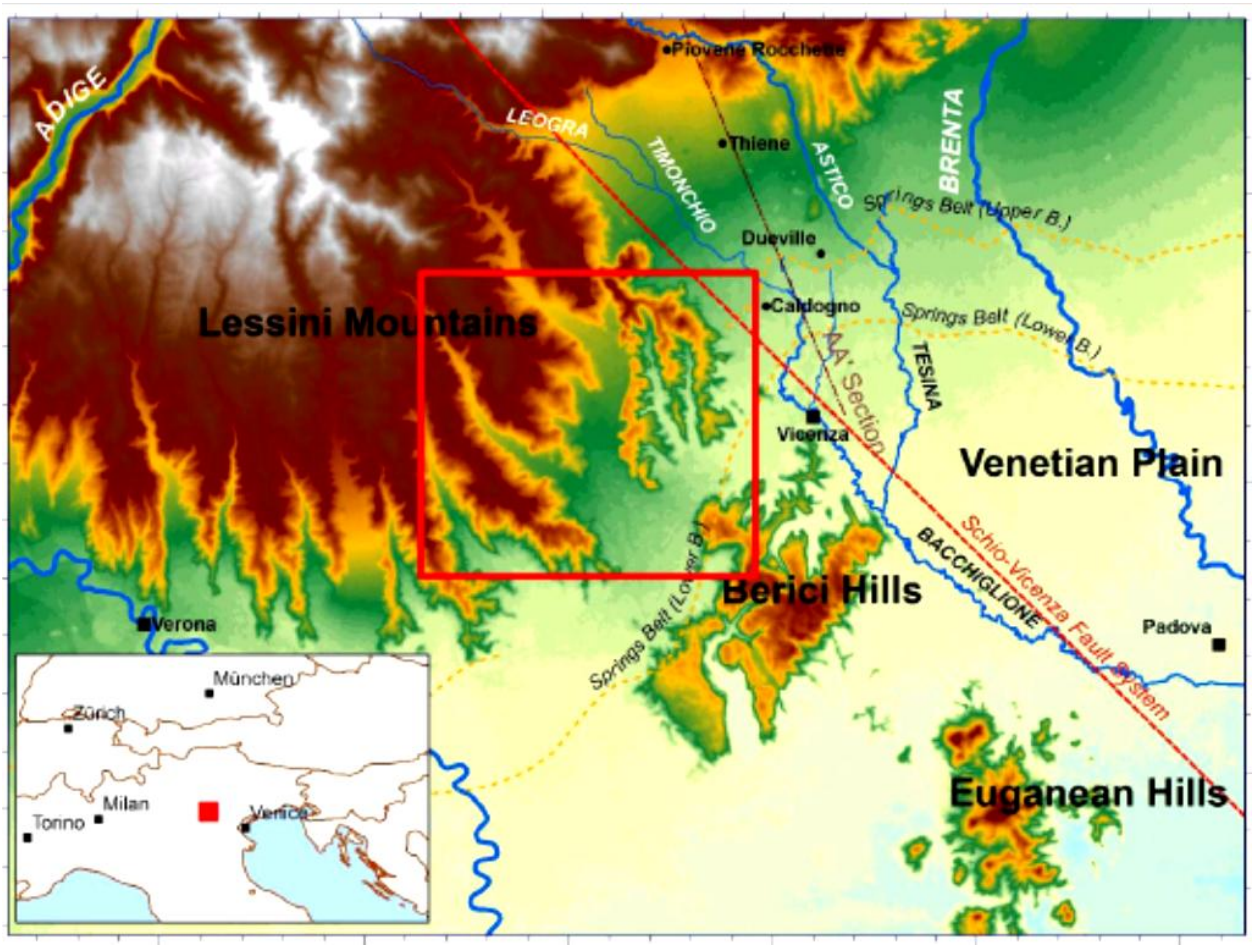


Figure 5 Study area. (ARPAV, Mazzola et al., 2018).

The PFASs contamination in tap water and surface waters was revealed in late spring 2013 by a study of the National Research Council (CNR) and was followed by an ARPAV environmental monitoring campaign that detected PFASs also in groundwater (ARPAV, 2017; Veneto region, 2019). The highest PFASs concentrations where observed in groundwater and PFOA was the most important congener for quantity (40% of the total amount of PFASs) and distribution, while PFOS was less than 5% of the total amount of PFASs (ARPAV, 2017; Veneto region, 2019).

A synthesis of knowledge on the geological and hydrogeological characteristics of the investigated area and a first conceptual model of the PFASs contamination in rivers and groundwater were proposed by ARPAV in 2013, immediately after the PFASs contamination detection (ARPAV, 2017; Veneto region, 2019).

A water flow model and a three-dimensional groundwater flow model of the deep multi-aquifer sedimentary basin in the contaminated area was developed by ARPAV (ARPAV, Mazzola et al., 2018) in 2018, according to the B4 actions of LIFE Phoenix EU project (ARPAV, Mazzola et al., 2018). The model has recently been calibrated.

According to the conceptual model developed by ARPAV (ARPAV, 2017; ARPAV, Mazzola et al., 2018), the factory producing fluoropolymer-based materials, located in the municipality of Trissino, was the main responsible for the PFASs contamination. In fact, the PFASs discharged by the factory in the near river quickly passed in groundwater because of the high permeability of the river bed. Also, the PFASs contamination of groundwater occurred through a waste disposal site located on the factory property ground.

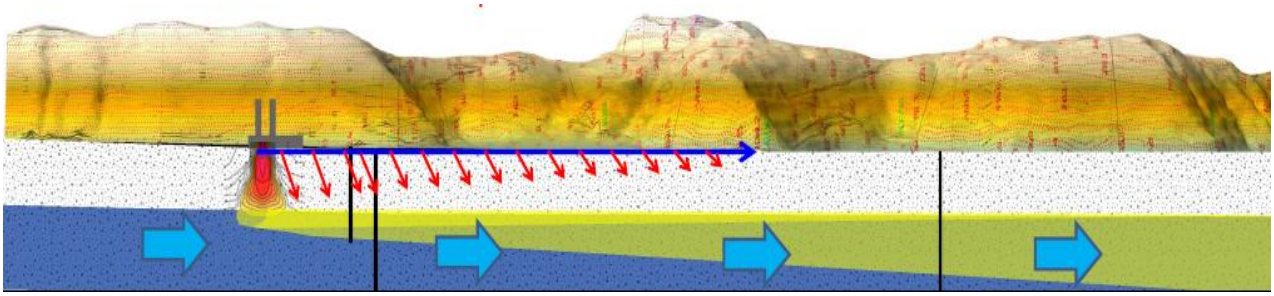


Figure 6 Schematic representation of the pollution from the source site. In yellow is highlighted the plume generated by the groundwater flow. The blue arrows show the groundwater direction movements while the orange ones explicit the pollution trigged by the dispersion of Poscola river (light blue) (ARPAV technical report, 05/2016).

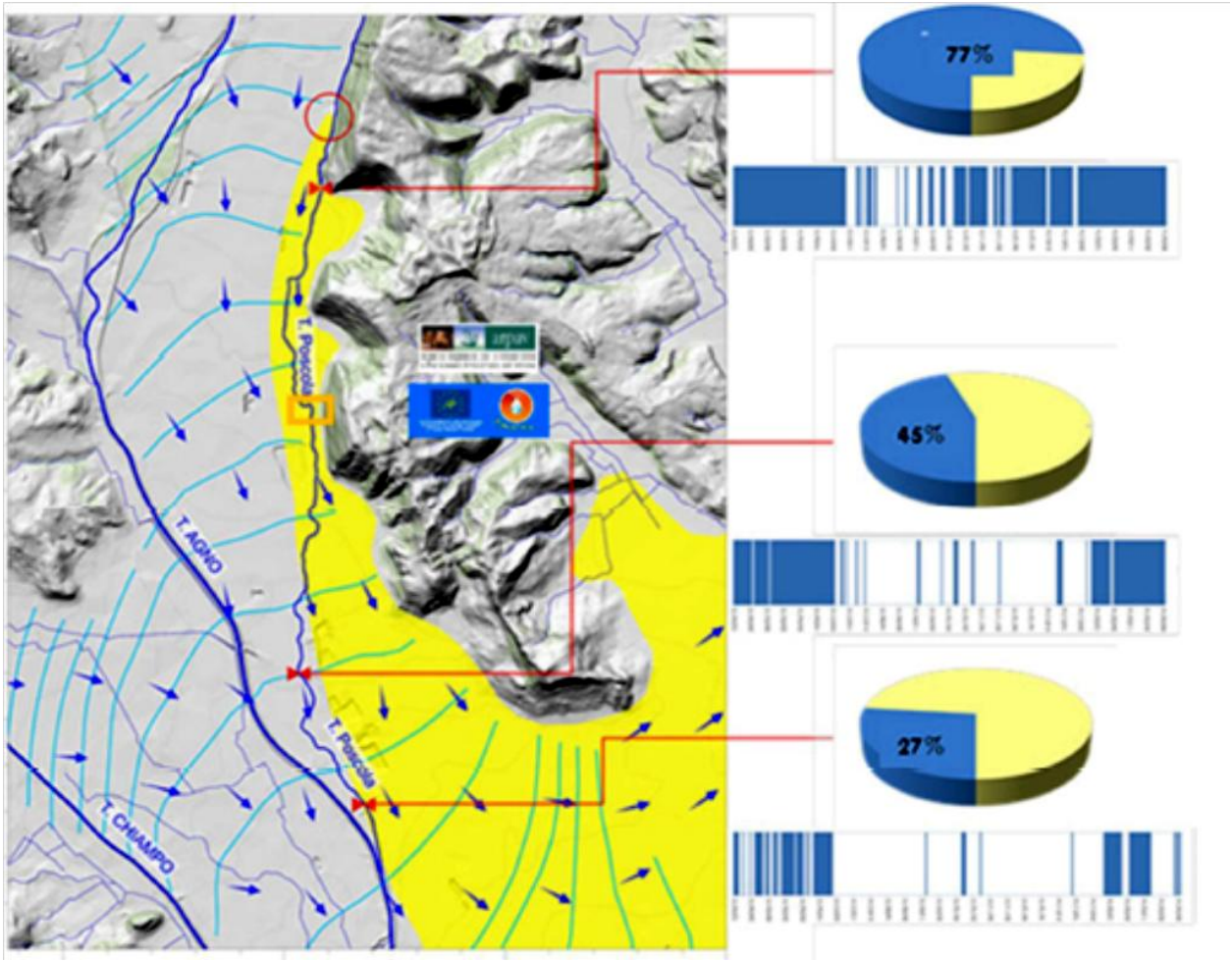


Figure 7Poscola river and location of the three sections (red arrows) analysed by digital hydrometers. Blue arrows represent the phreatic flow. The yellow area represents the plume (threshold of 500 ng/l PFAS). The bar graphs on the right side shows, with blue lines, the days with presence of runoff over the observation timeframe (April 2015 to April 2016). Pie charts show the time express as percentage with runoff (ARPAV technical report, 05/2016).

The simulated contamination plume was defined as the area where the PFASs concentration (sum of total PFASs) in groundwater was found higher than 500 ng/L and was more than 190 km^2 wide. From the contamination source PFASs seemed to

move in particular towards east (18 km) and towards south (35 km), according to the hydraulic gradient. (ARPAV, 2017; Veneto region, 2019).

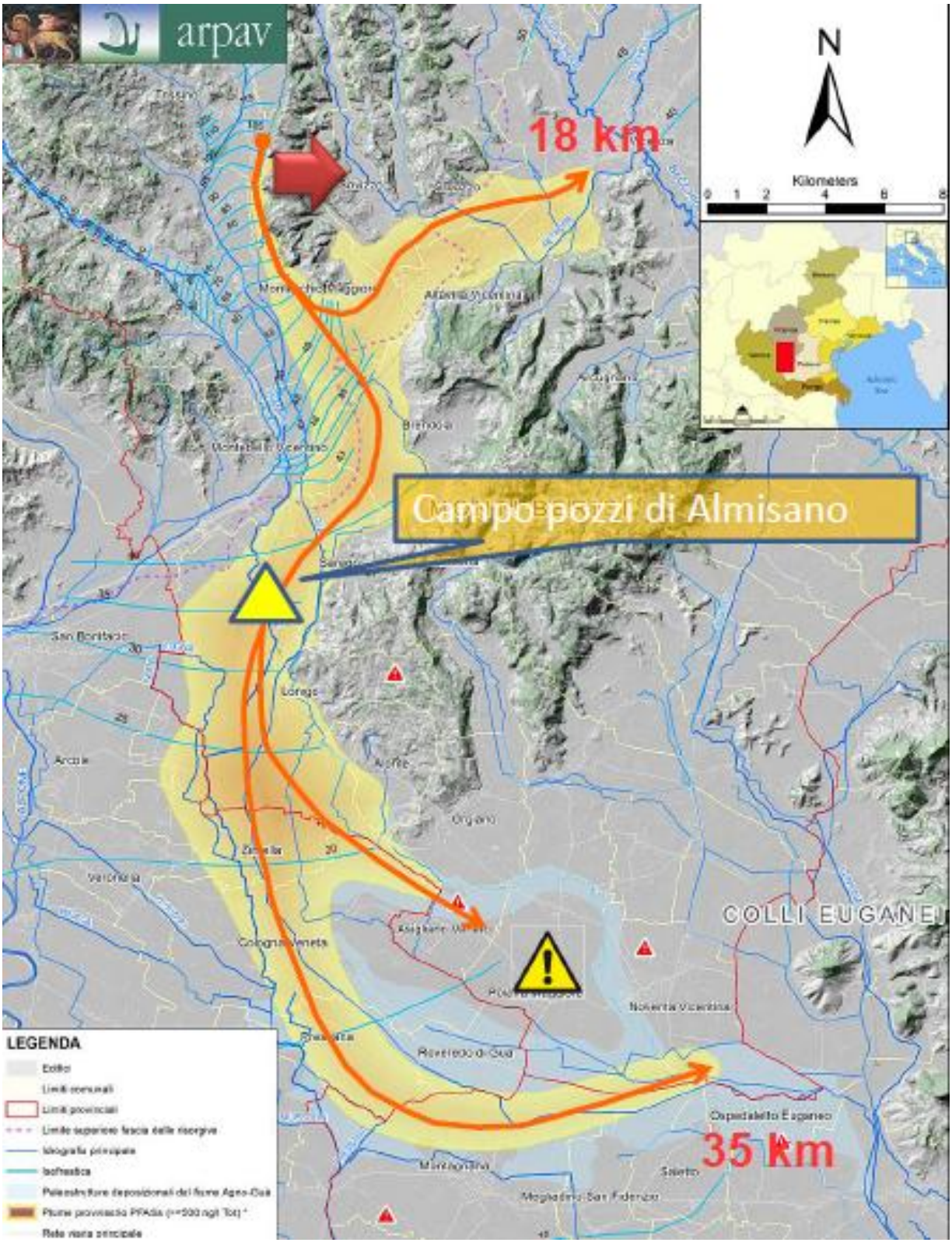


Figure 8 Contamination plume reconstruction (yellow area) March, 2016. The simulated plume was defined as the area where the PFASs concentration (sum of total PFASs) in groundwater was found higher than 500 ng/L. The plume lengths towards east and towards south are indicated by the numbers in red. The yellow triangle represents the location of the principal wells serving the population living in the study area (ARPAV, 2017; Veneto region, 2019).

The water model proposed by ARPAV in 2018 was tested for modelling all the relevant flow processes. As reported by the authors: "this model will help in the next

future our knowledge of the complex hydraulic situation in the contaminated area. The discretization process (meshing into triangle shape elements) keeps into account the model domain, rivers, and wells. The model domain is made of about 225 thousands elements with over 170 thousands nodes. [...] The domain surface is about 94 km^2 with a volume of roughly 6.6 km^3." (ARPAV, Mazzola et al., 2018).

According to the results of the ARPAV monitoring campaign (ARPAV, 2017), in the study area, the horizontal hydraulic permeability was found very heterogeneous (ranging between 10E-02 and 10E-05 m/s) and in some cases differed widely between near sampling points (ARPAV, 2017; ARPAV, Mazzola et al., 2018), while the vertical hydraulic permeability was assumed equal to 1/10 of the horizontal one, according to field surveys and bibliographic data (ARPAV, Mazzola et al., 2018). The calibrated hydraulic conductivity field obtained after the validation process of the water model developed in 2018 ranged between 7.6E-03 and 1E-06 m/s.

Thanks to the new and accurate ARPAV water model we hope that in the next future will be able to collect more important data on predicted PFASs concentrations in groundwater that will be extremely useful in the exposure assessment, in particular for the analysis at individual level. In fact, due to the heterogeneity of the horizontal and vertical permeability of the soil, the differences in PFASs concentrations.

THE HUMAN BIO-MONITORING STUDY

The people living in the study area were exposed to PFASs contamination in groundwater through drinking water from public waterworks (i.e. tap water) and private wells (i.e. direct exposure to groundwater) (ARPAV, 2017; Veneto region, 2019).

The fluorochemical factory definitely closed in November 2018, but many remediation works were carried out immediately after the PFASs detection in rivers

and groundwater. First environmental remediation works started at the end of summer 2013 to ensure the safety of the people living in the study area.

The exposure to PFASs in tap water started to decrease in September 2013 in many municipalities, when contaminated waterworks were supplied with the installation of Granular Activated Carbon (GAC) filters and PFASs levels in drinking water were markedly reduced. The direct exposure to PFASs in groundwater seemed to vary over time but without a clear trend (ARPAV, 2017).

A Human Bio-monitoring (HBM) study was developed in the context of the Health Surveillance Program (HSP) offered by the Veneto Region to the residents of the municipalities over and near the contamination plume (Pitter et al., 2020).

The principal aims of the HSP were the characterization of PFASs exposure of people living in contaminated areas and the prevention, early diagnosis and treatment of chronic disorders possibly associated to PFASs exposure (ARPAV, 2017; Veneto region, 2019).

The HBM study included a structured interview, blood and urine tests, and measurement of 12 PFASs in serum by high-performance liquid chromatography-tandem mass spectrometry. 18,345 participants born between 1978 and 2002 (age ranged between 14 and 39 years of age at recruitment) were studied and a multivariable linear regression was used to identify sociodemographic, lifestyle, dietary, and reproductive predictors of serum PFASs concentrations (Pitter et al., 2020). The subjects were recruited starting from January 2017 to June 2019, after the approval of the regional government in December 2016.

The PFASs with the highest serum concentrations were PFOA [median 44.4 ng/mL, interquartile range (IQR) 19.3-84.9 ng/mL], perfluorohexanesulfonic acid (PFHxS) (median 3.9 ng /mL , IQR 1.9-7.4 ng/mL), and PFOS (median 3.9 ng/mL, IQR 2.6-5.8 ng/mL). The major predictors of serum levels were gender, municipality, duration of residence in the affected area, and number of deliveries. Overall, the

regression models explained 37%, 23%, and 43% of the variance of PFOA, PFOS, and PFHxS, respectively (Pitter et al., 2020).

In the context of the health surveillance plan, the Veneto Region, on the basis of the exposure assessment and following the prevention of the risk for human health, analyzed the entire area where PFASs were detected in at least one environmental matrix (in particular in soil and rivers). Then, that area was divided in different sub-areas: the red area (further divided in the A-red area and the B-red area), the orange area, the yellow area and the green area.

The municipalities with high PFASs concentrations detected in tap water (i.e. served by contaminated waterworks) before the GAC filters installation were labelled as the "Red Area". The Red Area was identified as the area of maximum exposure and initially involved 21 municipalities in the provinces of Vicenza, Verona and Padova and was inhabited by 126000 people, but in 2018, 9 additional municipalities were added, even if some of them were only partially supplied by the contaminated waterworks. At present, the Red Area is 595 km^2 wide and has a total population of about 140,000 residents. (Pitter et al., 2020).

The Red Area was further divided into: the A-Red Area, including the municipalities in the Red Area that were located over the predicted groundwater contamination plume (so, also the water from the private wells was contaminated by PFASs), and the B-Red Area, including the municipalities in the Red Area that were located outside the predicted groundwater contamination plume (so, the water from the private wells was not contaminated).

The orange area was the area of the independent water harvesting, the yellow area was defined as a warning area, since PFASs were found in water for irrigation only. The green area was defined as an in-depth area since PFASs were found in environmental matrixes but population didn't appeared to be in danger, so new monitoring studies and inspections were needed.

Study	Population	PFOA [ng/mL]	PFOS [ng/mL]
HBM-Red Area	Age: 14-39, exposed, Red Area	44.4 (average value: 64.4)	3.9 (average value: 4.8)
Frisbee et al., 2009	Age: 20-39, exposed, Ohio	21.8	18.1
Ingelido et al. 2010	Age: 20-35, Italian general population	2.87	4.46
Ingelido et al. 2018	Age: 20-51, exposed, Veneto	13.77	8.69
Ingelido et al. 2018	Age: 20-51, not exposed, Veneto	1.64	5.84

Tabella 6 median PFAS serum concentration observed in the human bio-monitoring study for the subjects living in the Veneto region (HBM-Red Area) and for subjects from other studies on PFASs. (Pitter et al., 2020).

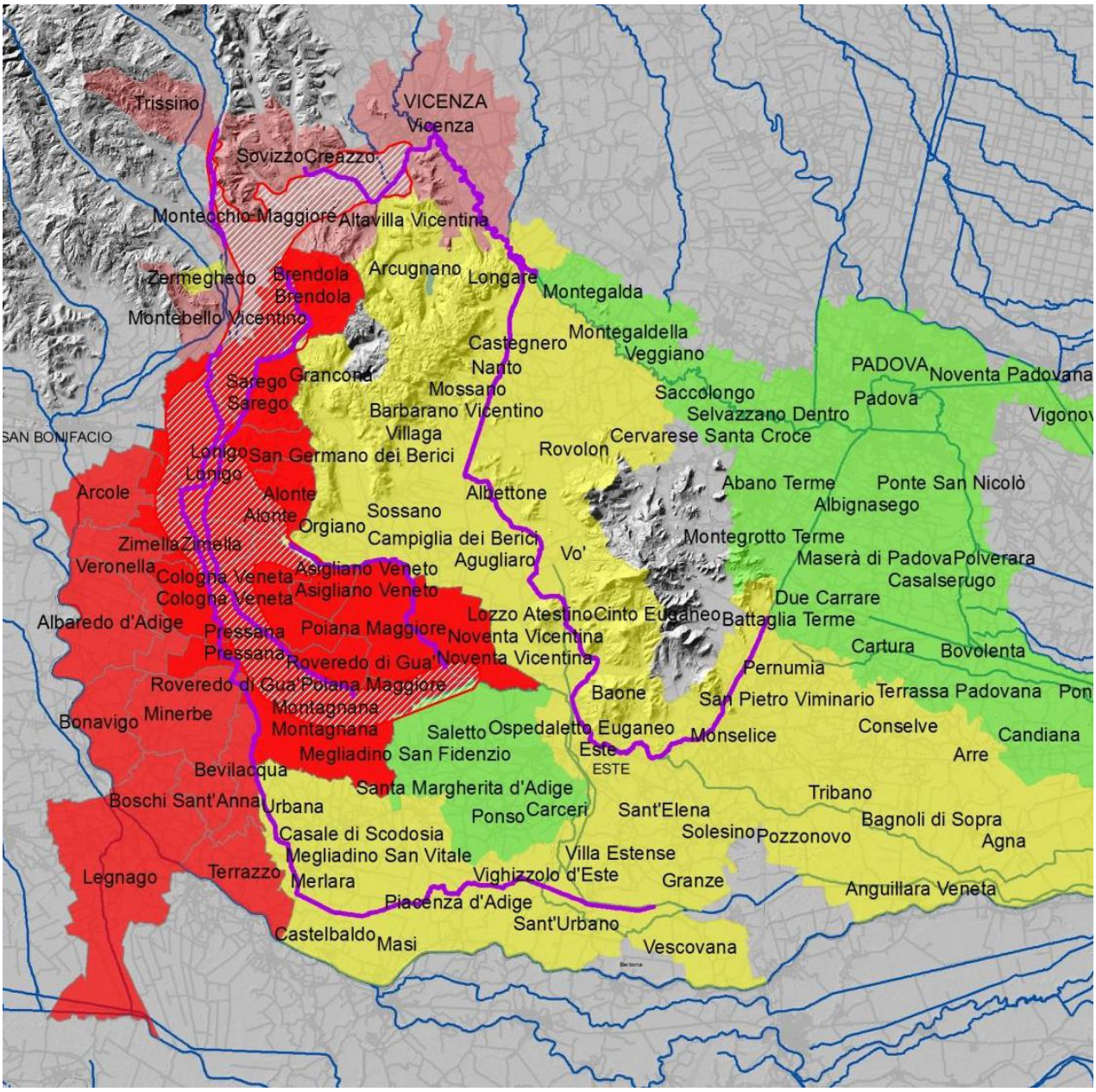


Figure 9 Areas of different exposure and different risk for health. Dashed area = contamination plume, red area = area of maximum exposure (divided in the A-red area: dark red, and the B-red area: light red), orange area = independent water harvesting, yellow area = warning area, green area = in-depth area (ARPAV, 2017; Veneto region, 2019).

In 2018, a cross-sectional epidemiological study on the association between internal dose of PFAS and anthropometric and bio-humoral parameters in the exposed Veneto population was proposed by University of Padova (UNIPD), ARPAV, the Regional Center for Biomarkers (CRIBT) and the London School of Hygiene and Tropical Medicine (LSHTM) and accepted by the Veneto Region. New important results on PFASs effect on human health are expected from this study at the end of this year.

Type	Variables
Sociodemographic	age, sex, country of birth (Italy vs HMPC countries), education level, occupation (farmer vs not farmer)
Residential/environmental factors	residential time in the Red area, prevalent area of residence (A or B), municipality of residency at recruitment, time-lag between the beginning of the study and blood sampling, private food production (breeding animals and growing vegetables)
Behavioral factors	alcohol, smoking, physical activity, diet (quartiles of meat, milk, dairy products, cereal, fish, egg, fruit and vegetable servings per week consumption), water intake
Anthropometry and health	Body Mass index (BMI), renal function (eGFR),current pregnancy (yes/no), number of deliveries
Drinking water sources (only restricted population)	consumption of bottled water consumption of well water

Table 7 Variables investigated with the questionnaire administered in the context of the HBM study (Pitter et al., 2020).

For the present study observed PFAS concentration data in tap water, groundwater and air and observed PFAS serum concentration data were provided by ARPAV and the Veneto region.

METHODS

ANALYSIS OF THE STUDIED POPULATION

For the present study 179 subjects (88 men and 91 women) between 14 and 39 years of age (average: 26) were selected among the people recruited in the HBM study. Observed average serum concentration in the enrolled subjects was found very high for PFOA (57.9 ng/mL) and quite low for PFOS (4.4 ng/mL), with a huge variability between subjects (both men and women) for PFOA (standard deviation: 62.3 ng/mL for PFOA, 3.4 ng/mL for PFOS). The median was found quite lower respect to the average value for both the PFAS (39.1 ng/mL for PFOA, 3.3 ng/mL for PFOS), according to the results found for the entire population analyzed in the HBM study. In the testing procedure were analyzed a similar number of men and women. Furthermore, sex was taken into account in each comparative analysis. Analysing separately the group of men and the group of women was necessary, since observed PFAS serum concentrations were found considerably higher in men (PFOA average value and standard deviation, men: 78.0 ± 71.4, women: 38.5 ± 44.4 ng/mL; PFOS average value and standard deviation, men: 5.2 ± 3.3, women: 3.7 ± 3.4 ng/mL) after a Wilcoxon rank sum test with continuity correction (for PFOA: W = 2269, p-value = 5.598E-07; for PFOS: W = 2406, p-value = 4.017E-06). The choice of the Wilcoxon test to analyze the PFAS serum concentrations was based on the size of the samples and on the result of a Shapiro-Wilk test, that showed a non normality for all the distributions of serum concentration data. Both the Shapiro-Wilk and the Wilcoxon tests were run with R software (RStudio Version 1.3.1093, R version 4.0.3). In women, the observed average concentration was found lower with respect to men (-51% for PFOA, -29% for PFOS). All selected female subjects were in fertile age to take into account also the role of menstruation in the analysis. The group of men and the group of women were chosen to be homogeneous for the time of exposure (that was 18 years for the entire population, 18.7 for men and 17.7 for

women). The average weight of the recruited subjects was 68.6 kg and the average consumption of water was 1.67 L/day. Men average weight was 77.22 kg while women average weight was 60.19 kg. Men average water consumption was 1.86 L/day while women average water consumption was 1.48 L/day. Men average age was 26 years while women average age was 27 years. Independent samples two-tailed t tests showed that age and exposure time were found not statistically different between men and women (p-value: 0.29 for age and 0.41 for exposure time) while the water intake rate and the body weight turned out to be higher for men (p-values: 0.29E-04 and 5.9E-15 respectively).

In summary, the group of men showed higher values respect to the group of women for water intake rate and body weight (as shown by the t tests). A consequence of drinking more contaminated water is surely a higher PFAS intake but a higher weight means less PFAS per kg of body weight. In conclusion the higher observed PFAS serum concentrations for men could be partly due to an higher water intake, but more likely, the higher water intake could be compensated by the higher weight. In the last case the higher observed PFAS serum concentrations for men would be due to other factors.

PFAS serum concentrations observed in the A-red area were found statistically higher than those observed in the B-red area after a Wilcoxon rank sum test with continuity correction (W = 5360, p-value = 2.46E-05 for PFOA; W = 5038, p-value = 1.04E-03 for PFOS) and independent samples two-tailed or one-tailed t tests showed no difference in the distributions of data for all the exposure variables investigated (age: p-value = 0.68, exposure time: p-value = 0.47, water intake: p-value = 0.26 and weight: p-value = 0.10). So, a comparison between the two different area was deemed necessary, since the difference in PFAS serum concentrations were not due to a difference in exposure variables, except for PFAS concentrations in water.

In conclusion, the statistical analysis showed that the higher average PFOA concentration observed for the A-red area respect to the B-red area (using the Wilcoxon test) was not due to differences in subjects physical characteristics or exposure between the two groups, as confirmed by the results of the t tests.

Summary of population characteristics (average value ± standard deviation)							
Sex	**C-PFOA (ng/mL)**	**C-PFOS (ng/mL)**	**Exp_t (years)**	**Age (years)**	**A-WIR (L/day)**	**A-BW (kg)**	**N**
M	78.0 ± 71.4 (m: 57.0)	5.2 ± 3.3 (m: 4.0)	18.7 ± 7.7	26 ± 8	1.9 ± 0.7	77 ± 16	88
W	38.5 ± 44.4 (m: 23.2)	3.7 ± 3.4 (m: 2.6)	17.7 ± 8.9	27 ± 7	1.5 ± 0.7	60 ± 11	91
Tot pop	57.9 ± 62.3 (m: 39.1)	4.4 ± 3.4 (m: 3.3)	18.2 ± 8.3	26 ± 8	1.7 ± 0.8	69 ± 16	179
A-red area	78.6 ± 67.0 (m: 61.9)	5.4± 4.1 (m: 4.6)	17.7 ± 8.7	26 ± 8	1.6 ± 0.8	66 ± 16	76
B-red area	42.6 ± 47.6 (m: 32.3)	3.6± 2.5 (m: 2.9)	18.6 ± 8.1	27 ± 7	1.7 ± 0.7	70 ± 16	103

Table 8 summary of population characteristics (average value ± standard deviation) for men (M), women (W) and total population (tot pop). C-PFOA = average observed PFOA serum concentration, C-PFOS = average observed PFOS serum concentration, Exp_t = exposure time, A-WI = average water intake, A-BW = average body weight, N = number of subjects. For PFAS serum concentrations also the median is reported (m).

	sex		**area**	
	W	**p-value**	**W**	**p-value**
C-PFOA	2269	5.598E-07	5360	2.46E-05

C-PFOS	2406	4.017E-06	5038	1.04E-03

Table 9 Outputs of the Wilcoxon rank sum test with continuity correction run to compare the group of men versus the group of women (sex) and the group of subjects living in the A-red area versus the group of subjects living in the B-red area (area) for observed PFAS serum concentrations. W = statistic, p-value.

	age	**expt**	**water intake**	**weight**
p-value	0.29	0.41	2.85E-04	5.92E-15
Null hypothesis	Not rejected	Not rejected	Rejected	Rejected

Table 10 outputs of the t-tests run to compare the group of men versus the group of women for age, exposure time, (expt), water intake and weight.

	age	**expt**	**water intake**	**weight**
p-value	0.68	0.47	0.26	0.10
Null hypothesis	Not rejected	Not rejected	Not rejected	Not rejected

Table 11 outputs of the t-tests run to compare the group of subjects living in the A-red area versus the group of subjects living in the B-red area for age, exposure time, (expt), water intake and weight.

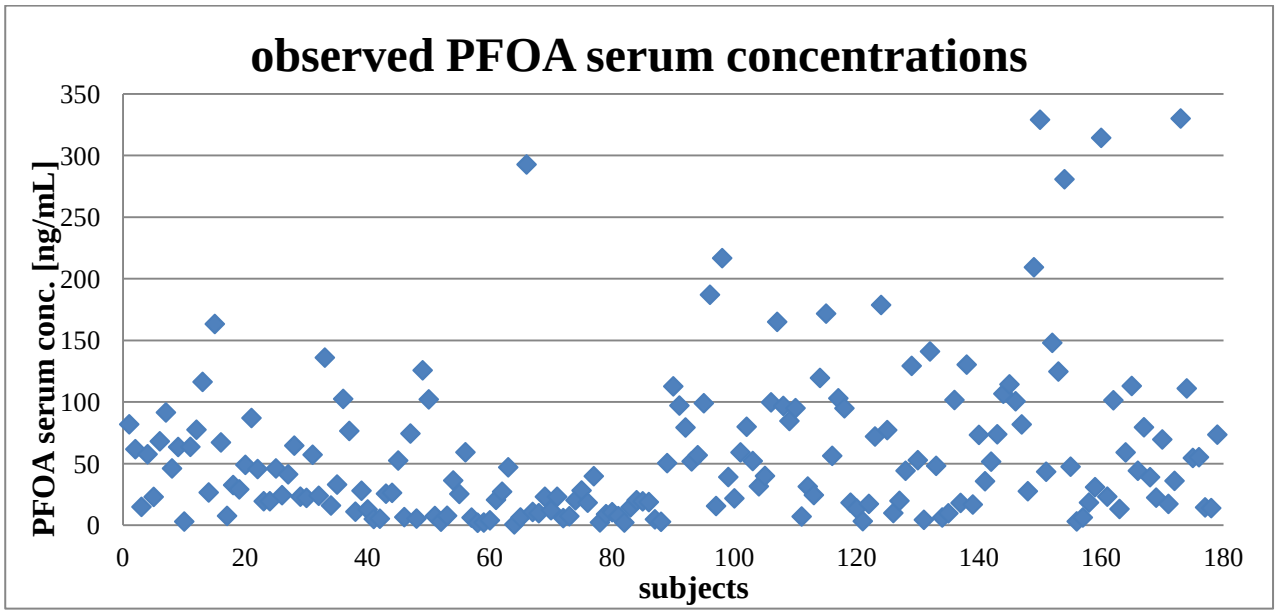


Figure 10 observed PFOA serum concentrations for the studied population.

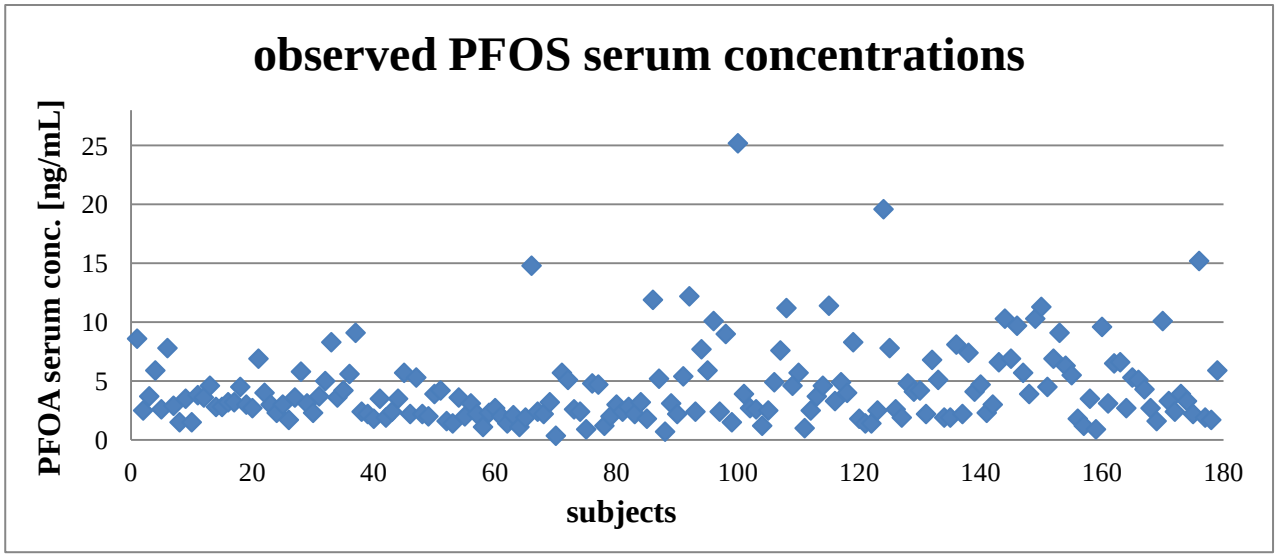


Figure 11 observed PFOS serum concentrations for the studied population

The selected subjects lived in 5 different municipalities: Sarego, Lonigo, Legnago, Veronella, Albaredo d'Adige. The municipalities of Sarego and Lonigo are located in the A-red area, therefore the groundwater extracted through the private wells was contaminated. The municipalities of Legnago, Veronella and Albaredo d'Adige are located in the B-red area so they faced a PFAS contamination in tap water only. 25 subjects of the studied population lived in Sarego municipality, 51lived in Lonigo, 71 in Legnago, 15 in Veronella and 17 in Albaredo d'Adige for a total of 76 subjects in the A-red Area (42% of the total) and 103 subjects in the B-red area (58% of the total). In the choice of these five municipalities the PFAS concentrations in subjects, in groundwater and in tap water were taken into account to test the PBPK models reliability in different exposure scenarios. The subjects living in the municipality of Sarego showed the highest PFAS serum concentrations (average value: 96.7 ng/mL for PFOA; 5.7 ng/mL for PFOS) while those living in the municipality of Legnago showed the lowest concentrations (average value: 32.2 ng/mL for PFOA; 3.4 ng/mL for PFOS).

	Sarego [ng/mL]	**Lonigo [ng/mL]**	**Veronella [ng/mL]**	**Albaredo D'Adige [ng/mL]**	**Legnago [ng/mL]**

PFOA	96.7 ± 93.5	69.7 ± 54.0	71.6 ± 78.2	60.8 ± 40.7	32.2 ± 42.9
PFOS	5.7 ± 3.1	5.3 ± 4.6	4.6 ± 3.7	3.8 ± 2.0	3.4 ± 2.4

Table 12 Average PFAS serum concentrations ± standard deviation for each municipality considered.

In conclusion, the municipalities were chosen for their different characteristics. In fact, the subjects living in Sarego had the highest serum concentrations and were exposed to PFAS both in tap water and groundwater, while those living in Lonigo also were exposed to PFAS both in tap water and groundwater, but had lower serum concentrations. The subjects living in Veronella and Albaredo D'Adige had serum concentrations similar to those found in Lonigo but were exposed to PFAS in tap water only. Also the subjects living in Legnago were exposed to PFAS in tap water only, but they had the lowest serum concentrations. For each municipality considered in the present study, only a part of all the subjects collected in the context of the HBM study (Pitter et al., 2020) were analyzed. Subjects with very different age, weight, BMI, exposure period, dietary habits, drinking habits (e.g. drinking water intake and sources) and PFAS serum concentrations were requested in order to validate the models for a wide variety of situations.

A subsample of the studied population was created to see if several parameters like age, exposure time and pregnancy could affect the analysis. In order to achieve this aim in the subsample were only selected the subjects with the following characteristics: age equal or higher than 21 years old, time of exposure equal or higher than 10 years and no pregnant women. PFAS predicted values in serum obtained from this subsample were then compared to those obtained from the total population and to observed values. The subsample consisted of 105 people, 52 men and 53 women.

EXPOSURE ASSESSMENT

EXPOSURE ROUTES

PFAS INTAKE THROUGH WATER

PFAS concentration data in groundwater, raw water and treated water were provided by ARPAV and checked through a comparison with the data obtained by the local water company "Acque Veronesi". The term 'raw water' indicates the tap water before the treatments in the water treatment plant where it is forced to pass through the GAC filters, while the treated water is the tap water after passing the GAC filters. Subjects were exposed to the PFAS in groundwater through the private well and to the PFAS in tap water first through the raw water (before the GAC filter installation in 2013) and then through the treated water.

Tap water

The concentration trend in the tap water was divided into several different time periods (Pi), varying from a municipality to another. The aim was to build a more accurate exposure curve to give better input data to the Loccisano, ML1 and ML2 models. In fact, thanks to this division into different time periods, PFAS concentration values given as input data in the model varied in time (because varied from one period to another). While in the creation of the PBPK models considered in this study the input concentration value was one, constant in time, in this study the sensible PFAS concentration decrease in tap water during the last years of exposure was taken into account and the division into different time periods was essential to increase the accuracy of the exposure assessment. In fact, the time periods were essentially exposure periods created to define better the exposure of the subjects to PFAS in tap water.

The first selected time period (P1) indicates the period of time in which the PFAS concentration trend reached the highest values and therefore in the simulations it starts at the subject birth and ends with the first stable decrease in concentration due

to the effect of the GAC filters or other remediation actions. The reference value of PFAS concentration for this period was chosen as the average of the values observed in the first few months after the detection of PFAS in the groundwater. The concentration values observed in the time period P1were considered also as representative of the time period before July 2013, when there were no PFAS measurements available. This choice was supported by the analysis of the groundwater flux carried out by the ARPAV (ARPAV, 2017). This analysis showed that steady concentration levels were likely present from many decades in the study area, since the contamination started in the Sixties and the groundwater velocity was high due to the high permeability of the soil.

For example in Sarego municipality the first available data for PFOA was in August 2013 and the average concentration values remained high (but data range was wide) until July 2014. So, these dates were chosen as the beginning date and the end date for the time period P. The same considerations were adopted for the other municipalities. The starting and the final dates for the time periods (Pi), that follows the time period P1, were chosen on the basis of the PFAS concentration trend. The reference value for the PFAS concentration at the period Pi was chosen as the average of the values observed in that period. The chosen time periods for the different municipalities and the corresponding average PFOA concentration values are reported in the following tables:

Municipality	**P1**	**P2**	**P3**	**P4**	**First sample-last sample collected > 5**
Sarego	BD-7/14	8/14-10/17	11/17-BS		8/13-10/17
Lonigo	BD-2/14	3/14-4/16	5/16-10/17	11/17-BS	8/13-10/17
Legnago	BD-11/16	12/16-BS			11/16-11/16
Veronella	BD-10/14	11/14-10/17	11/17-BS		7/13-8/17

Albaredo d'Adige	BD-2/15	3/15-10/17	11/17-BS		7/13-8/17

Table 13 Selected time periods (Pi) for PFOA average concentration values in tap water. BD= birth date of the subject. BS=date of the blood sampling.

Municipality	**A-PFOA, P1 [ng/L]**	**A-PFOA, P2 [ng/L]**	**A-PFOA, P3 [ng/L]**	**A-PFOA, P4 [ng/L]**	**A-PFOA, (until the last value higher than 5 ng/L)**	**Number of samples collected (until the last value higher than 5 ng/L)**
Sarego	99	27	5		38	185
Lonigo	249	89	61	5	114	182
Legnago	27	5			27	1
Veronella	184	116	5		148	26
Albaredo d'Adige	255	124	5		192	23

Table 14 Average PFOA concentrations in tap water (A-PFOA) for the different time periods (Pi).

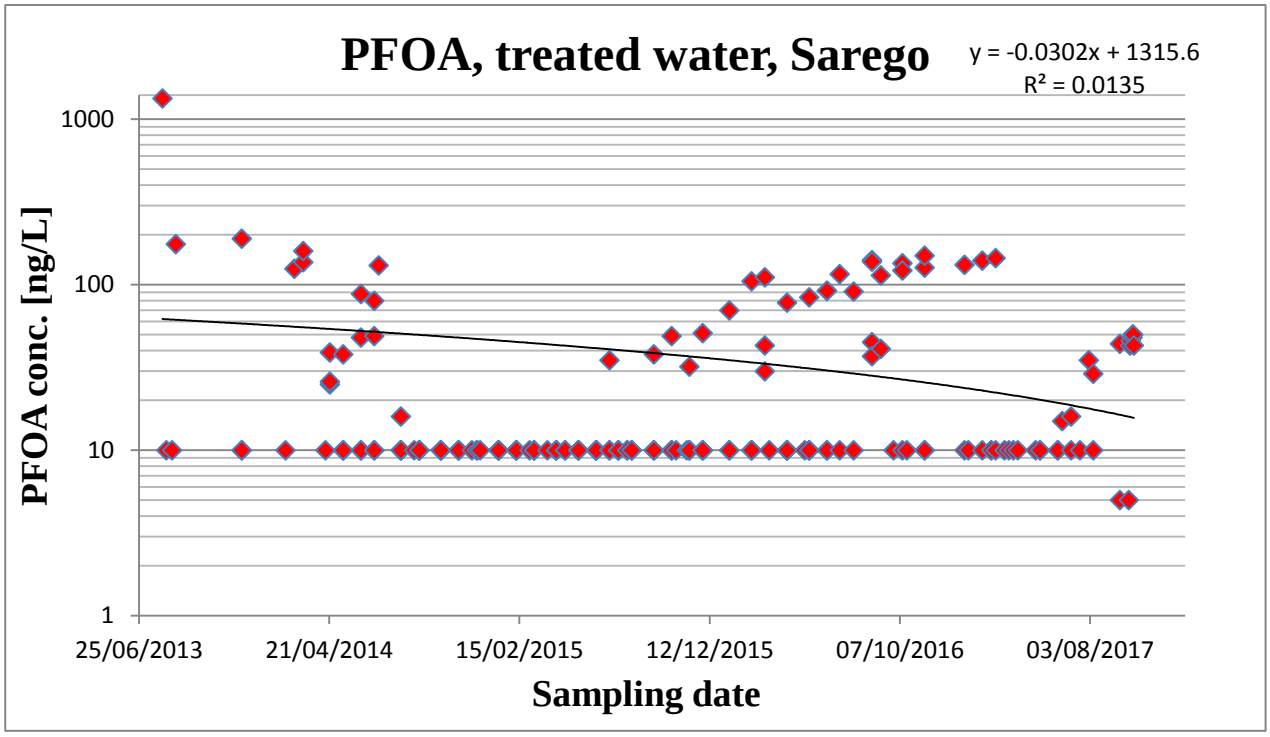


Figure 12 observed PFOA concentrations in treated water [ng/L] over time in the municipality of Sarego

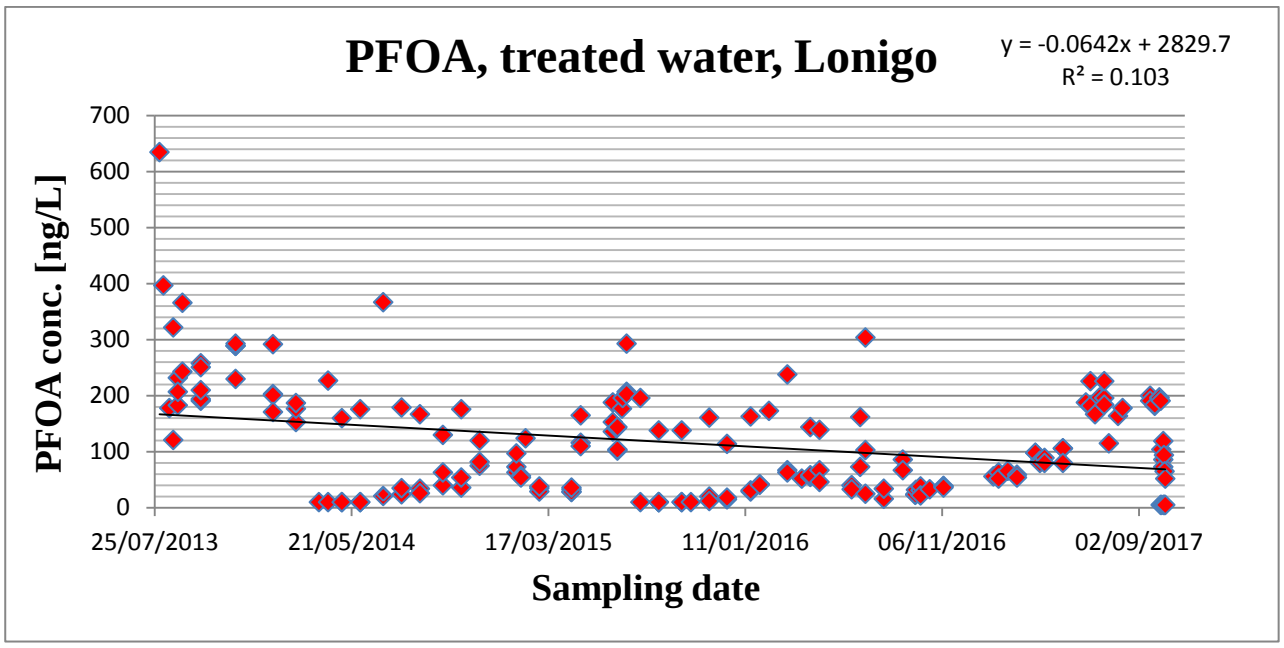


Figure 13 observed PFOA concentrations in treated water [ng/L] over time in the municipality of Lonigo.

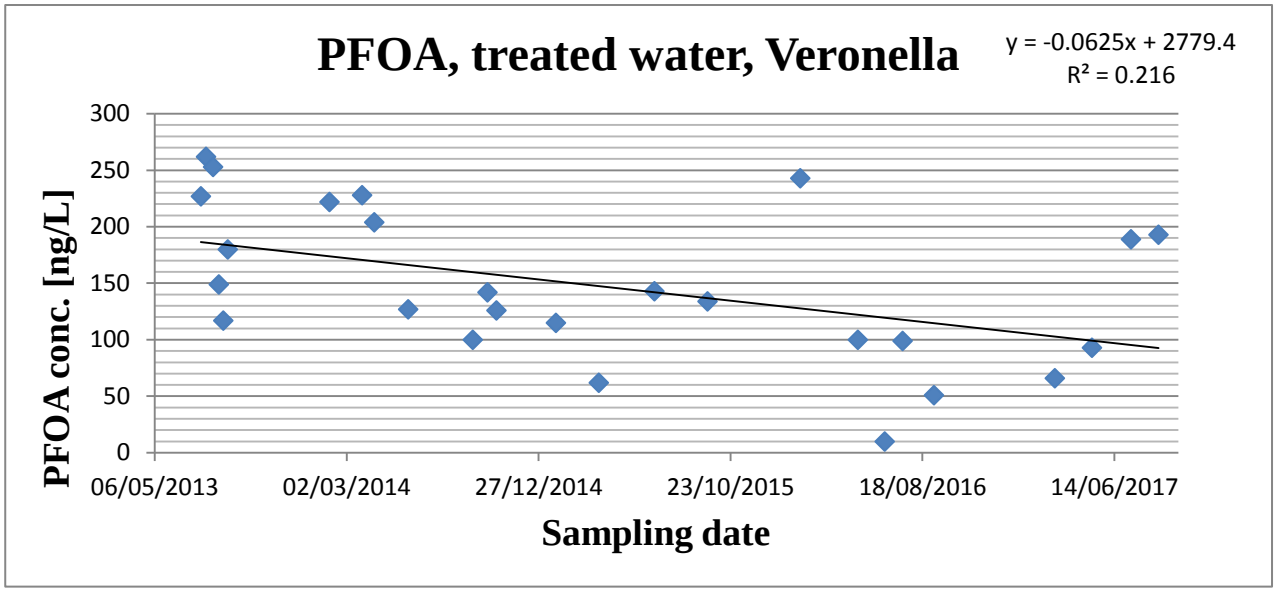


Figure 14 observed PFOA concentrations in treated water [ng/L] over time in the municipality of Veronella

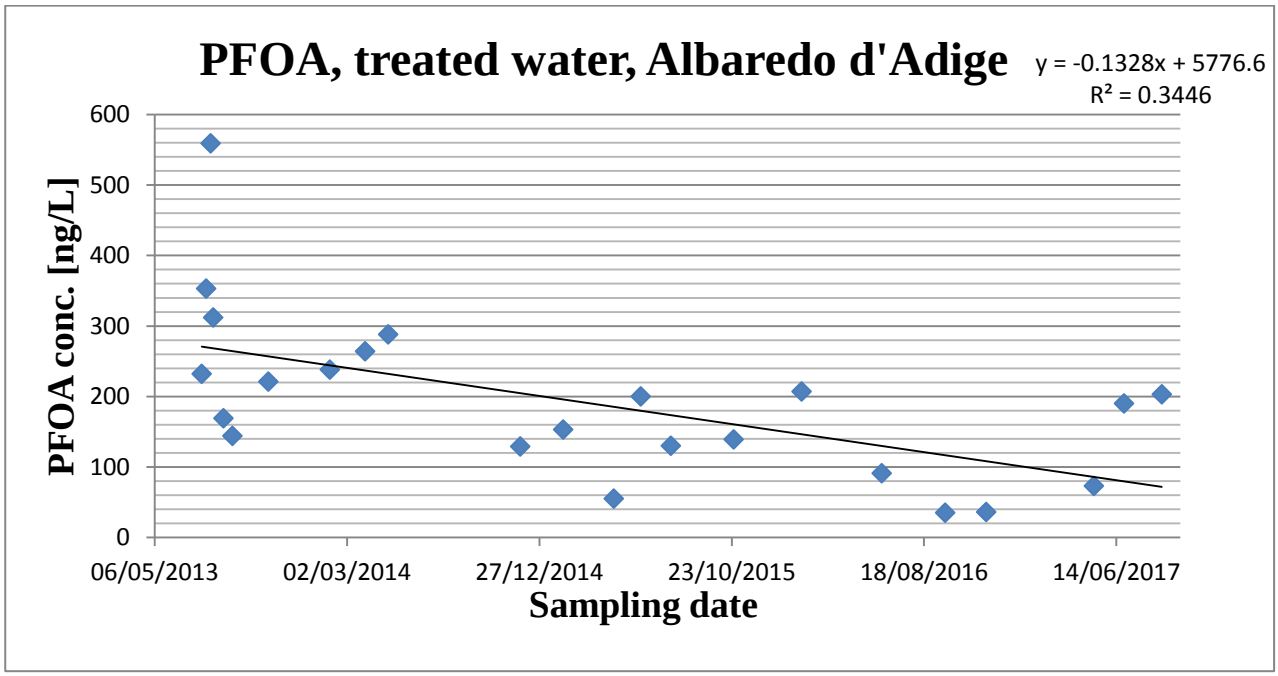


Figure 15 observed PFOA concentrations in treated water [ng/L] over time in the municipality of Albaredo d'Adige.

The chosen time periods for the different municipalities and the corresponding average PFOS concentration values are reported in the following table:

Municipality	P1	P2	P3	A-PFOS, P1	A-PFOS, P2	A-PFOS, P3

				[ng/L]	[ng/L]	[ng/L]
Sarego	BD-7/14	8/14-BS	-	38	10	-
Lonigo	BD-2/14	3/14-10/17	11/17-BS	37	25	18
Legnago	BD-BS	-	-	5	-	-
Veronella	BD-BS	-	-	13	-	-
Albaredo d'Adige	BD-6/16	7/16-BS	-	18	10	-

Table 15 Selected time periods (Pi) for PFOS average concentrations in tap water (A-PFOS) and average PFOS concentrations in tap water (A-PFOS) for the different time periods (Pi). BD= birth date of the subject. BS=date of the blood sampling.

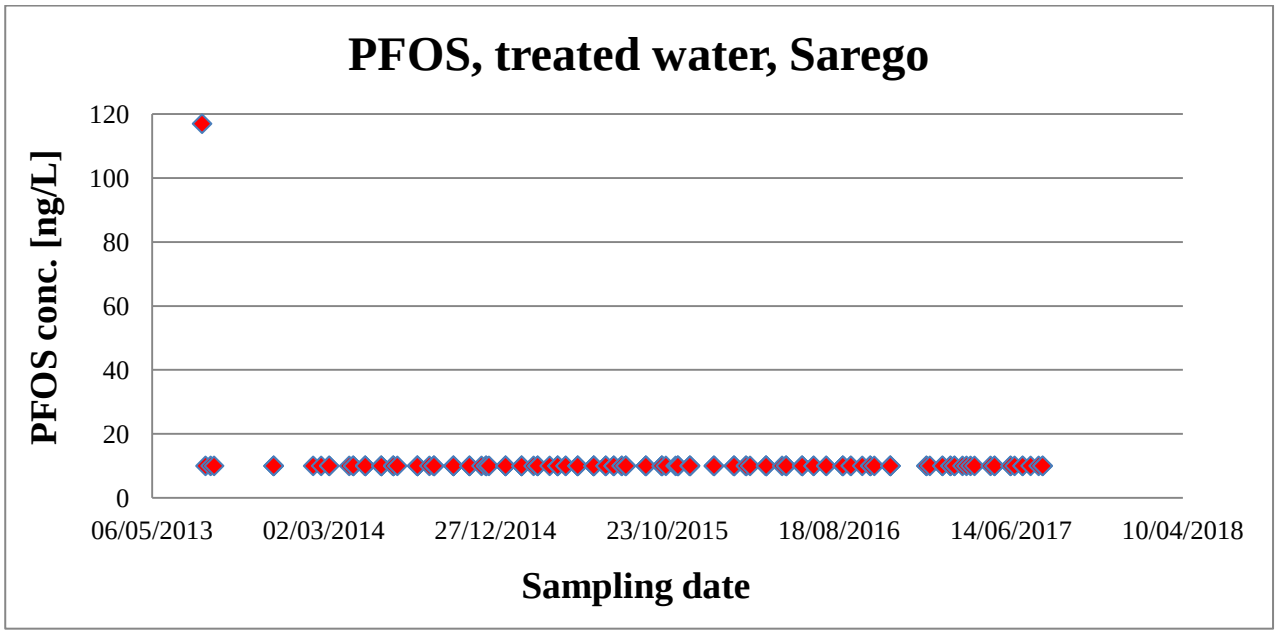


Figure 16 observed PFOS concentrations in treated water [ng/L] over time in the municipality of Sarego.

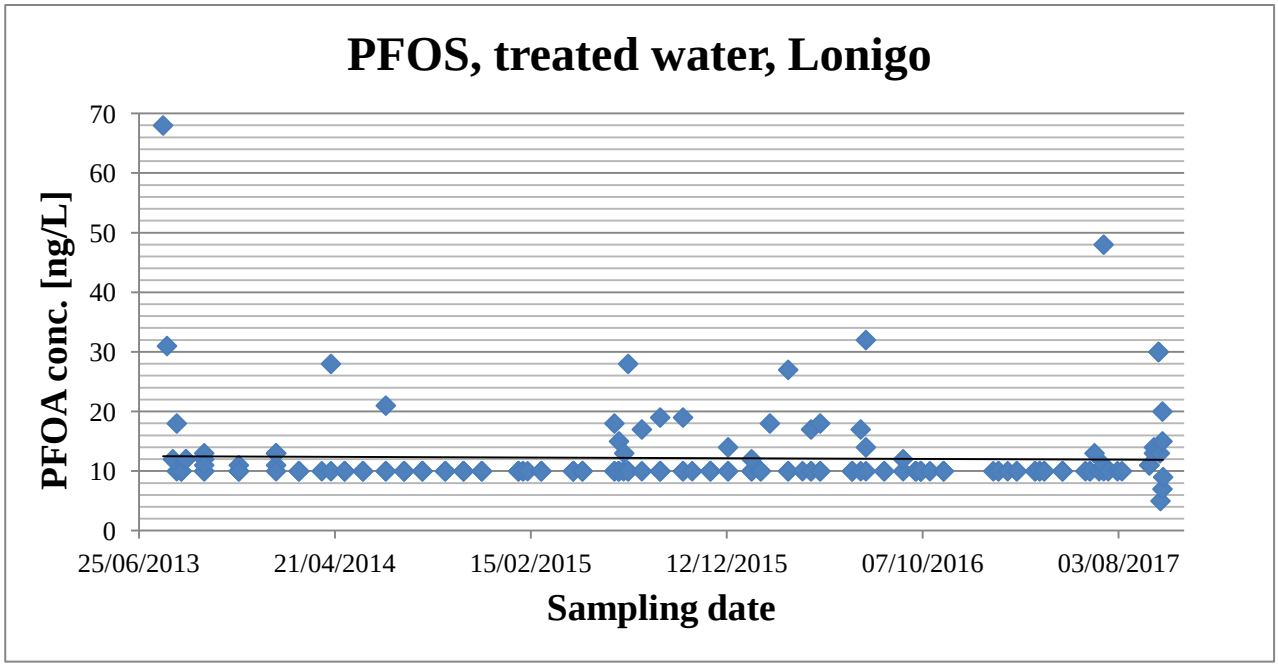


Figure 17 observed PFOS concentrations in treated water [ng/L] over time in the municipality of Lonigo

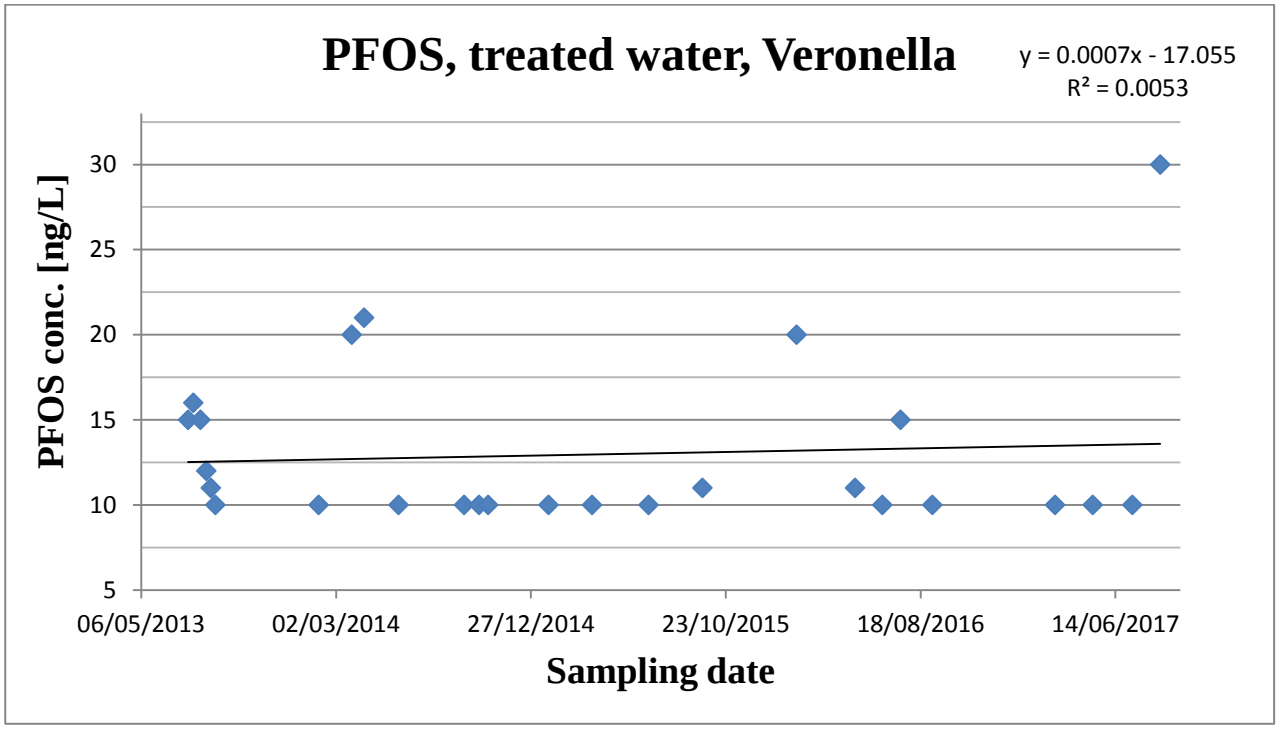


Figure 18 observed PFOS concentrations in treated water [ng/L] over time in the municipality of Veronella

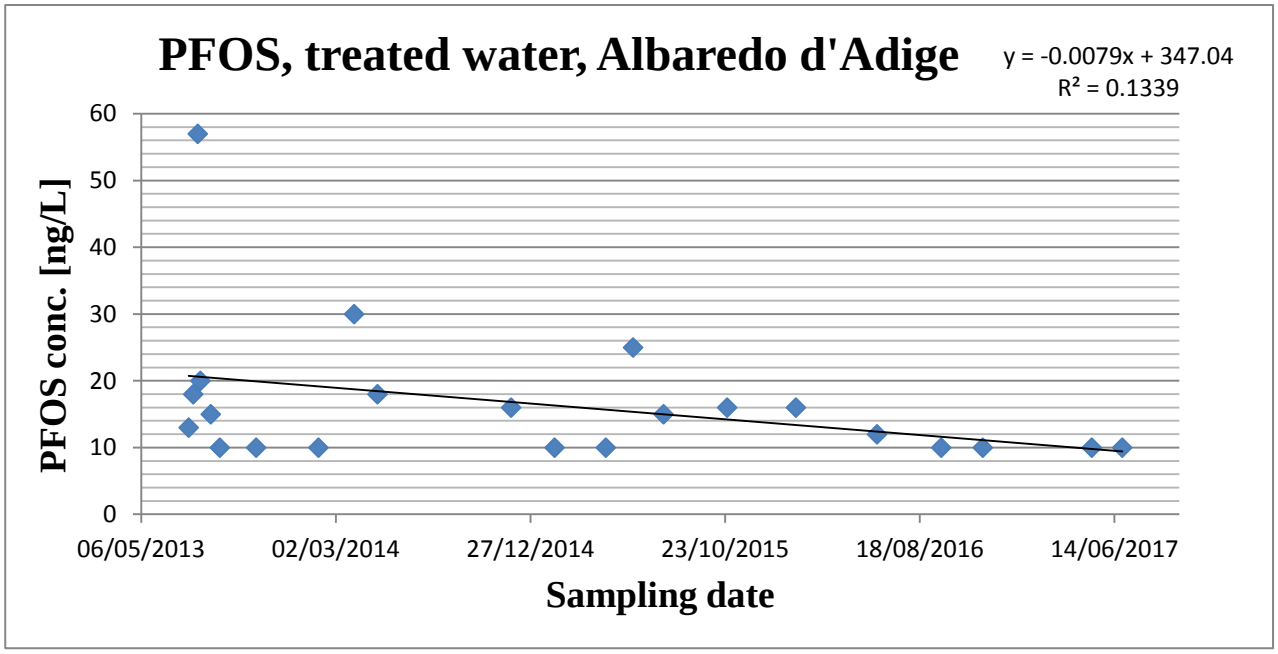


Figure 19 observed PFOS concentrations in treated water [ng/L] over time in the municipality of Albaredo d'Adige.

The assumptions made regarding the period before the PFAS detection in rivers and groundwater were confirmed by the analysis of the raw water and the groundwater data. In fact the PFAS concentrations found in these two type of waters suggested a trend that could perfectly explain the one found in the treated water.

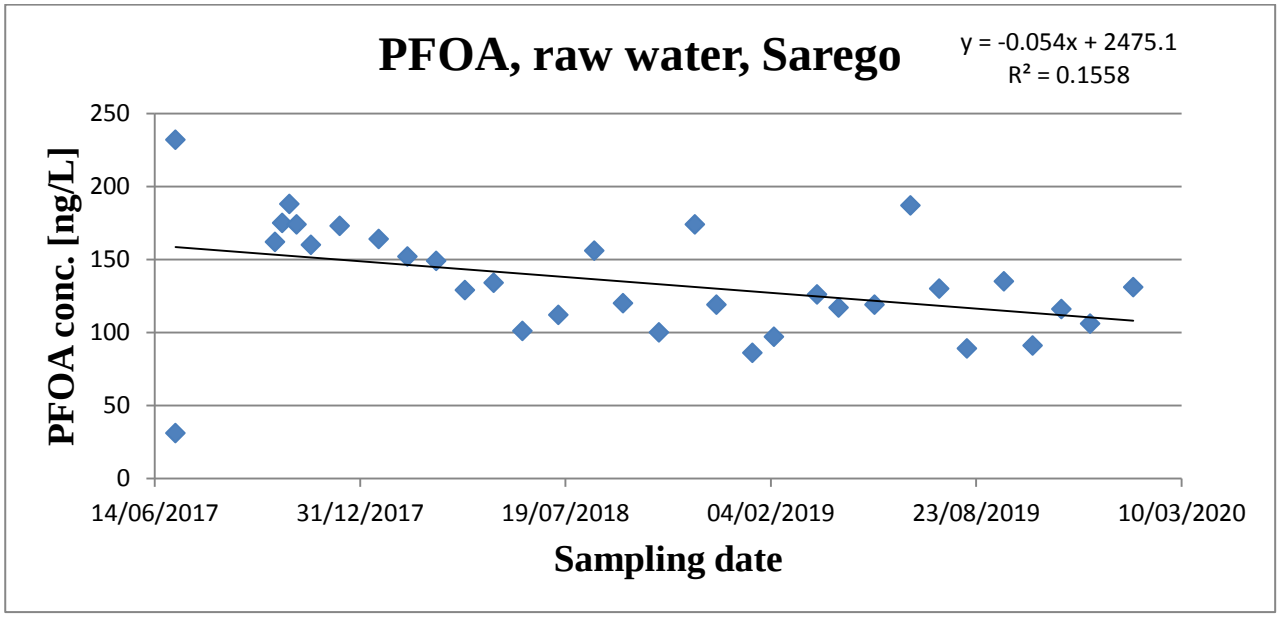


Figure 20 observed PFOA concentrations in raw water [ng/L] over time in the municipality of Sarego

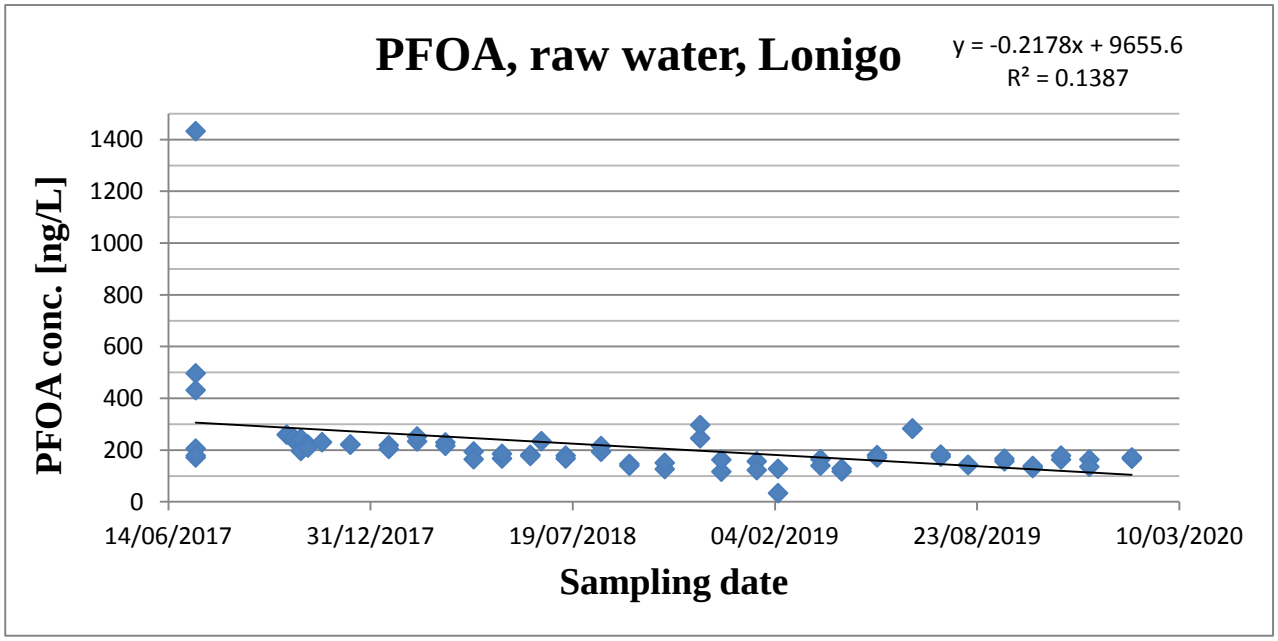


Figure 21 observed PFOA concentrations in raw water [ng/L] over time in the municipality of Lonigo.

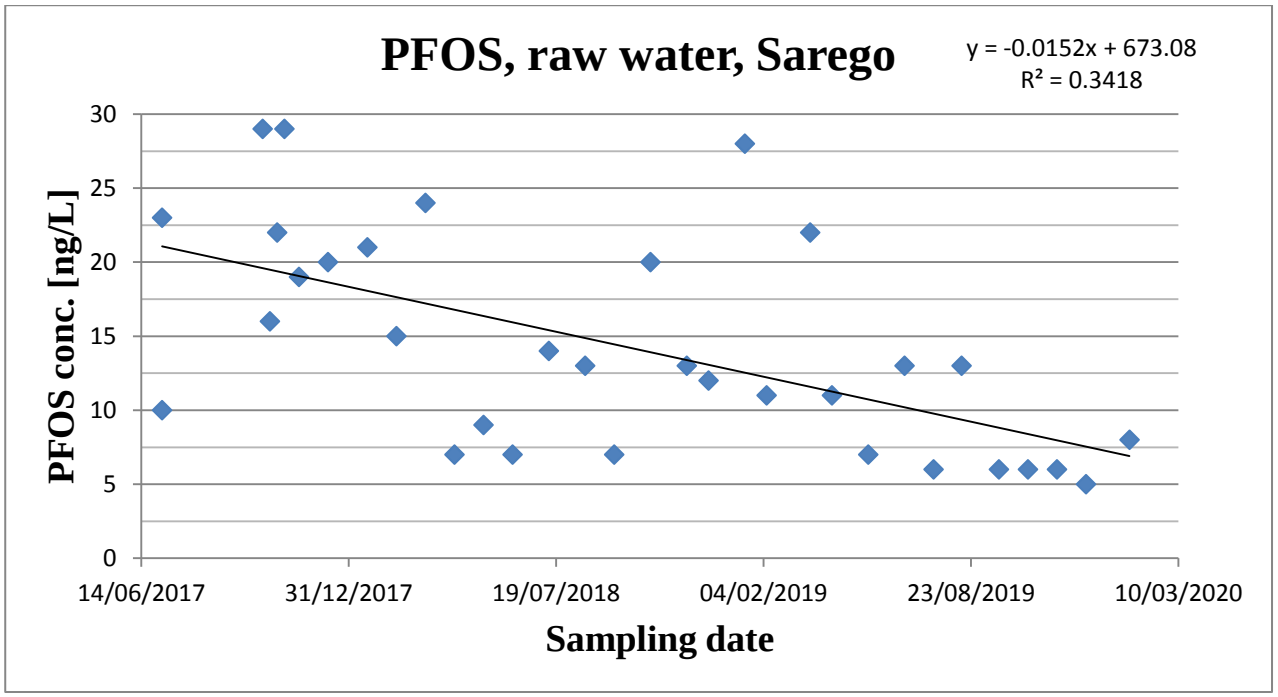


Figure 22 Observed PFOS concentrations in raw water [ng/L] over time in the municipality of Sarego.

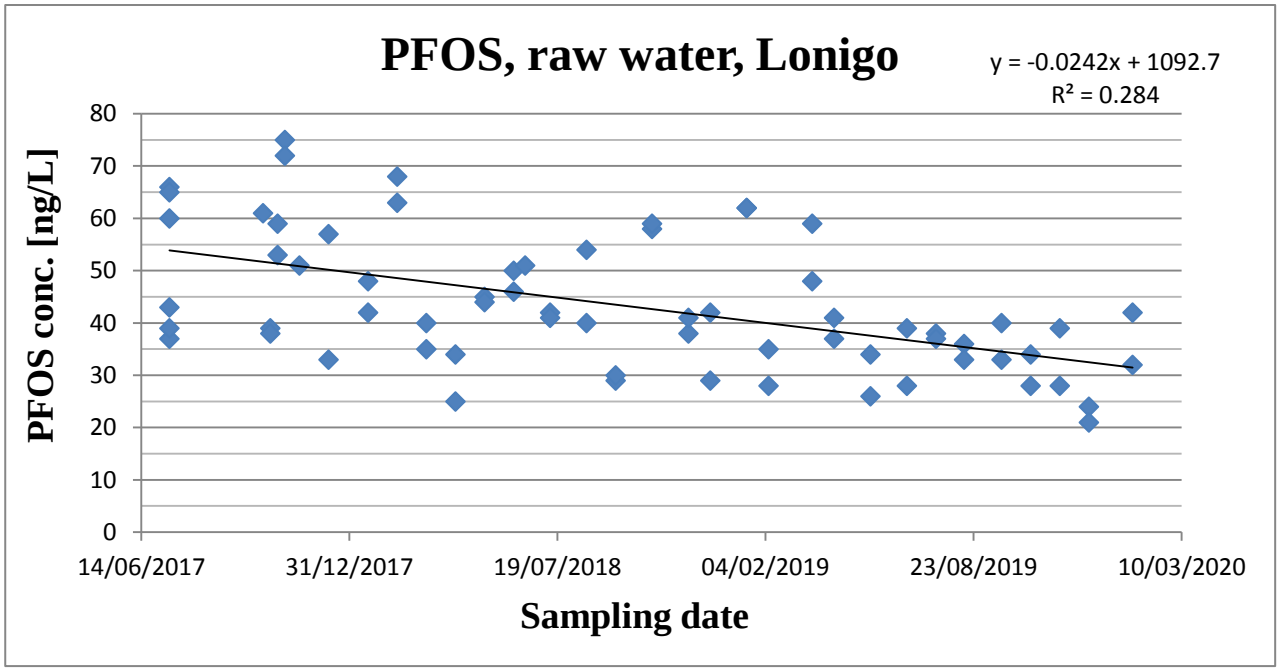


Figure 23 Observed PFOS concentrations in raw water [ng/L] over time in the municipality of Lonigo.

Looking at the PFAS concentration decreasing trend in tap water and raw water after 2013 and at the history of PFAS discharges from the industrial plant (ARPAV 2017; Veneto region, 2019), the values observed in the few months of the time period P1 were likely similar or a bit lower than before. That's the reason why all the values below the PFAS concentration limit of detection in tap water and raw water (10 ng/L, and in the last time periods: 5 ng/L) were assumed to be equal to the limit of detection. In this way the underestimation due to the short PFAS time series of PFAS measurements (since 2013) was counterbalanced. A clear example of the underestimation of PFAS concentration in treated water can be seen looking at the PFOA concentrations in the municipality of Sarego. The average value of PFOA concentrations in raw water for the period 6/17-3/20 was equal to 134 ng/L with a decreasing trend while the average PFOA concentration in tap water in the period P1 was 99 ng/L. That's the reason why the reference value for PFOS concentration in tap water at period P1 in the municipality of Sarego was estimated starting from the PFOS concentrations in raw water observed in the same municipality in the period 6/17-3/20. A direct proportion was used to estimate the value at 8/13, following the straight line fitted by the method of the least squares. The value

obtained in this way was 38 ng/L. PFOS concentrations average value in raw water was 14 ng/L but the decreasing trend was quite marked (from the 20 ng/L or more at the end of 2017 to the 6 ng/L at the end of 2019). This procedure was necessary since the available data for PFOS concentrations in treated water at period P1 in Sarego were likely low because of the effect of the remediation measurements and so not indicative of the time period before 2013.

Groundwater

The PFAS concentration trend over time was considered non significant in the groundwater analysis, since the huge spatial variability (in all the 3 dimensions) of the sampling affected the time trend (different samples in time were taken in different places). The proper representative value of PFOA concentration in groundwater flowing under the municipalities of Sarego and Lonigo was assumed to be the median of the distribution of the observed data, since the PFOA concentration distribution found in these municipalities showed the majority of the values below the average value. Moreover all the values below the PFAS limit of detection (10 ng/L, and in the last time periods: 5 ng/L) were assumed to be equal to the limit of detection and this had led to an overestimation. However, also the average value was taken into account in the simulation step, since the exact location of the subjects was unknown. The representative value of PFOA concentration in groundwater for the municipality of Legnago was assumed to be the average value. 19 samples were collected in the municipality of Albaredo d'Adige and 18 of them measured less than the limit of detection (10 ng/L), one 39 ng/L. So, 0 ng/L was the chosen representative value for the PFOA concentration in groundwater in the municipality of Albaredo D'Adige. No samples were collected in the vicinity of the municipality of Veronella, since the ARPAV studies showed that the PFAS plume was far from that area. So, 0 ng/L was the chosen value for the PFOA concentration in groundwater in the municipality of Veronella.

	A-PFOA	M-PFOA	SD-	5-95	D_{first}-D_{last}	N

	[ng/L]	[ng/L]	PFOA [ng/L]	percentile [ng/L]		
Sarego	3754	680	5432	84-14886	7/13-10/19	138
Lonigo	1657	400	2490	18-6895	7/13-10/19	237
Legnago	19	10	20	10-46	4/16-9/19	118
Albaredo d'Adige	<10	<10	-	-	12/15-9/17	19

Table 16 PFOA average concentration in groundwater (A-PFOA), median (M-PFOA), standard deviation (SD-PFOA), 5-95 percentile of the distribution, date of the first-last collected sample (D_{first}-D_{last}) and number of samples collected (N).

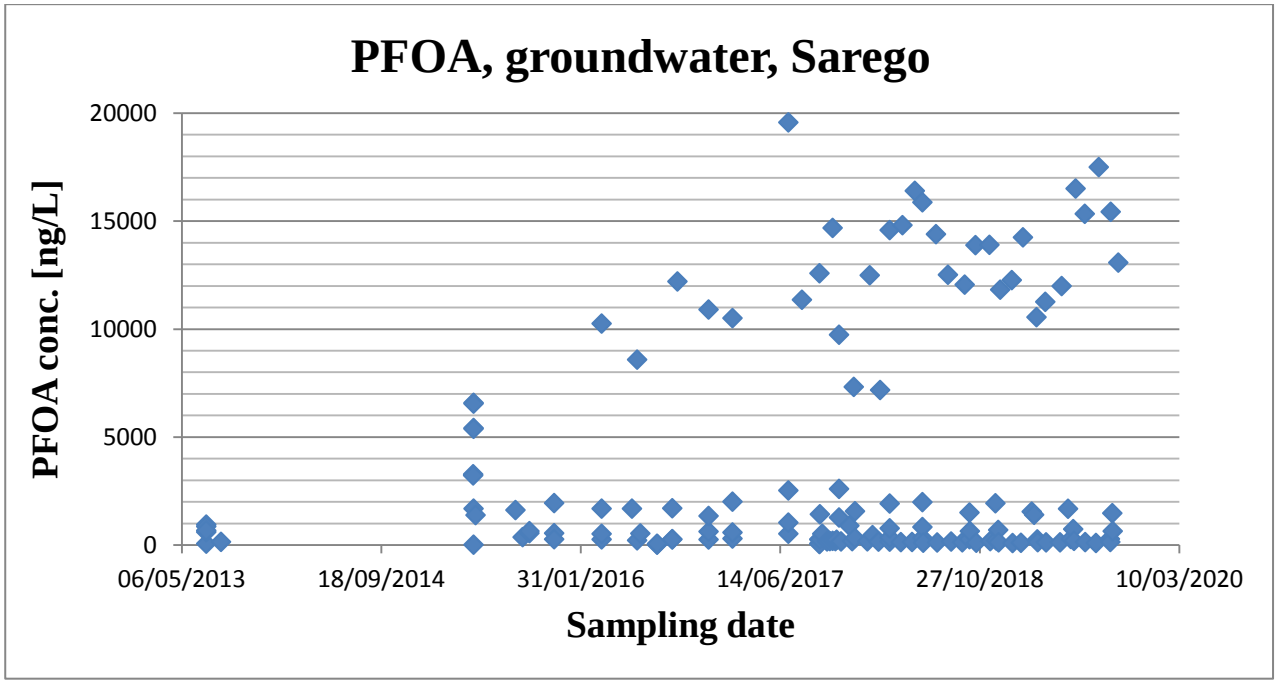


Figure 24 Observed PFOA concentrations in groundwater [ng/L] over time in the municipality of Sarego.

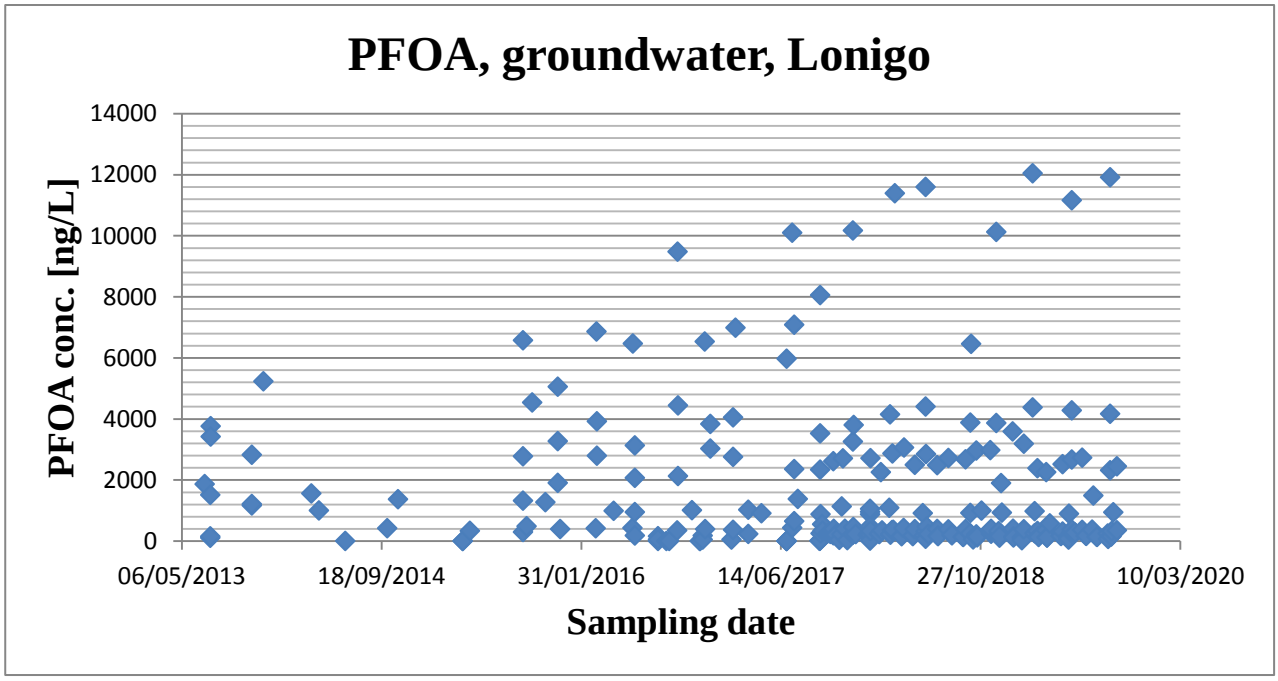


Figure 25 Observed PFOA concentrations in groundwater [ng/L] over time in the municipality of Lonigo.

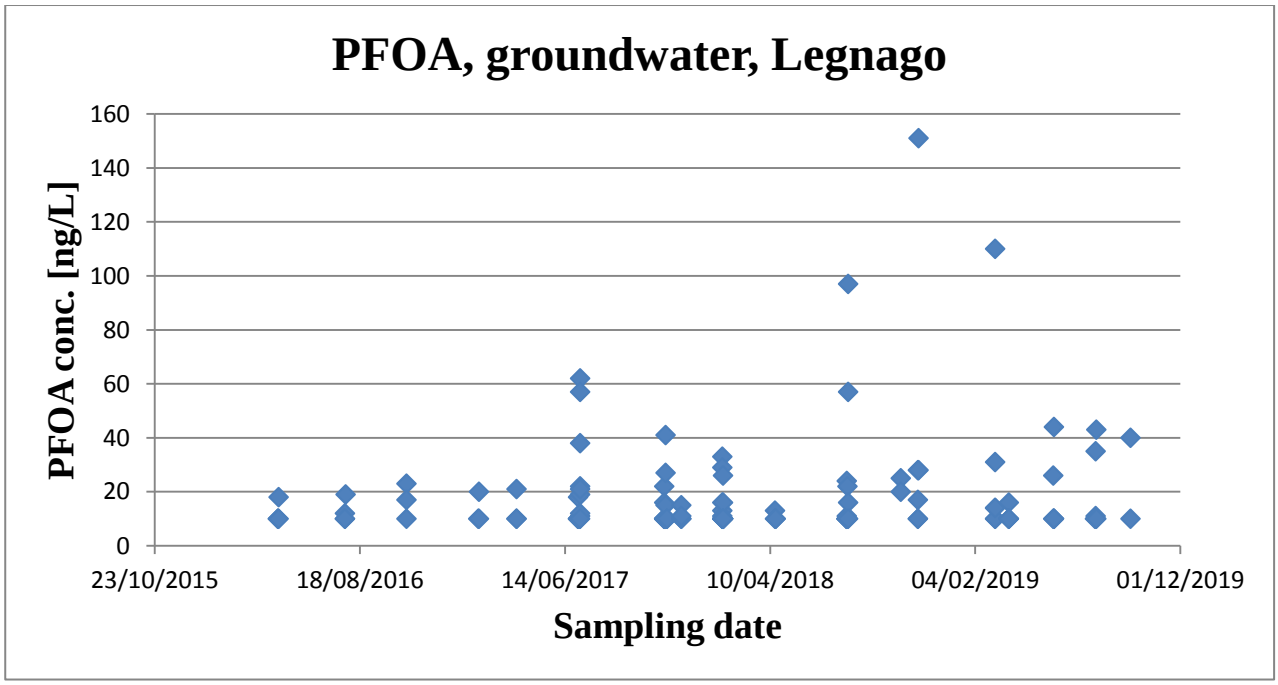


Figure 26 Observed PFOA concentrations in groundwater [ng/L] over time in the municipality of Legnago.

For PFOS, 19 samples were collected in the municipality of Albaredo d'Adige and all measured less than the limit of detection (10 ng/L). So, the half of the limit of detection (5 ng/L) was the chosen representative value for the PFOS concentration in groundwater in this municipality. The same considerations were made for the

municipality of Legnago, where the PFOS concentration was under the limit of detection in all the 118 samples taken. No samples were collected in the vicinity of the municipality of Veronella for the same reason explained before for PFOA, so, 5 ng/L was the chosen representative value for the PFOS concentration in groundwater for this municipality. The exposure to PFOS in groundwater of the subjects living in the municipality located in the B-red area was assumed to last until the date of the blood sampling to compensate the underestimation due to the adoption of a concentration value lower than the limit of detection. The representative value of PFOS concentration in groundwater flowing under the municipalities of Sarego and Lonigo was assumed to be the average of the distribution of the observed data.

	A-PFOS [ng/L]	M-PFOS [ng/L]	SD-PFOS [ng/L]	5-95 percentile [ng/L]	D_{first}-D_{last}	N
Sarego	29	21	23	10-83	7/13-10/19	138
Lonigo	37	37	23	10-77	7/13-10/19	237
Legnago	<10	<10	-	-	4/16-9/19	118
Albaredo d'Adige	<10	<10	-	-	12/15-9/17	19

Table 17 PFOS average concentration in groundwater (A-PFOS), median (M-PFOS), standard deviation (SD-PFOS), 5-95 percentile of the distribution, date of the first-last collected sample (D_{first}-D_{last}) and number of samples collected (N).

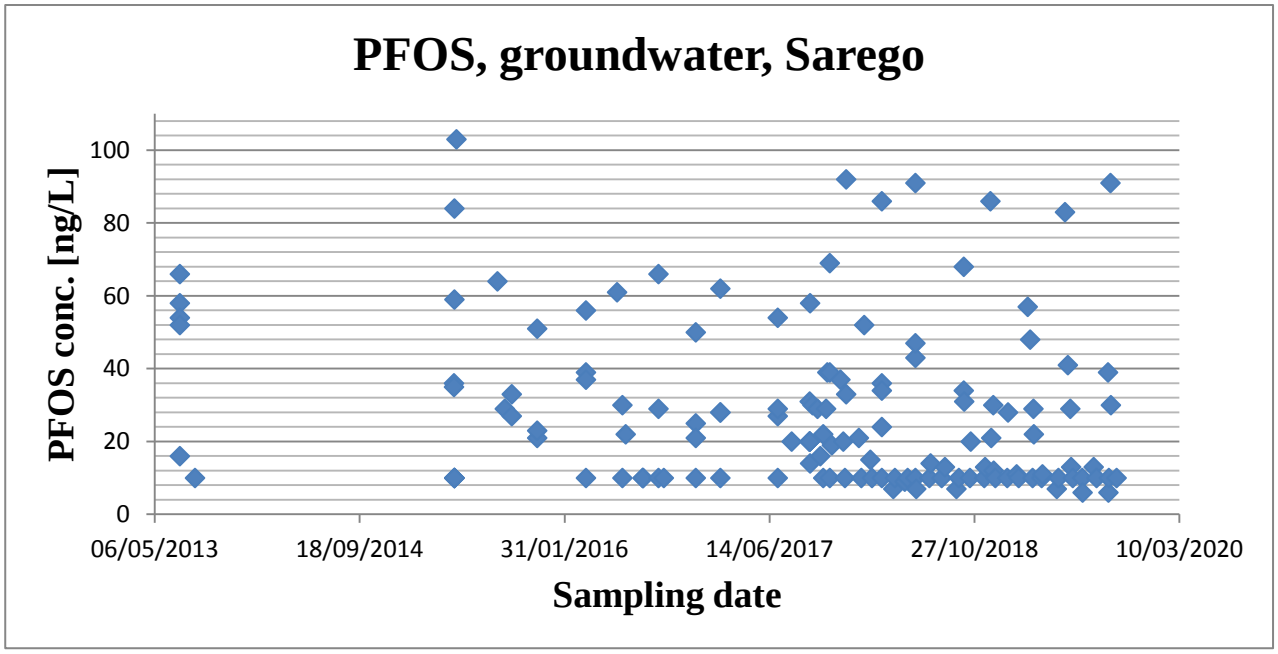


Figure 27 Observed PFOS concentrations in groundwater [ng/L] over time in the municipality of Sarego.

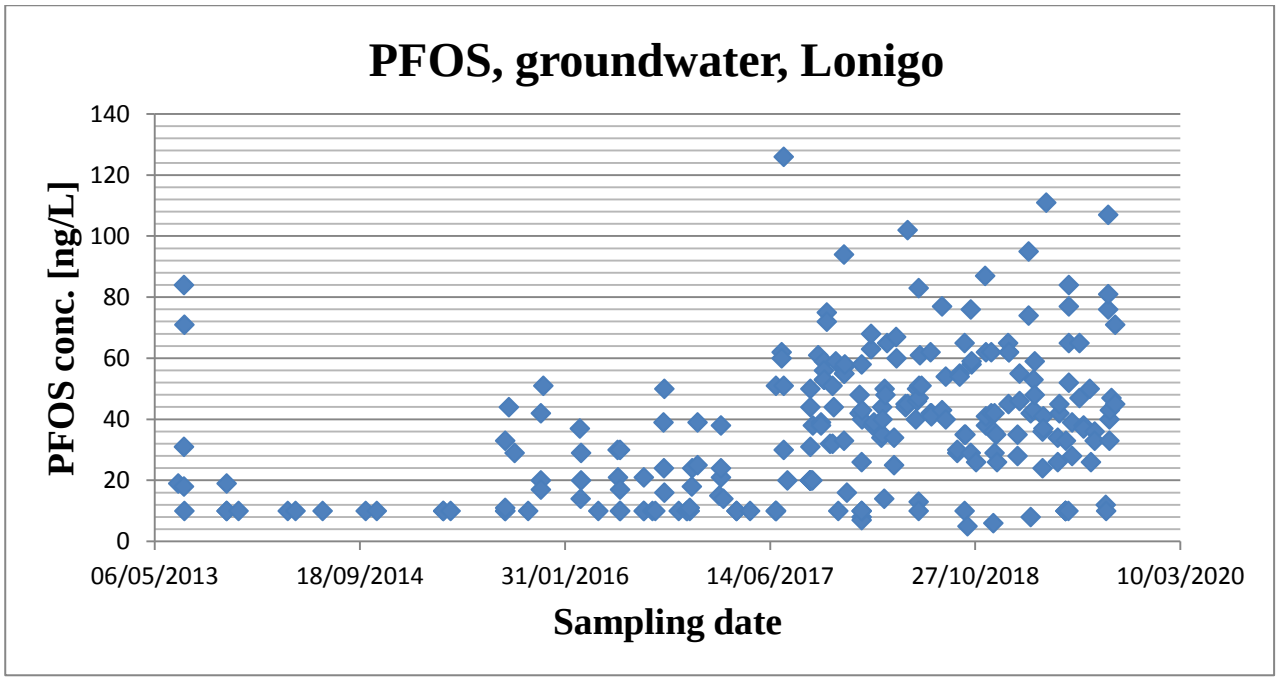


Figure 28 Observed PFOS concentrations in groundwater [ng/L] over time in the municipality of Lonigo.

Considerations, details and solved issues on water exposure assessment

Two main issues had to be solved in the exposure assessment step. The first one was the lack of some useful questions and the presence of some inaccuracies in some others in the administered questionnaire. In facts, important questions like the

consumption of tea (often prepared with tap water) were not asked. Moreover, in the first type of administered questionnaire (before August 2018) all the questions about the water use did not specify the time period to which they referred and in the second type (after August 2018) the question refers to the period before the GAC filters installation only (2013). Therefore the information about the water use after the GAC filters installation (2013) was missing.

The third issue was the low reliability in subjects answer on owning a private well. This issue arose from the fact that many wells had not been declared to the competent authority and so many people could have denied owning the well.

These issues led to a poor knowledge on drinking habits. This problem concerned mainly the people who lived in the municipalities of the A-area (Sarego and Lonigo) because they were exposed to high PFOA concentrations present in the private well. This problem was in part solved with the creation of three exposure scenarios for the municipalities of Sarego, Lonigo and Legnago.

The three scenarios created were:

- Worst-case scenario (WCS), with the following characteristics:

  water intake: 1/3 from private well, 1/3 from company water, 1/3 from bottled water. Exposure to private well ends at the time of the sample collection. In this scenario a change in water use after the detection of PFAS in groundwater in 2013 was not supposed to happened.

- Most likely scenario (MLS), with the following characteristics:

  water intake: 1/3 from private well, 1/3 from company water, 1/3 from bottled water. Exposure to private well ends at the end of the time period P1, when the PFAS contamination was discovered and the Veneto population was certainly well informed about the risk in drinking the water from the private well. This scenario took into account a change in the

water use after the detection of PFAS in groundwater in 2013. This was considered to be the most likely scenario.

- Best-case scenario (BCS), with the following characteristics:

  No exposure to the private well was considered in this scenario. Water intake was assumed to be 1/3 from company water and 2/3 from bottled water.

So, water intake, that assumed different values according to the considered municipality and sex, was divided in clean water intake rate and in contaminated water intake rate (equal to 1/3 of the water intake).

The municipality of Legnago was in B-red area but was considered in the three scenarios since the analysis of groundwater from ARPAV showed PFOA concentrations slightly over the limit of detection (average value: 19 ng/L).

For PFOA, other two scenarios were created for the subjects living in the municipalities of Sarego and Lonigo, both at individual and at average level: the median scenario (MS) and the average scenario (AS). The two scenarios differed for the reference value fixed for PFOA concentration in groundwater. In the MS the reference value was the median of the distribution of PFOA concentration data in groundwater, while in the AS the reference value was the average value. The creation of these two scenarios aimed to find a range of uncertainty in the final output for the PBPK models. Looking at the shape of the distribution curve, the MS was assumed to be the most reliable scenario, since the median seemed to be the most appropriate value to adopt, while the AS was judge to be a worst case scenario.

Another issue we faced during the exposure assessment was whether or not to consider the few highest PFAS concentrations data as outliers. Eventually they were not considered as outliers, since the reason of their high value was likely that they were taken in different places or in different time periods. Spatial and temporal variability of PFAS concentrations in groundwater and temporal variability of PFAS

concentrations in tap water were so considered the main cause. These assumptions were confirmed by the study of the contaminated plume pathway over time and by the study of PFAS concentration trend in raw water.

The PFAS daily intake through drinking water was computed multiplying the PFAS concentration in water (equal to the sum of the PFAS concentration values in tap water and in groundwater) by the contaminated water intake rate as shown in the following equation:

$$PWI_n = PCTW_m \cdot TWIR_n + PCGW_m \cdot GWIR_n$$

Where:

PWI_n = PFAS daily intake through drinking water for the n-subject [ng/day];

$PCTW_m$ = PFAS concentration in tap water in the m-municipality [ng/L];

$TWIR_n$ = tap water intake rate for the n-subject [L/day];

$PCGW_m$ = PFAS concentration in groundwater in the m-municipality [ng/L];

$GWIR_n$ = groundwater intake rate for the n-subject [L/day];

Since:

$$TWIR_n = GWIR_n = CWIR_n = 1/3 \cdot WIR_n$$

Where:

$CWIR_n$ = contaminated water intake rate (for each drinking water source) for the n-subject [L/day].

WIR_n = water daily intake rate for the n-subject [L/day]

The previous equation can also be rewritten as follow:

$$PWI_m = (PCTW_m + PCGW_m) \cdot CWIR_n$$

$$PWI_m = PCW_m \cdot CWIR_n$$

Where:

PCW_m = total PFAS concentration in water (sum of PFAS concentration in tap water and PFAS concentration in groundwater) in the m-municipality [ng/L].

	PFOA A-WI, tot pop [ng/day]	**PFOA A-WI, men [ng/day]**	**PFOA A-WI, women [ng/day]**	**PFOA A-CW [ng/L]**	**A-CWIR [L/day]**	**A-WIR [L/day]**
Sarego	381.7 (MS) 1888.0 (AS)	402.5 (MS) 1990.7 (AS)	368.7 (MS) 1823.8 (AS)	779 (M) 3853 (A)	0.49	1.47
Lonigo	359.1 (MS) 1054.7 (AS)	395.9 (MS) 1162.7 (AS)	324.5 (MS) 953.0 (AS)	649 (M) 1906 (A)	0.55	1.66
Legnago	26.2	28.2	24.1	46	0.57	1.71
Veronella	98.1	134.9	79.7	184	0.53	1.60
Albaredo d'Adige	159.0	181.9	117.3	255	0.62	1.87

Table 18 PFOA average contaminated water intake rate (PFOA A-WI) and average PFOA concentration in water (PFOA A-CW), that is the sum of PFOA concentration in tap water and PFOA concentration in groundwater, average contaminated water intake rate (A-CWIR) and average water intake rate (A-WIR). M=median, A= average value. MS = median scenario, AS = average scenario.

	PFOA A-WI, tot pop [ng/day]	PFOA A-WI, men [ng/day]	PFOA A-WI, women [ng/day]	PFOA A-CW [ng/L]	A-CWIR [L/day]	A-WIR [L/day]
Total pop. (MS)	188.4	196.3	182.3	349.9	0.55	1.67
A-red area (MS)	366.5	397.8	340.3	691.8	0.53	1.60
B-red area	58.3	69.4	46.9	100.1	0.57	1.72
Total pop. (AS)	594.7	585.9	608.6	1133.0	0.55	1.67
A-red area (AS)	1328.8	1406.2	1264.0	2546.5	0.53	1.60

Table 19 PFOA average contaminated water intake rate (PFOA A-WI) and average PFOA concentration in water (PFOA A-CW), that is the sum of PFOA concentration in tap water and PFOA concentration in groundwater, average contaminated water intake rate (A-CWIR) and average water intake rate (A-WIR). MS = median scenario, AS = average scenario.

	PFOS WI, tot pop [ng/day]	PFOS WI, men [ng/day]	PFOS WI, women [ng/day]	PFOS CW [ng/L]	A-CWIR	A-WIR
Sarego	32.8	34.6	31.7	67	0.49	1.47
Lonigo	27.1	29.9	24.5	49	0.55	1.66
Legnago	5.7	6.1	5.2	10	0.57	1.71
Veronella	9.6	13.2	7.8	18	0.53	1.6
Albaredo d'Adige	14.3	16.4	10.6	23	0.62	1.87
Total pop.	16.7	17.5	15.9	30.9	0.55	1.67

A-red area	29.0	31.3	27.1	54.9	0.53	1.60
B-red area	7.7	8.9	6.4	13.3	0.57	1.72

Table 20 PFOS average contaminated water intake rate (PFOA A-WI) and average PFOS concentration in water (PFOS A-CW), that is the sum of PFOS concentration in tap water and PFOS concentration in groundwater, average contaminated water intake rate (A-CWIR) and average water intake rate (A-WIR). M=median, A= average value. MS = median scenario, AS = average scenario.

The PFAS exposure assessment was considered particularly accurate for the municipalities of Legnago, Veronella and Albaredo d'Adige, since they were located in the B-red area. In fact all the subjects living in the same municipality were probably exposed to the same PFAS concentration in tap water. On the contrary, PFAS (especially PFOA) concentrations in groundwater were very different from one place to another in the same municipality, as shown by the collected samples. This huge spatial variability of the PFAS concentration in groundwater led to a huge difference in PFAS exposure of subjects living in the same municipality but drinking from different private wells. For these reasons a moderate-high uncertainty was associated to the predictions of the exposure assessment for the municipalities of Sarego and Lonigo, located in the A-red area, while a low uncertainty was associated to the predictions for the subjects living in the municipalities of Legnago, Veronella and Albaredo d'Adige, located in the B-red area. However, while the PFAS special variability in groundwater was surely an issue when the analysis was at individual level, it was not such a problem when the analysis was at aggregate level. In fact at aggregate level the uncertainty due to PFAS spatial variability decreases sharply, since the PFOA average value for the municipality become an appropriate data to represent a group of subjects located in different areas of the same municipality. These considerations led to conclude that testing the Loccisano model at individual level was mostly significant for the municipalities of Veronella, Albaredo d'Adige and Legnago.

When the accuracy in the exposure assessment process is high then the model validation process can be considered more accurate and useful. In this work the accuracy in the exposure assessment process was medium or high in every scenario

except in those created for PFOA for the subjects living in the municipalities of Sarego and Lonigo at individual level.

In the following table are summarized the considerations on the exposure assessment concerning PFOA intake through water.

	Sarego	Lonigo	Legnago	Veronella	Albaredo d'Adige
Level of contamination in tap water	4	2	5	3	1
Level of contamination in groundwater	1	2	3 (low)	4 (not detected)	4 (not detected)
Uncertainty at aggregate level	low/medium	low/medium	low	low	low
Uncertainty at individual level	high	high	low	low	low

Table 21 Contamination level ranking (1 is the highest and 5 is the lowest) and uncertainty levels for PFOA for the people living in that municipality.

PFAS INTAKE THROUGH FOOD

Data on number food portions taken weekly were collected in the HBM questionnaire. The "standard portion" for each type of food was taken from the Italian society of human nutrition (SINU) that defines the quantitative standards for portions in Italy in accordance with consumer expectations (Table 22).

Type of food	Standard portion	Unit
Fruit	150	g

Raw vegetable	80	g
Cooked vegetable	200	g
Milk, yogurt	125	mL
Meat	100	g
Egg	50	g

Table 22 quantitative standards for portions in Italy according to the Italian society of human nutrition (SINU).

The number of portions of food per week taken for each subject from the questionnaire was multiplied by the weight of the "standard portion" for each type of food to obtain the weight of food ingested weekly.

PFOA and PFOS concentrations in food were taken from studies on Veneto population exposed to PFAS contamination conducted by the ISS. The concentration values were referred to local food (A-red area) because people lived in a rural area and many subjects owned a vegetable garden (71) and animals (26). Some types of food were missing but likely the ones with the highest PFAS concentrations (eggs, milk) were analysed. In the ISS study many samples from different type of food were analyzed for each food category and 3 scenarios were developed. The chosen PFOA and PFOS concentration value for each food category was the average value of the data in the medium scenario, as reported in the following table:

Food category	**Type of food**	**PFOA conc. [µg/kg]**	**PFOS conc. [µg/kg]**	**PFOA chosen value[µg/kg]**	**PFOS chosen value[µg/kg]**
Fruit	Apricot	0.080	0.140	0.058	0.065
	Cherry	0.050	0.050		
	Apple	0.050	0.050		
	pear	0.050	0.050		
	peach	0.050	0.050		

	grapes	0.067	0.050		
Vegetable	Asparagus	0.063	0.050	0.064	0.050
	Onion	0.050	0.050		
	Corn	0.119	0.050		
	Lettuce	0.050	0.050		
	Potato	0.050	0.051		
	Tomato	0.050	0.050		
Meat (muscle)	Cow	0.057	0.052	0.080	0.050
	Pig	0.252	0.050		
	Duck	0.050	0.050		
	Broiler	0.050	0.050		
	Guinea fowl	0.050	0.050		
	Hen	0.050	0.050		
	Turkey	0.050	0.050		
Milk				0.051	0.050
Egg				1.171	0.929

Table 23 PFAS concentrations in food according to the ISS (Medium scenario) and PFAS chosen values.

Since few important food categories were missing in the ISS study (e.g. fish), these values, referred to a consumption of the local food only, were chosen for all the subjects to avoid an underestimation of PFAS exposure through food.

The predicted PFAS intake through a food category was computed according to the following equation:

$$PIF_i = \frac{DP \times PCF \times SP}{1000}$$

Where:

PIF_i = PFAS daily intake through food category i [ng/day];

DP = Daily portion of food category i [1/day];

PCF = PFAS concentration in food category i [ng/kg];

SP = Standard portion of food category i [g];

and 1000 is the correction factor [g/kg].

The total intake of PFAS through food was then computed for each subject as follow:

$$PTIF_n = \sum_{i=1}^{5} PIF_i$$

Where:

$PTIF_n$ = PFAS total daily intake through food estimated for the n-subject [ng/day].

The average PFOA total daily intake through food (A-PTIF) was quite similar for all the municipalities, ranging from 37.1 ng/day (Albaredo d'Adige) to 51.0 ng/day (Lonigo). A-PTIF showed very similar values for men and women (men: 42.1; women: 44.5).

Municipality	**A-PTIF [ng/day]**	**A-$PIF_{fruit\&veg}$ [ng/day]**	**A-PIF_{milk} [ng/day]**	**A-PIF_{meat} [ng/day]**	**A-PIF_{egg} [ng/day]**
Sarego	42.9	20.5	5.4	5.6	11.4
Lonigo	51.0	24.2	4.7	6.2	15.9
Legnago	40.4	17.5	4.2	6.3	12.4
Veronella	39.1	18.4	3.4	5.6	11.7
Albaredo d'Adige	37.1	15.7	3.8	6.7	10.8
Tot pop	43.3	19.7	4.4	6.2	13.0
Men	42.1	16.0	4.4	7.4	14.2

Women	44.5	23.3	4.4	4.9	11.9
A-red area	48.3	23.0	4.9	6.0	14.4
B-red area	39.7	17.3	4.0	6.3	12.0

Table 24 Average total PFOA daily intake through food (A-TPI) and average PFOA intake for each food category (A-PIi) for the subjects living in each municipality, for total population (tot pop), men , women, the A-red Area and the B-red area.

The average PFOS total daily intake through food (A-PTIF) for the total population was a little lower respect to PFOA (PFOS: 37.2 ng/day; PFOA: 43.3 ng/day). Also A-PTIF for PFOS was quite similar for all the municipalities, ranging from 31.4 ng/day (Albaredo d'Adige) to 44.0 ng/day (Lonigo). A-PTIF for PFOS, like A-PTIF for PFOA, showed very similar values for men and women (men: 35.4 ng/day; women: 38.9 ng/day).

Municipality	**A-PTIF [ng/day]**	**A-PIF$_{fruit\&veg}$ [ng/day]**	**A-PIF$_{milk}$ [ng/day]**	**A-PIF$_{meat}$ [ng/day]**	**A-PIF$_{egg}$ [ng/day]**
Sarego	37.3	19.4	5.3	3.5	9.0
Lonigo	44.0	22.9	4.6	3.9	12.6
Legnago	34.5	16.6	4.1	4.0	9.8
Veronella	33.5	17.4	3.3	3.5	9.3
Albaredo d'Adige	31.4	14.8	3.8	4.2	8.6
Tot pop	37.2	18.7	4.3	3.9	10.3
Men	35.4	15.2	4.3	4.7	11.2
Women	38.9	22.0	4.3	3.1	9.5
A-red area	41.8	21.7	4.8	3.8	11.4
B-red area	33.8	16.4	3.9	4.0	9.5

Table 25 Average total PFOS daily intake through food (A-TPIF) and average PFOS intake for each food category (A-PIFi) for the subjects living in a municipality, for total population (tot pop), men , women, the A-red Area and the B-red area.

These data showed a similar contribution of the food to the PFAS exposure for the different municipalities and the PFOA average intake was just a bit higher compared

to the PFOS one. Both PFOA and PFOS average total intakes through food were slightly lower in men respect to women and quite higher for the subjects living in the A-red area respect to those living in the B-red area. These facts led to the following conclusion: the differences in PFAS serum concentrations observed between men and women were not attributable to food while those observed between the A-red area and the B-red area were partly attributable to food. Looking at the PFAS concentrations in water, it was clear that the contribution from food to the total exposure was very low compared to the contribution from water for PFOA, especially in the A-red area, while it was high for PFOS, especially in the B-red area (and in particular in the municipalities of Legnago and Veronella).

The exposure of subjects to the PFAS in food was assumed to start at the date of birth of the subject and to end at the date of the blood sampling. Therefore the food route contribution to the total exposure was expected to be quite relevant especially for PFOS, since the PFOS daily intakes through drinking water were similar to those through food. The same considerations were valid for PFOA in the municipalities located in the B-red area, where there PFOA was not present in groundwater, but in tap water only.

AIR ROUTE AND DERMAL ROUTE

Looking at PFASs physico-chemical characteristics (e.g. physical state, vapour pressure, solubility) and at the exposure scenario, the principal matrix for long-range environmental transport can be definitely considered water. However, also air route can be a relevant route of exposure for PFASs, as claimed by the scientific literature (Shin et al., 2011; Frisbee et al., 2009; US-EPA, 2014).

In this study air route was considered negligible for the subjects living in the contaminated area. This decision was taken after an analysis carried out by ARPAV (ARPAV, 2017; Veneto region, 2019).

Starting from 2013, ARPAV measured PFASs concentrations at the chimney in the fluoropolymer plant inside the factory considered the main responsible for the

PFASs found in rivers and groundwater. The sampling procedure involved 12 different PFASs and the use of PUF (polyurethane foams) high volume air samplers and standard filters (C13), while the extracting procedure involved the adoption of three extraction cycles with ultrasound (ARPAV, 2017; Veneto region, 2019). PFOA concentration at the chimney was found to be higher respect to PFOS.

A simulation of PFOA dispersion in the atmosphere was carried out by ARPAV to predict the contribution of the air route to the total exposure using a non-steady-state Lagrangian puff dispersion model (CALPUFF; Scire et al., 2000) supported by a meteorological pre-processor (CALMET) to create a fall-out map for the area around the municipality of Trissino (ARPAV, 2017; Veneto region, 2019).

The simulation area was centred on the fluoropolymer plant and was 8x8 km^2 wide. The grid cell was 250x250 m^2 wide. PFASs were considered as fine particulate and the emission was assumed constant and without interruptions for the simulation period (year 2016).

source	H [m]	D [m]	V [m/s]	T [C°]	Q [m^3/s]	PFOA mass flow [g/s]
Chimney E17	20	0.24	6.5	48	0.29	7.4E-08
Cooling tower 1	8	4	1.9	25	23.65	1.3E-07
Cooling tower 2	8	4	1.9	25	23.65	1.3E-07

Table 26 characteristics of the modeled sources of PFAS in the fluoropolymer plant (H=height of the chimney, D=diameter of the chimney), characteristics of the emitted plumes (V=velocity,T=temperature,Q=flow) and PFOA mass flow in the plumes.

The CALPUFF output showed the PFOA highest concentration in atmosphere near the sources, inside the fluoropolymer plant (for chimney E17: 60 m; for cooling towers: 202 m and 203 m for the annual average, 54 m and 57 m for the daily

maximum) with a daily average deposition flux equal to 239 [pg/(m^2day)] for the chimney E17 and equal to 380 [pg/(m^2day)] for the cooling towers. Looking at the outputs and at the fall-out maps produced, an extremely rapid decrease in PFOA concentration with the increase of the distance from the point of the highest concentration was predicted by CALPUFF. The PFOA daily highest concentration predicted for the nearest receptor, located in the proximity of the fluoropolymer plant, was 1.6E-02 [ng/m^3], but looking at the fall-out maps, it was clear that three kilometres from the fluoropolymer plant the PFOA annual average concentration was already very low (<9E-06 [ng/m^3]). This fact was likely due to the low height of the chimneys. The contribution of the plant predicted by CALPUFF to PFOA daily and annual average concentrations few kilometres far from the plant (in the red area) was very low, since the values observed in samples collected in a city (Vicenza) near the municipalities under study were found extremely higher respect to those attributable to the plant (0.03 [ng/m^3] for PFOA and 0.01 [ng/m^3] for PFOS). The values predicted by CALPUFF also were significantly lower respect to those found around the world in areas near a fluoropolymer plant where air was considered an important route of exposure (e.g. “6 out of 28 samples collected in the study in West Virginia were between 70 and 170 ng/ m^3 for PFOA”, (ARPAV, 2017; referring to contamination from Du Point company: Shin et al., 2011; Frisbee et al., 2009). Also, PFOA concentrations in the atmosphere observed in the contaminated area were very lower respect to the concentration found in samples from many urban areas around the world (ARPAV, 2017).

For all these reasons and after having analyze the size of PFAS contribution through diet to the exposure, air route was considered negligible and was not taken into account, since a very slight underestimation in the predicted result could be attributed in not having considered air route in the exposure assessment.

	Mass flow	**Red area [ng/m^3]**	**Max nearest**	**Vicenza (samples)**	**urban areas**	**J, UK (samples)**

	[g/year]		receptor [ng/m^3]	[ng/m^3]	(samples) [ng/m^3]	[ng/m^3]
PFOA	10.5	<9E-06	1.6E-02	3E-02	1.6E-03-1.5E-02	1.0E-01-5.5E-01

Table 27 PFOA mass flow yearly emitted by the fluoropolymer plant and PFOA average annual concentration predicted few kilometers far from the fluoropolymer plant (in the red area), PFOA daily highest concentration predicted at the nearest receptor, PFOA concentration observed in samples collected in Vicenza, in urban areas around the world (urban areas) and in Japan and United Kingdom (J,UK).

For the present study, also dermal contact was considered a negligible route of exposure for PFAS, since subjects were exposed to high concentrations of PFAS through diet while there were no reasons to consider the PFAS exposure through dermal contact different from that for the general population.

PFAS TOTAL DAILY INTAKE

PFAS total daily intake was calculated for each subject according to the following equation:

$$PTI_n = PWI_m + PTIF_n$$

Where:

PTI_n = PFAS total daily intake for the n-subject [ng/day];

PWI_m = PFAS intake through drinking water for the subjects living in the m-municipality [ng/day];

$PTIF_n$ = PFAS total daily intake through food estimated for the n-subject [ng/day].

The average PFAS total daily intake calculated for the subjects living in the A-red area was very higher respect to the subjects living in the B-red area (414.9 ng/day (MS) and 1377.1 ng/day (AS) vs 98.0 ng/day, respectively, for PFOA and 70.8 ng/day (MS) vs 41.5 ng/day, respectively, for PFOS), as expected. On the contrary,

the average PFAS total daily intakes calculated for men and women were found very similar (238.4 ng/day (MS) vs 226.8 (MS) ng/day, respectively for PFOA and 54.0 ng/day (MS) vs 53.8 ng/day, respectively, for PFOS). The average total daily intake was found very higher for PFOA (231.8 ng/day) respect to PFOS (53.9 ng/day).

	PFOA [ng/day]			PFOS [ng/day]		
Municipality	**A-PTI**	**A-PTI, men**	**A-PTI, women**	**A-PTI**	**A-PTI, men**	**A-PTI, women**
Sarego	424.6 (1930.9, AS)	445.0	411.6	70.1	71.3	69.3
Lonigo	410.1 (1105.7, AS)	449.7	373.0	71.2	75.5	67.2
Legnago	66.6	64.5	69.2	40.2	36.3	44.5
Veronella	137.2	171.6	119.9	43.1	43.1	43.1
Albaredo d'Adige	196.1	220.4	151.8	45.8	49.1	39.7
Tot pop	231.8 (638.1, AS)	238.4	226.8	53.9	54.0	53.8
A-red area	414.9 (1377.1, AS)	448.3	386.8	70.8	74.1	67.9
B-red area	98.0	106.2	89.7	41.5	39.4	43.5

Table 28 PFAS total average daily intake (A-PTI) in [ng/day] for the different municipalities and for the total population (median scenario, MS). AS = average scenario.

PBPK MODELS TESTING PROCEDURE

In this study an advanced and complex multi-compartment PBPK model developed for PFAS (Loccisano et al., 2011) supported by a very detailed exposure assessment was tested with the observed data from the HBM study and compared with two simpler and user-friendly one-compartment models (Thompson et al., 2010; Bartell, 2017) that require a quicker and more direct simulation setting and less detailed information from the exposure assessment. The code of the multi-compartment model for humans developed by Loccisano (Loccisano et al., 2011), and so reported here as the "original Loccisano model", was slightly modified to improve the accuracy of the exposure description. So, the original Loccisano model with this implementation was called in this study the "Loccisano model". The Loccisano model was tested both at individual and at aggregate level. The two less complex models were developed by Thompson (Thompson et al., 2010) and Bartell (Bartell, 2017) and so they were called in this study the "Thompson model" and the "Bartell model", respectively. Thompson and Bartell models were tested at aggregate level only. A new PBPK model was created slightly modifying the input parameters and the equation terms in the code of the Loccisano model to better fit the observed data. This model was named the "modified Loccisano" model (two different versions of this model were implemented: Version 1 and Version 2). The modified Loccisano model optimization was achieved using parameters derived from the scientific literature to introduce only credible and accurate values under the physiological point of view.

The testing procedure was based on the comparison of the observed PFAS serum concentrations at the time of the blood sampling (since observed data from the HBM study were available at this time only) with the PFAS concentrations in plasma compartment (for all the new models based on the original Loccisano model) or PFAS serum concentrations (for Thompson and Bartell models) predicted by the PBPK models. Comparing PFAS concentration in plasma and serum is equivalent, since PFAS are bound to albumin in plasma, so serum to plasma ratios for PFAS is

1:1 and this ratio is independent of the level of concentrations measured (Ehresman et al., 2007). Moreover, PFASs are not metabolised in the human body, so they can be easily found and measured in serum, without looking for metabolites.

Observed PFAS serum concentrations were measured as ng/mL in the HBM study while the PFAS serum concentrations predicted by the Loccisano model were reported in µg/L. Since the two units are equivalent, in this study all the concentrations, when they are compared, are reported as ng/mL.

It is essential to underline that data obtained from a very accurate exposure description were given as input in the models derived from the original Loccisano model, while less accurate data were used for both the Thompson and the Bartell model. In fact the two simple one-compartment models allowed to provide a constant exposure to tap water over time only, while the models from the original Loccisano model, allowed to consider the PFAS concentration decrease in tap water over the last years before the blood sampling. This difference resulted in a slightly overestimation for PFAS serum concentration predicted by the one-compartment models.

The models tested in this study were chosen for their ability to predict PFAS serum concentrations in humans accurately. In fact they were all created exclusively to simulate PFAS only (so they are compound specific PBPK models). The Loccisano model predicts the PFAS uptake in several tissues of the human body through a system of differential equations, taking into account the renal resorption process, that seems essential to predict PFAS pathway in human body at best. Thompson and Bartell model are one-compartment models based on equations for a one-compartment PBPK model with a constant exposure.

THE THOMPSON MODEL

The model proposed by Thompson (Thompson et al., 2010), identified in this study as the "Thompson model", is a simple, one-compartment, first order pharmacokinetic model developed to predict PFAS serum concentrations starting from few data: the dose, the elimination rate, and the volume of distribution. The model equation is the following:

$$\frac{dCA}{dt} = \frac{D(t)}{Vd} - k \cdot CA(t)$$

Where:

CA = PFAS serum concentration [ng/mL]

D = PFAS daily absorbed dose [ng/(kg (BW)*day)]

Vd = volume of distribution [mL/kg (BW)]

k = PFAS serum elimination rate constant [1/day]

Thompson assumed the existence of steady-state conditions, so the equation became the following:

$$CA = \frac{D}{Vd \cdot k}$$

The Thompson model and other similar models for PFASs were firstly used adopting the backward approach (i.e. from concentrations in human body to concentrations in exposure media) (e.g. Trudel et al., 2008; Thompson et al., 2010). In this and other (Fromme et al., 2007) studies the Thompson model was used

adopting the forward approach (i.e. from concentrations in exposure media to concentrations in human body).

Standard data collected for the United State population or taken by the literature were also suggested by Thompson (Thompson et al., 2010) to expedite the analysis. In this study the data collected by the HBM study for the Veneto population were used.

Using the model equation in this form did not allow to vary the exposure over time. So, the PFAS concentrations in tap water used in the Thompson model were those used for time period P1 in the Loccisano model, since they were the most representative for the entire period of exposure. The impossibility to vary the exposure over time resulted in an overestimation for the Thompson model since only the highest PFAS concentration value was considered. The overestimation was higher for the subjects living in the municipalities of Veronella, Albaredo d'Adige and Legnago, since in the B-red area the tap water was the principal route of exposure. In the A-red area the principal route of exposure was the groundwater, especially for PFOA, so the overestimation was less relevant. The exactly same considerations were also true for the Bartell model.

To derive the data requested by the Thompson model, first the PFAS average total daily intake for each municipality and sex (calculated adding the PFAS daily intake through food and through water) was divided by the average body weight of that municipality and sex to find the PFAS exposure per kg of body weight.

$$E_m = \frac{(A)PTI_m}{(A)BW_m}$$

Where:

E_m = daily average exposure to PFAS per kg of body weight for the subjects living in the m-municipality [ng/(kg(BW) day)];

(A)BW_m = average body weight for the subjects living in the m-municipality.

Then, the PFAS exposure per kg of body weight was multiplied by the gastrointestinal absorption fraction of 0.91 (Thompson et al., 2010; Trudel et al., 2008) to obtain the PFAS daily absorbed dose (D).

$$D_m = E_m \cdot 0.91$$

Where:

D_m = PFAS daily average absorbed dose for the subjects living in the m-municipality [(ng/kg (BW))/ day].

Finally the equation of the Thompson model was applied to obtain the PFAS serum concentration:

$$CA = \frac{D_m}{(Vd_m \cdot k)}$$

Where:

CA = PFAS average serum concentration for the subjects living in the m-municipality [ng/mL];

Vd_m = average volume of distribution for the subjects living in the m-municipality [mL/kg (BW)]; it is equal to 170 for PFOA and 230 for PFOS (Thompson et al., 2010);

k = PFAS serum eliminate constant [1/day]; it is equal to $8 \cdot 10^{-4}$ for PFOA and $3 \cdot 10^{-4}$ for PFOS (Thompson et al., 2010).

THE BARTELL MODEL

The model proposed by Bartell (Bartell, 2017), identified in this study as the "Bartell model", consisted in an implementation of a one-compartment PBPK model for PFAS in an online platform, to create an online PFASs serum calculator (https://www.ics.uci.edu/~sbartell/pfascalc.html, Lu S, Bartell SM. Serum PFAS Calculator for Adults, Version 1.2, 2020). This PFASs calculator was developed to predict serum concentrations of several PFASs (PFOA, PFOS, PFHxS, PFNA) quickly and with few available data. The PFASs calculator was used in this study to predict PFOA and PFOS serum concentrations.

The Bartell model is based on a modified one-compartment exponential decay model with adjustment for background exposures to describe the relationship between PFOA intake and serum concentrations in adults (Olsen et al., 2007; Bartell et al., 2010; Bartell, 2012).

The Bartell model was based on the mathematical solution for a one-compartment PBPK model with a constant exposure (Thuresson et al.2006; Bartell 2012):

$$C_t = C_\infty + (C_0 - C_\infty)e^{-kt}$$

Where:

C_t = PFAS serum concentration at time t (ng/mL);

C_∞ = PFAS serum concentration at steady state (i.e. after enough time has passed for the serum concentration to stabilize after continuous exposure);

C_0 = initial PFAS serum concentration;

k = elimination rate constant = $\frac{\ln(2)}{t_{1/2}}$ (1/years). $t_{1/2}$ = half-life = 2.3 (years) for PFOA based on a prospective sub-cohort of 200 participants from the C8 Science Panel studies (Frisbee et al., 2009) and 3.4 (years) for PFOS (Li et al., 2018).

For individuals consuming PFOA-contaminated water, the steady state serum PFOA concentration is calculated by the online calculator as follows:

$$CA_{\infty} = \frac{PCW_m \cdot S}{1000} + B$$

Where:

CA_{∞} = average PFAS serum concentration at steady state (i.e. after enough time has passed for the serum concentration to stabilize after continuous exposure) for the m-municipality;

PCW_m = total PFAS concentration in water: average for the m-municipality (ng/L);

S = steady-state ratio of serum:water PFAS concentrations = 114 (unitless) for PFOA, (Hoffman et al., 2011; CDC, 2017);

B = background serum PFOA concentration (ng/mL) contributed by sources other than local drinking water = 1.67 ng/mL for PFOA and 5.20 ng/mL for PFOS (Hoffman et al., 2011; CDC, 2017).

The online calculator takes into account sex and if the women are in their fertile age or not.

The value for the volume of distribution (V_d) was set by the Bartell model developers equal to the value chosen in the Thompson model: 0.17 (L/kg) for PFOA and 0.23 (L/kg) for PFOS (Thompson et al., 2010).

The online serum calculator required two parameters as input. First of all, the starting serum PFAS concentration (C_0) was required as initial condition. The value for this parameter was set equal to 2 ng/mL for PFOA and equal to 5 ng/mL for PFOS, as suggested by the calculator developers for a person without PFAS in his/her water. Then, the PFAS concentration in water [ng/L] was set for each municipality according to the values calculated in this study (PCW_m).

The required parameter "water ingestion rate" (WINR) was fixed by the calculator but it was set to the value predicted by the following equation:

$$WINR_m = \frac{(A)CWIR_m}{(A)BW_m}$$

Where:

$WINR_m$ = average water ingestion rate for kg of body weight for the subjects living in the m-municipality (L/(day*kg));

$(A)CWIR_m$ = average contaminated water intake rate for the subjects living in the m-municipality (L/day);

$(A)BW_m$ = average body weight for the subjects living in the m-municipality (kg).

THE ADAPTED LOCCISANO MODEL

In this study the original Loccisano model was adapted to simulate at best the PFAS intake of the selected subjects. In particular few lines of the model code were modified, using the Berkeley-Madonna program language (8.3.9 version), to

simulate the rapid decrease in PFAS exposure during the last years due to the decrease in PFAS concentration levels in the tap water. So, first of all, the number of the parameters defined in the section of the model "exposure parameters" was extended, adding all the necessary times (tchng, T2, T3, T4) corresponding to the number of hours from the beginning of the exposure (i.e. the birth of the subject) to the end of the time periods (P1, P2, P3 and P4).

The following lines define the exposure parameters (i.e. times Ti) in the Loccisano model code for PFOA for the subject n. 24113, living in the municipality of Lonigo:

"

; Exposure parameters

tchng = 251004

; number of hours from birth to the end of P1 (February 2014)

T2 = 269400

; number of hours from birth to the end of P2 (P2 goes from Feb 2014 to Apr 2016)

T3 = 282540

; number of hours from birth to the end of P3 (P3 goes from Apr 2016 to Ott 2017, from Ott 2017 the concentration in tap water is 5)

T4 = 284700

; number of hours from birth to the time of the blood sampling, Nov 2018

".

Secondly, the lines of the code to turn the dose on and off were modified to describe the beginning and the end of the exposure to PFAS concentrations in groundwater and the beginning and the end of the different concentrations of PFAS in tap water.

This operation was essential to attribute the right PFAS concentration to the corresponding time period (Pi).

For example the following lines were written to turn the dose on or off in the Loccisano model for PFOA for the subject n. 24113, living in the municipality of Lonigo in the MLS:

“

;turn dose on/off

DoseOn20142016 = IF time>tchng THEN 1.0 ELSE 0.0

DoseOn20162017 = IF time>T2 THEN 1.0 ELSE 0.0

DoseOn2017 = IF time>T3 THEN 1.0 ELSE 0.0

DoseOnpozzo = IF time<tchng THEN 1.0 ELSE 0.0

”.

In the MLS the end date for the exposure to PFAS from the private well was chosen as the end date of the time period P1, so the “dose on” mode for the private well was selected before the time = tchng only.

The same lines were changed in the WCS but in this case the “dose on” mode for the private well was selected before the time of the blood sampling (T4):

“

DoseOnpozzo = IF time<T4 THEN 1.0 ELSE 0.0

”.

The line was removed in the BCS since it was unnecessary (no exposure to the PFAS in groundwater was considered in this scenario).

Then, in the “oral exposure” section the following parameters were given as input in the model:

- the PFAS total daily intake through food estimated for the n-subject in µg/day (= $PTIF_n$ /1000);
- the PFAS concentration in tap water in the m-municipality in µg/day (= $PCTW_m$ /1000) for the time period P1;
- the difference between the PFAS concentration in tap water in the m-municipality in µg/day (= $PCTW_m$ /1000) for the time period P1and the PFAS concentration in tap water in the m-municipality in µg/day (= $PCTW_m$ /1000) for the time period P2;
- the difference between the PFAS concentration in tap water in the m-municipality in µg/day (= $PCTW_m$ /1000) for the time period P2 and the PFAS concentration in tap water in the m-municipality in µg/day (= $PCTW_m$ /1000) for the time period P3;
- the difference between the PFAS concentration in tap water in the m-municipality in µg/day (= $PCTW_m$ /1000) for the time period P3and the PFAS concentration in tap water in the m-municipality in µg/day (= $PCTW_m$ /1000) for the time period P4;
- the PFAS concentration in groundwater in the m-municipality in µg/day (= $PCGW_m$ /1000);
- the contaminated water intake rate for the n-subject [L/day] (= $CWIR_n$), that is equal to the tap water intake rate for the n-subject [L/day]; (= $TWIR_n$) and equal to the groundwater intake rate for the n-subject [L/day]; (= $GWIR_n$).

For example the following lines were written to describe the oral exposure in the Loccisano model for PFOA for the subject n. 24113, living in the municipality of Lonigo in the MLS, AS:

“

; Oral exposure

; Oral uptake (ug/kg/day), MB average values (average of LB and UB in the ISS document)

Oraldose = 0.021

; (ug/day), PFAS total daily intake through food estimated for the n-subject in µg/day (= ;PTIFn /1000)

Drinkconcpozzo = 1.657

; PFAS concentration in groundwater in the municipality of Lonigo, AS, (µg/L)

Drinkconcreteal2014 = 0.249

; PFAS concentration in tap water in the m-municipality in µg/L (= ;PCTWm /1000) for all the time period P1

Drinkconcrete20142016 = 0.160

; difference in PFAS concentration in tap water between P1 and P2 (µg/L)

Drinkconcrete20162017 = 0.028

; difference in PFAS concentration in tap water between P2 and P3 (µg/L)

Drinkconcretedal2017 = 0.056

; difference in PFAS concentration in tap water between P3 and P4 (µg/L)

Drinkrate = 1.5/3

; WIR_{24113} = water daily intake rate for the subject 24113 (L/day)

Drinkdosepozzo = Drinkconcpozzo*Drinkrate

; PFAS daily intake through groundwater (ug/day)

Drinkdoserete2014 = Drinkconcreteal2014*Drinkrate

; PFAS daily intake through tap water during the time period P1 (ug/day)

Drinkdoserete20142016 = Drinkconcrete20142016*Drinkrate

; difference in PFAS daily intake through tap water between P1 and P2 (ug/day)

Drinkdoserete20162017 = Drinkconcrete20162017*Drinkrate

; difference in PFAS daily intake through tap water between P2 and P3 (ug/day)

Drinkdoserete2017 = Drinkconcretedal2017*Drinkrate

; difference in PFAS daily intake through tap water between P3 and P4 (ug/day)

".

The differences between the PFAS concentration in tap water in the m-municipality in µg/day (= $PCTW_m$ /1000) for the time period Pi and the PFAS concentration in tap water in the m-municipality in µg/day (= $PCTW_m$ /1000) for the following time period (P(i+1)) were calculated since that was the correct way to give the actual PFAS daily intake as input in the model, as explained later in the following sections.

The next section described the creation of the input to be given to the model compartment and it was written as follows:

"

Tinput = 24

; to spread the exposure over a period of 24 hours

;oral

Inputcibo = IF MOD(time,24) <=Tinput THEN Oraldose/Tinput ELSE 0.0

; input created to describe the PFAS hourly intake through food (ug/h)

;drinking water

Inputpozzo = IF MOD(time,24) <= Tinput THEN Drinkdosepozzo/Tinput ELSE 0.0

; input created to describe the PFAS hourly intake through groundwater (ug/h)

Inputrete1 = IF MOD(time,24) <= Tinput THEN Drinkdoserete2014/Tinput ELSE 0.0

; input created to describe the PFAS hourly intake through tap water during the time period P1(ug/h)

Inputrete2 = IF MOD(time,24) <= Tinput THEN Drinkdoserete20142016/Tinput ELSE 0.0

; input created to describe the PFAS hourly intake through tap water during the time period P2 (ug/h)

Inputrete3 = IF MOD(time,24) <= Tinput THEN Drinkdoserete20162017/Tinput ELSE 0.0

; input created to describe the PFAS hourly intake through tap water during the time period P3 (ug/h)

Inputrete4 = IF MOD(time,24) <= Tinput THEN Drinkdoserete2017/Tinput ELSE 0.0

; input created to describe the PFAS hourly intake through tap water during the time period P4 (ug/h)

”.

The inputs created were added in the differential equation for the gut compartment as shown here:

“

; Gut compartment

AG' = QG*(CA*Free-CG*FreeG) + Inputcibo + Inputpozzo*DoseOnpozzo + Inputrete1 - Inputrete2*DoseOn20142016 - Inputrete3*DoseOn20162017 - Inputrete4*DoseOn2017

init AG = 0.0

CG = AG/VG ; Concentration in gut (ug/L)

CVG = CG/PG ; Concentration leaving gut (ug/L)

”.

Where:

AG' = PFAS rate of change in the gut compartment (ug/h)

QG = plasma flow to gut (L/h)

CA = total concentration of PFAS in plasma for the n-subject (µg/L)

Free = free fraction of PFAS in plasma for the n-subject (dimensionless)

CG = concentration in gut (µg/L)

FreeG = Free/PG (dimensionless)

PG = gut/plasma partition coefficient (dimensionless)

As shown in the differential equation for the gut compartment, the differences in the PFAS intake between the time periods Pi and P(i+1): Inputrete2, Inputrete3 and Inputrete4, due to the changes in the PFAS concentrations in tap water, were subtracted to the PFAS intake in the period P1 during the effective time periods thanks to the “dose on” mode to obtain the right PFAS intake for each time period Pi.

Moreover in the original code there were errors in the values of Tmc and Kt as suggested by the (EFSA, 2018) Panel on Contaminants in the Food Chain (EFSA, 2018). “As described in Table 1 of Loccisano et al. (2011) and consistent with Andersen et al. (2006), the units are in mg, so when expressed in μg, to be consistent with other parameters in the codes, they should be” (EFSA, 2018):

• for PFOA, Tmc = 6000 μg/h/kg$^{0.75}$ and Kt = 55 μg/L (and not 6 and 0.055)

• for PFOS, Tmc = 3500 μg/h/kg$^{0.75}$ and Kt = 23 μg/L (and not 3.5 and 0.023)

In the original model two values were proposed for PFOA half-life and both appeared to be probable. In this model the chosen value for the half-life was 2.3 years (equal to: Tmc = 6000 μg/h/kg$^{0.75}$), in accordance with the considerations expressed by (EFSA, 2018) Panel (EFSA, 2018). In addition, the value of the cardiac output to liver (QLC) was corrected for the cardiac output to the gut and so was changed from 0.25 to 0.069 and the duration of dose (expressed by the parameter Tinput) of 0.6 hours was replaced to a value equal to 24 hours “assuming that exposure was spread over a longer period” (EFSA, 2018).

The Stiff method was chosen, as in (EFSA, 2018).

The measured body weight (BW), the predicted exposure time, the predicted PFAS oral intake, the average PFAS concentration in tap water and groundwater for each period and the predicted water intake of the specific subject were used in the simulations at individual level while the average value of the parameters were used at aggregate level.

The Loccisano model modified as described in this paragraph was named “the adapted Loccisano model”.

Few examples of scripts created for the Berkeley Madonna software to develop simulations using the adapted Loccisano model are presented in Annex A.

MODIFIED LOCCISANO MODEL VERSION 1 AND VERSION 2

Some adjustments to the code of the Loccisano model at aggregate level were made in order to improve its ability in predicting PFAS serum concentrations in the selected subjects. This model optimization was based on the use of values found in the scientific literature for some parameters, to maintain the validity of the new model under the physiological point of view. The changes were not individual specific, so the Loccisano model at individual level was not modified.

First of all the partition coefficient proposed by Loccisano (Loccisano et al., 2011) were replaced with the partition coefficients proposed by Fàbrega (Fàbrega et al., 2014) for the common compartments between the two models. So, the values of liver/plasma, fat/plasma and kidney/plasma partition coefficients were modified, while the values of skin/plasma, rest of the body/plasma and gut/ blood plasma partition coefficients remained the same.

This operation improved the final result of the model proposed by Fàbrega (Fàbrega et al., 2014) since the partition coefficients proposed in that study are human-based, while those from Loccisano model are rat-based. The model with the partition coefficients proposed by Fàbrega was called in this study the "modified Loccisano 1" (ML1). The aim of this model was to quantify the improvement in the predicted values provided by the human-based partition coefficients.

Model (PFOA)	PL	PF	PK	PSk	PR	PG
Loccisano	2.2	0.04	1.05	0.1	0.12	0.05
ML1	1.03	0.47	1.17	0.1	0.12	0.05

Table 29 partition coefficients for PFOA (liver/plasma: PL, fat/plasma: PF, kidney/plasma: PK, skin/plasma: PSk, rest of the body/plasma: PR and gut/blood plasma: PG) in the original Loccisano model (Loccisano) and in Modified Loccisano model 1 (ML1).

Model (PFOS)	PL	PF	PK	PSk	PR	PG
Loccisano	3.72	0.14	0.8	0.29	0.2	0.57

ML1	2.67	0.33	1.26	0.29	0.2	0.57

Table 30 partition coefficients for PFOS (liver/plasma: PL, fat/plasma: PF, kidney/plasma: PK, skin/plasma: PSk, rest of the body/plasma: PR and gut/blood plasma: PG) in the original Loccisano model (Loccisano) and in Modified Loccisano model 1 (ML1).

A second model was then created for the two PFAS under study. In this model, called the "Modified Loccisano 2" (ML2), more changes than in the ML1 were made in order to obtain predicted values closer to observed ones. So, the aim was even in the creation of the ML2 the optimization of the Loccisano AL model to reduce the difference between the observed and predicted PFAS serum concentrations (i.e. to reduce the error committed by the Loccisano AL model).

So, first of all, in the ML2 the same values used in the ML1 were adopted for partition coefficients. Secondly, the maximum resorption rate parameter, T_m, was changed from 6000 μg/h/kg$^{0.75}$ (Bartell et al., 2010) to 10000 μg/h/kg$^{0.75}$ (Olsen et al., 2007) for PFOA and from 3500 μg/h/kg$^{0.75}$ (Loccisano et al., 2011) to 3270 μg/h/kg$^{0.75}$ (Dzierlenga et al., 2020) for PFOS. For PFOA, a maximum resorption rate equal to 6000 μg/h/kg$^{0.75}$ corresponds to a 2.3 years half-life, while a maximum resorption rate of 10000 μg/h/kg$^{0.75}$ corresponds to a 3.8 years half-life. Both the values appears to be probable (EFSA, 2018) and even if the a half-life of 2.3 seems reliable and supported by other studies (Li et al., 2018), it's the smallest value found in literature for PFOA half-life. Moreover in the ML2 for PFOS the free fraction of PFOS in plasma was changed from 0.025 to 0.03 (Fàbrega et al., 2014). The choice to modify the parameters: Tm and Free derived first of all, from the fact that in the Fàbrega model, based on Loccisano, they were, together with Kt, the variables with the highest sensitivity (Fàbrega et al., 2015; Fàbrega et al., 2016). But there was another important reason: different values were found in the literature for these parameters, in particular for Tm, as reported also in the section of this work: "PBPK MODELLING DEVELOPED FOR PFASs: A comprehensive review"; paragraph: "collected information and parameters". This could due to a high variability in humans (or from one population to another) or to some errors in estimating the

actual value. In any case the probability to get closer to the true value of these parameters adopting these new values is quite high.

A further adjustment of the ML2 model code for PFOS was implemented for women. This new adjustment derived from the conclusion of a recent study (Gomis et al., 2017) which claimed that menstruation losses reduced the concentration of PFOA in serum by up to 18 % and the concentration of PFOS in serum by up to 29 %. In the mentioned study, the difference in intrinsic elimination half-life for PFOS between Australian men and women and for PFOA between American and Australian men and women could be explained by menstruation losses for those percentages.

To take into account the menstruation losses the ML2 model was modified to simulate better women at aggregate level. The PFAS values predicted by the ML2 model for women at aggregate level were so reduced by 18 % and 29 % respectively for PFOA and PFOS, according to the Gomis study (Gomis et al., 2017). While the PFOA reduction of 18 % due to menstruation losses explained only in part the 51% less observed in PFOA serum concentration for women in the Veneto population, the difference in PFOS concentration (29%) was entirely explained to the percentage found for menstruation losses in the Gomis study (Gomis et al., 2017). The values found in the Gomis study for PFOS (29%) was taken as a reference value for this study and from this value were derived the further adjustments implemented in the ML2 model for women, also called the “modified Loccisano 29%” (ML29) model. The adjustments were implemented in the model code of the ML2 for PFOS only, since PFOA predicted concentrations were found lower than the observed ones. So, that kind of implementation would have decreased the accuracy of the model output and that would not have been an optimization of the model. However, in the PBPK model comparison at aggregate level the value of a hypothetical model for PFOA designed for women taking into account menstruation losses to produce a -18% less than men was considered and called the “modified Loccisano 18%” (ML18) model.

So, the equation describing the plasma compartment in the ML2 model code for PFOS and for every group of women in every municipality was adjusted to achieve the 29 % less in the average predicted concentrations. Practically the adjustment consisted in adding a term in the equation that described the mass of PFAS in time in the plasma compartment. We added the term: "$\mathbf{-N \cdot CA \cdot QCP}$" (µg/h), called "menstruation term", in both models for PFOA and PFOS. The value of the coefficient N was predicted running the simulations to obtain the 29 % less of the original predicted value obtained with the ML2 model for women without adding the term: "$\mathbf{-N \cdot CA \cdot QCP}$" . The added term represented the PFOS loss due to menstruation in women in fertile age. The term: "$\mathbf{-N \cdot QCP}$" (L/h) represented the average plasma flux lost every hour due to menstrual cycle by the woman during the exposure period.

In the menstruation term: "$\mathbf{-N \cdot CA \cdot QCP}$", the free fraction of PFOS in plasma compartment (Free) was not included in the menstruation coefficient N, since a menstruation loss during the period causes a loss of the all plasma, and so the loss of the free PFOS but also the loss of PFOS bound to albumin.

So the equation for the plasma compartment became the following:

$$APlas' = \mathrm{QF} \cdot \mathrm{CF} \cdot \mathrm{FreeF} + (\mathrm{QL} + \mathrm{QG}) \cdot \mathrm{CL} \cdot \mathrm{FreeL} + \mathrm{QR} \cdot \mathrm{CR} \cdot \mathrm{FreeR} + \mathrm{QSk} \cdot \mathrm{CSk} \cdot \mathrm{FreeSk} + \mathrm{QK} \cdot \mathrm{CK} \cdot \mathrm{FreeK} - \mathrm{QCP} \cdot \mathrm{CA} \cdot \mathrm{Free} - \mathbf{N \cdot CA \cdot QCP}$$

N assumed the following values:

$N = 7.40 \cdot 10^{-7} \pm 0.05 \cdot 10^{-7}$, for the municipality of Sarego

$N = 7.90 \cdot 10^{-7} \pm 0.05 \cdot 10^{-7}$, for the municipality of Lonigo

$N = 8.20 \cdot 10^{-7} \pm 0.05 \cdot 10^{-7}$, for the municipality of Veronella

$N = 8.35 \cdot 10^{-7} \pm 0.05 \cdot 10^{-7}$, for the municipality of Albaredo d'Adige

$N = 8.75 \cdot 10^{-7} \pm 0.05 \cdot 10^{-7}$, for the municipality of Legnago.

The weighted average value of N for the women of the all five municipalities was equal to $N = 8.2 \cdot 10^{-7} \pm 0.05 \cdot 10^{-7}$. The values for N for every municipalities were obtained through an iteration process using the Berkeley-Madonna software and the uncertainty associated to the prediction was due to the precision of the PFOS serum concentration values (= 0.1 ng/mL).

Starting from the value of N, the average blood flux and the average plasma flux lost during each menstruation cycle were predicted for each municipality through the following set of equations.

First of all, the plasma volume hourly lost with menstruation was predicted as follows:

$$Q_{p,h} = N \cdot QCP$$

Where:

$Q_{p,h}$ = average plasma volume hourly lost with menstruation (L/h), according to the predicted value of N;

N = menstruation coefficient (dimensionless);

QCP = plasma flow in the human body (L/h).

Then, the plasma flow (QCP) was rewritten as:

$$QCP = QCC \cdot (1 - Htc) \cdot BW^{0.75}$$

Where:

QCC = cardiac blood output = 12.5 (L/(h*kg^0.75)), according to Loccisano model;

Htc = hematocrit = 0.44 (dimensionless);

BW = body weight (kg).

So, the previous equation was rewritten as follows:

$$Q_{p,h} = N \cdot QCC \cdot (1 - Htc) \cdot BW^{0.75}$$

The plasma volume hourly lost with menstruation was multiplied by the number of hours in a day and the number of days during a menstrual cycle to obtain the plasma volume lost during a menstrual cycle:

$$Q_{p,mc} = N \cdot QCC \cdot (1 - Htc) \cdot BW^{0.75} \cdot 24 \cdot 29.2 \cdot 1000$$

Where:

$Q_{p,mc}$ = average plasma volume lost during a menstrual cycle (mL/cycle);

24 = number of hours in one day (h/day);

29.9 = number of days per menstrual cycle (according to Verner et al., 2015 and Gomis et al., 2017 that proposed 12.5 menstruation cycles per year), (day/cycle);

1000 = number of mL in one L (mL/L);

This equation did not take into account that the simulation was run over the all time of exposure of the subjects. This fact produced an error, since the fertile age in a

woman begins at 12 years of age (Verner, 2015; Gomis 2017), and so the menstrual cycles. The previous equation was so corrected adding a correction term for the fertile age, as shown here:

$$Q_{p,mc,fert} = N \cdot QCC \cdot (1 - Htc) \cdot BW^{0.75} \cdot 24 \cdot 29.2 \cdot 1000 \cdot \frac{exp_t}{age_{fert}}$$

Where:

$Q_{p,mc,fert}$ = average plasma volume lost with menstruation during one cycle, corrected for fertile age, (L/cycle);

24 = number of hours in one day (h/day);

29.9 = number of days per menstrual cycle (according to Verner, 2015 and Gomis, 2017; that proposed 12.5 menstruation cycles per year), (day/cycle);

1000 = number of mL in one L (mL/L);

Exp_t = time of exposure for women living in that municipality (years);

Age_{fert} = fertile age in women until the blood sampling (years), it was calculated as the difference between the average age of women in that municipality and the beginning of the fertile age (12, according to Verner, 2015):

$$age_{fert} = age - 12$$

The term: $\frac{exp_t}{age_{fert}}$ was the correction term for the fertile age. This term took into account that the value of N was found starting from simulations run over the all time

of exposure and not over the period of time that begins at fertile age and ends at the time of the blood sampling.

The total volume of plasma lost in one year was obtained applying the following equation:

$$Q_{p,year,fert} = N \cdot QCC \cdot (1 - Htc) \cdot BW^{0.75} \cdot 24 \cdot 29.2 \cdot 1000 \cdot \frac{exp_t}{age_{fert}} \cdot 12.5$$

Where:

$Q_{p,year,fert}$ = average plasma volume lost with menstruation during one year, corrected for fertile age, considering a number of 12.5 cycles per year (L/year).

The predicted values for the plasma flux were compared with those obtained in the Verner study (Verner, 2015). In this study, the value for the plasma flux lost with menstruation during one cycle was 69.4 mL, whereas the plasma flux lost with menstruation during one year was 868 mL. The corresponding average menstrual blood volume per cycle was 43.4 mL. This value was surely in an acceptable range for this parameter but it could vary a lot from women to another and to a group of women to another, mostly if the sample is small. These values for the total serum equivalent volume in menstrual fluid were obtained according to the following equation:

$$Q_{p,mc,fert} = Q_{mc,fert} \cdot 0.5 \cdot \left(1 - \frac{Htc}{100}\right) + Q_{mc,fert} \cdot 0.5$$

Where:

$Q_{mc,fert}$ = average menstrual fluid volume per cycle, corrected for fertility age (mL/cycle).

In the Verner study (Verner, 2015) the menstrual bood volume per cycle was assumed equal to the half of the menstrual fluid volume per cycle (Q_{mc}):

$$Q_{b,mc,fert} = Q_{mc,fert} \cdot 0.5$$

Where:

$Q_{b,mc,fert}$ = average blood volume lost with menstruation during one cycle, corrected for fertile age (mL/cycle).

Thanks to this set of equations the menstrual blood volume per cycle corrected for fertility age was calculated also in this study through the following equation:

$$Q_{mc,fert} = \frac{2 \cdot Q_{p,mc,fert}}{2 - \frac{Htc}{100}}$$

And consequently:

$$Q_{b,mc,fert} = \frac{Q_{p,mc,fert}}{2 - \frac{Htc}{100}}$$

The shared assumption in comparing the values predicted in this study using the previous set of equations and the value proposed by Verner (Verner, 2015) was that

the flux of the non-blood portion of the menstrual fluid was equal to the flux of the blood portion as assumed in the Verner study (Verner, 2015). Moreover we assumed that the half of the volume that is not blood has an albumin concentration equal to that in plasma, as claimed in the Verner study (Verner, 2015). The value of hematocrit parameter (Htc) used in this study was the one proposed by Loccisano: 0.44, and not the one proposed by Verner (40). So, the previous equation was rewritten as follows:

$$Q_{b,mc,fert} = \frac{Q_{p,mc,fert}}{2 - Htc}$$

Municipality	$Q_{b,mc,fert}$ (mL/cycle)	$Q_{p,year,fert}$ (mL/year)	$Q_{p,mc,fert}$ (mL/cycle)	$Q_{p,mc}$ (mL/cycle)	$Q_{p,h}$ (mL/h)	Age_{fert} (years)	Age (years)	Exp_t (years)
Sarego	58.6	1143	91.4	73.9	1.06E-04	14.8	26.8	18.3
Lonigo	60.1	1173	93.8	84.3	1.20E-04	15.0	27.0	16.7
Veronella	78.0	1521	121.7	86.8	1.24E-04	14.2	26.2	19.9
Albaredo	96.9	1890	151.2	81.6	1.16E-04	9.5	21.5	17.6
Legnago	67.7	1320	105.6	96.0	1.37E-04	16.0	28.0	17.6
Women (total population)	67.0	1307	104.5	86.9	1.24E-04	14.9	26.9	17.7

Table 31 Values of parameters associated to menstrual cycle calculated starting from the menstruation coefficient (N) calculated for women in each municipality. Qb,mc,fert = average blood volume lost with menstruation during one cycle, corrected for fertile age; Qp,year,fert = average plasma volume lost with menstruation during one year, corrected for fertile age, considering a number of 12.5 cycles per year; Qp,mc,fert = average plasma volume lost with menstruation during one cycle, corrected for fertile age; Qp,mc = average plasma volume lost during a menstrual cycle; Qp,h = average plasma volume hourly lost with menstruation. Agefert = fertile age in women until the blood sampling (years), it was calculated as the difference between the average age of women in that municipality and the beginning of the fertile age (12, according to Verner, 2015). Age =average age for the women living in that municipality. Expt = time of exposure for women living in that municipality.

This result demonstrated that the values for the menstruation coefficient N found with the process described before had also a strong physiological relevance, since they were only quite higher (51%) to those assumed in the Verner study (Verner et al., 2015), but reliable, considering the huge variability range (Verner et al., 2015; Wong et al. 2014; Lorber et al, 2015) and all the assumptions and uncertainties in the system of equations. The following table compares the results found in this study and those reported in the Verner study:

Municipality	**$Q_{b,mc,fert}$ (mL/cycle)**	**$Q_{p,year,fert}$ (mL/year)**	**$Q_{p,mc,fert}$ (mL/cycle)**	**$Q_{p,mc}$ (mL/cycle)**	**$Q_{p,h}$ (mL/h)**
This study	67.0	1307	104.5	86.9	1.24E-04
Verner, 2015	43.4	868	69.4	-	-

Table 32 comparison between the values found in this study and those reported in the Verner study for parameters associated to menstrual cycle. Qb,mc,fert = average blood volume lost with menstruation during one cycle, corrected for fertile age; Qp,year,fert = average plasma volume lost with menstruation during one year, corrected for fertile age, considering a number of 12.5 cycles per year; Qp,mc,fert = average plasma volume lost with menstruation during one cycle, corrected for fertile age; Qp,mc = average plasma volume lost during a menstrual cycle; Qp,h = average plasma volume hourly lost with menstruation.

Values for N with a stronger physiological relevance were found using the ML2 for the total population (so without the correction in the plasma compartment for women) to predict PFOS from the beginning of the exposure until 12 years of age (= beginning of the fertile age) and then using the ML2 corrected for women from 12 years of age to the actual age of the female subjects to obtain the correct value of N predicted considering the real average fertile period for women. So, the ML2 for the total population with the average data for the women living in each municipality were run over the $Exp_{t,non\text{-}fert}$ period only and the predicted PFOS concentration values until 12 years of age were obtained for the all compartments simulated (CA_{12} for plasma, CG_{12} for gut, CF_{12} for fat, CK_{12} for kidney, CL_{12} for liver and CR_{12} for the rest of the body). Then, those concentration values were assumed as the initial concentrations in the respective compartments in the second model applied (ML2 for women from 12 years of age until the age at the blood sampling). So, in the

second model all the periods Pi (and the respective times: tchng, T1, T2, T3 and T4) were subtracted to the number of hours used in the first model and the ML2 for women were run over the $Exp_{t,fert}$ period only. The new menstruation coefficients (N_{fert}) found following this method were the coefficients for the fertile age and from these coefficient new corrected fluxes of plasma and blood lost with menstrual cycle were calculated.

Of course, the relation between the different exposure times was the following:

$$exp_{t,fert} = exp_t - exp_{t,non-fert}$$

Where:

$exp_{t,fert}$ = average time of exposure for women living in a certain municipality during the fertile age (years);

$exp_{t,non\text{-}fert}$ = average time of exposure for women living in a certain municipality until the fertile age (= 12 years of age).

	Concentrations in tissues in women at 12 years of age						**$exp_{t,non\text{-}fert}$**
Municipality	**CA_{12}**	**CG_{12}**	**CL_{12}**	**CF_{12}**	**CK_{12}**	**CR_{12}**	**(years)**
Sarego	4.7	2.7	12.5	1.5	7.0	0.9	3.5
Lonigo	2.3	1.3	6.1	0.8	3.5	0.5	1.7
Veronella	3.8	2.2	10.1	1.3	5.7	0.8	5.7
Albaredo	4.7	2.7	12.5	1.6	7.1	0.9	8.1
Legnago	1.4	0.8	3.7	0.5	2.1	0.3	1.6

Table 33 Predicted PFOS concentrations in tissues in women of 12 years of age by the ML2 model for the total population for the following compartments: plasma (CA_{12}), gut (CG_{12}), liver (CL_{12}), fat (CF_{12}), kidney (CK_{12}), rest of the body (CR_{12}). expt,non-fert = average time of exposure for women living in a certain municipality until the fertile age (= 12 years of age).

In the new code the predicted PFOS concentration values in compartments for the women subjects until 12 years of age (CA_{12}, CG_{12}, CF_{12}, CK_{12}, CL_{12}, CR_{12}) were multiplied by the volume of the corresponding compartment (VPlas, VG, VF, VL, VK, VR) to find the initial value of the PFOS mass in the compartment (init APlas, init AG, init AF, init AL, init AK, init AR) to give as input in the new model. For example, for the plasma compartment the following equation was added:

$$init\ APlas\ =\ CA_{12} * VPlas$$

Where:

init APlas = initial value of the PFOS mass in the plasma compartment [µg];

CA_{12} = PFOS concentration values until 12 years of age predicted by the ML2 model for total population [µg/L];

VPlas = volume of the plasma compartment [L].

Similar equations were added in all the other compartments of the ML2 model.

In the following table are reported the corrected values of N taking into account the fertile age (N_{fert}) for the different municipalities:

Municipality	**N_{fert}**	**N**	**$exp_{t,fert}$ (years)**	**exp_t (years)**
Sarego	7.50E-07±0.25E-07	7.40E-07±0.05E-07	14.8	18.3
Lonigo	8.00E-07±0.25E-07	7.90E-07±0.05E-07	15.0	16.7
Veronella	8.30E-07±0.25E-07	8.20E-07±0.05E-07	14.2	19.9
Albaredo	9.30E-07±0.25E-07	8.35E-07±0.05E-07	9.5	17.6
Legnago	9.10E-07±0.25E-07	8.75E-07±0.05E-07	16.0	17.6
Women	8.44E-07±0.25E-07	8.19E-07±0.05E-07	14.9	17.7

(total population)				

Table 34 corrected values of menstruation coefficient taking into account the fertile age (Nfert) and associated precision compared to the values of menstruation coefficient not corrected (N) and associated precision for women living in a certain municipality and for total population of women. expt,fert = average time of exposure for women living in a certain municipality during the fertile age. expt = average time of exposure for women living in a certain municipality.

The values of N_{fert} and N were very similar. This fact showed that using the two different methods did not lead to different conclusions, probably because the steady state was achieved for PFOS serum concentration and so the first years of the exposure period had a minor importance. This fact led to the following conclusion: taking into account menstruation losses during the all time of exposure does not lead to big errors even if the women studied were very young at the beginning of the exposure. Moreover, the precision of N_{fert} values cannot be higher than $0.25*10^{-7}$ if the PFOS serum concentration has the precision of 0.1 µg/L. The difference between N and N_{fert} precision was interesting and was better understandable looking at the different shape of the PFOS serum concentration curves in the Loccisano model: the curve in the model to predict N was steeper and this fact led to a smaller range of values to predict N. So, the new values of average blood and average plasma fluxes lost with menstruation predicted starting from N_{fert} were very similar to those previously predicted starting from N.

Municipality	**$Q_{b,mc,fert}$ (mL/cycle)**	**$Q_{p,year,fert}$ (mL/year)**	**$Q_{p,mc,fert}$ (mL/cycle)**	**$Q_{p,mc}$ (mL/cycle)**	**$Q_{p,h}$ (mL/h)**	**Age_{fert} (years)**	**Age (years)**	**Exp_t (years)**
Sarego	59.4	1158	92.7	74.9	1.07E-04	14.8	26.8	18.3
Lonigo	60.9	1188	95.0	85.3	1.22E-04	15.0	27.0	16.7
Veronella	79.0	1540	123.2	87.9	1.25E-04	14.2	26.2	19.9
Albaredo	107.9	2105	168.4	90.9	1.30E-04	9.5	21.5	17.6
Legnago	70.4	1372	109.8	99.8	1.42E-04	16.0	28.0	17.6
Women	75.7	1476.5	118.1	88.0	1.28E-04	14.9	26.9	17.7

(total population)								

Table 35 Values of parameters associated to menstrual cycle calculated starting from the corrected values of menstruation coefficient taking into account the fertile age (Nfert) calculated for women in each municipality. Qb,mc,fert = average blood volume lost with menstruation during one cycle, corrected for fertile age; Qp,year,fert = average plasma volume lost with menstruation during one year, corrected for fertile age, considering a number of 12.5 cycles per year; Qp,mc,fert = average plasma volume lost with menstruation during one cycle, corrected for fertile age; Qp,mc = average plasma volume lost during a menstrual cycle; Qp,h = average plasma volume hourly lost with menstruation. Agefert = fertile age in women until the blood sampling (years), it was calculated as the difference between the average age of women in that municipality and the beginning of the fertile age (12, according to Verner, 2015). Age =average age for the women living in that municipality. Expt = time of exposure for women living in that municipality.

Using the reverse process a value of the menstruation coefficient ($N_{fert,V}$) for the women population in each municipality was calculated starting from the average plasma concentration proposed by Verner ($Q_{p,mc,fert,V}$), using the following equation:

$$N_{fert,V} = \frac{Q_{p,mc,fert,V}}{QCC \cdot (1 - Htc) \cdot BW^{0.75} \cdot 24 \cdot 29.2 \cdot 1000 \cdot \frac{exp_t}{age_{fert}}}$$

The value of hematocrit parameter (Htc) used in this equation was the one proposed by Loccisano: 0.44 (also used in the previous equations), and not the one proposed by Verner (40). Thanks to this equation another reliable set of values for the menstruation coefficient parameter was predicted and, using those values for the menstruation coefficient ($N_{fert,V}$), simulations with ML2 for women were run to find the predicted PFOS concentration values using the plasma flux proposed by Verner ($Q_{p,mc,fert,V}$). The model implemented with these adjustments was called the "modified Loccisano-Verner" (MLV) model.

	$N_{fert,V}$	**N_{fert}**	**N**	**$Exp_{t,fert}$ (years)**
Sarego	5.62E-07	7.50E-07	7.40E-07	14.8
Lonigo	5.84E-07	8.00E-07	7.90E-07	15.0

Veronella	4.68E-07	8.30E-07	8.20E-07	14.2
Albaredo	3.83E-07	9.30E-07	8.35E-07	9.5
Legnago	5.75E-07	9.10E-07	8.75E-07	16.0
Women (total population)	5.51E-07	8.44E-07	8.19E-07	14.9

Table 36 comparison between the menstruation coefficient calculated starting from the average plasma concentration proposed by Verner (Nfert,V), the menstruation coefficient taking into account the fertile age (Nfert) and the menstruation coefficient not corrected (N) for women living in a certain municipality and for total population of women. expt,fert = average time of exposure for women living in a certain municipality during the fertile age.

PERFORMED ANALYSIS

More than 500 simulations were carried on and several different analysis were performed in the context of the PBPK models testing procedure, since many aims were pursued. From the scenarios created in the exposure assessment (WCS, MLS, BCS, AS and MS), combined scenarios were designed to take into account all the possible situations. The combined scenarios were: the worst case scenario + the average scenario (WCS+AS), the most likely scenario + the average scenario (MLS+AS), the best case scenario + the average scenario (BCS+AS), the most likely scenario + the median scenario (MLS+MS).

Of course, the created scenarios differed only for the values predicted for the subjects living in all the municipalities located in the A-red area and in the municipality of Legnago and for PFOA only. In fact the created scenarios differed only for the PFOA value chosen as representative of PFOA concentrations observed in groundwater (AS and MS) and for the conditions of exposure to PFOA in groundwater (WCS, MLS, BCS).

In the evaluation of the reliability of the PBPK models, the comparison of the results was made for the MLS+MS (that was assumed to be the most reliable scenario).

Analysis features and purpose are briefly described in the paragraph below.

ANALYSIS AT INDIVIDUAL LEVEL

Several analysis were developed at individual level using the adapted Loccisano model (named: the "Loccisano IL" model).

Predicted vs observed PFOA serum concentration

First of all a comparison between the observed and the predicted PFOA serum concentrations was carried out through a statistical analysis.

A Shapiro-White test was run for all the predicted distributions of data obtained running the Loccisano IL model. The data were found not normally distributed. Thus, a Wilcoxon rank sum test with continuity correction was run to analyze if predicted and observed PFOA serum concentration data could come from the same population. The comparisons involved the total population, the group of men, the group of women, the group of subjects living in the A-red area and the group of subjects living in the B-red area and were made for the combined scenario: MLS+AS. Then, the Pearson correlation index was calculated for each comparison to test the prediction ability of the model as the subjects' physical characteristics (i.e. weight) and habits (i.e. water and food intake), other than the exposure parameters (i.e. PFAS concentration in tap water and groundwater and time of exposure), change. Finally, the average values and standard deviations were calculated for each distribution of data to analyze the deviation of the predicted concentrations from the observed data.

The analysis at individual level with the adapted Loccisano model was useful to test the ability of the model in predicting PFOA serum concentrations for the single subject. On the contrary, for PFOS, an analysis at individual level was judged less reliable, since the standard deviation of the PFOS medium-low concentrations observed in the environmental matrixes was comparable to the value of the uncertainty associated to the predicted PFOS serum concentrations.

PFOA concentrations in other tissues

Not only PFOA serum concentrations, but also PFOA concentrations in several tissues were predicted at individual level by the Loccisano IL model (and by the ML2 at the aggregate level). The aim of collecting predicting PFAS concentrations for important tissues of the human body was to compare these values with observed data that will be likely collected in the next future for the Veneto population or that are or will be available for other populations. The tissues investigated corresponded to the compartments simulated by the Loccisano model: plasma, gut, fat, kidney, liver, filtrate and the rest of the body. So, PFOA concentrations in gut (CG), fat (CF), kidney (CK), liver (CL), filtrate (CFil) and the rest of the body (CR) were predicted at the date of the blood sampling at individual level and compared with the PFOA serum concentrations (CA).

Analysis of a population subsample

All the analysis, except the one described in the present paragraph, involved all the subjects (179) of the total population analysed and some of them were very young (between 14 and 20 years old) at the time of the sampling and/or lived for few years in the contaminated area. So, an analysis was performed to avoid any huge error in the comparisons made for all the simulations and to analyse the influence of age and time of exposure on PFOA serum concentration. This analysis consisted in selecting the subjects only with more than 20 years of age and living in the contaminated area for more than 10 years (i.e. time of exposure > 10 years). Moreover no pregnant women were included (only one pregnant women was present in the total population sample). The selected subjects were 105 out of 179, with an equal number of men and women (52 men and 53 women) and the same proportion of subjects from the A-red area (43, i.e. the 41% of the total) and the B-red area (62, i.e. the 59% of the total). For these subjects the average value of the PFOA serum concentrations predicted using the adapted Loccisano model at individual level was compared to the observed average concentration and to the average value of the PFOA serum concentrations predicted using the Loccisano IL model for the total population (i.e. for all the 179 subjects).

A further analysis was performed selecting only the subjects with more than 20 years of age and comparing the predicted and the observed PFOA serum concentrations. Similarly, another comparative analysis of the predicted and observed concentrations was carried out for the subjects living in the contaminated area for more than 10 years.

Comparison of the created scenarios

Combined scenarios were created by the assumptions made in the exposure assessment on the average PFOA concentration values used in the simulations and on the time of exposure for the different routes.

All the created combined scenarios were compared at the individual level using the Loccisano IL model and were the following: WCS+AS, MLS+AS, BCS+AS, MLS+MS. The values for the municipalities of Albaredo D'Adige and Veronella remained the same in the different scenarios since no exposure to PFAS in groundwater was expected for the subjects living in these municipalities. For the municipality of Legnago only the MLS+AS was adopted, since the average value was considered the best value to represent the distribution of PFOA concentrations in groundwater.

This analysis aimed at give an idea of the predicted range of uncertainty for PFOA serum concentration.

The scenarios adopted for the analysis at aggregate level were the MLS+ML and the MLS+AS, but the most reliable scenario was assumed to be the MLS+ML, since the median was considered the best value to represent the distribution of PFOA concentrations in groundwater for the municipality in the A-red area.

ANALYSIS AT AGGREGATE LEVEL

The analysis at aggregate level consisted in comparing the PFAS average concentrations predicted by the different PBPK models with the observed PFAS average concentration collected in the HBM study.

This analysis pursued the aim to indicate the best model (and establish a ranking) to predict PFAS average concentration for the subjects living in the Veneto region. In the model choice was evaluated not only the accuracy of the final result but also the time required to set the software parameters and run the simulations other than the level of expertise required to set up the simulations.

The MLS+MS was the combined scenario used for the analysis at aggregate level, since it was judged the most reliable scenario, but many analysis were performed also using the combined scenario: MLS+AS.

In all the analysis at aggregate level, the values for the total population, men, women, A-red area and B-red area were obtained calculating the weighted average over the number of subjects living in each municipality.

LOCCISANO AL VS LOCCISANO IL

A comparison of the results obtained using the adapted Loccisano model at individual (Loccisano IL) and at aggregate level (Loccisano AL) was made in order to understand the differences in the predicted average PFOA concentrations. This analysis pursued the aim to suggest one of the two approaches evaluating not only the accuracy of the final result but also the time required to set the software parameters and run the simulations.

In this analysis were considered advantages and disadvantages in using the approach at individual level and at aggregate level for the most reliable combined scenarios (i.e. MLS+AS and MLS+MS).

ANALYSIS OF THE CONCENTRATION TREND OVER TIME

Considerations were made about the shape of PFAS concentration curve in tissues over time relying on the outputs of the most reliable model (according to the results of the testing procedure). The considerations produced on the PFAS concentration trend over time were necessary to predict the probable value assumed by the PFAS serum concentration in the next years, especially when the steady state will be

achieved. This value was also compared to the highest PFAS serum concentration reached over time to investigate the maximum difference. The analysis of the trend over time was essential also to understand the difference between the highest PFAS serum concentration reached over time and the PFAS serum concentration at the date of the blood sampling. This difference is very important, since it gives the idea of the value that would have been obtained using the original Loccisano model, without the corrections implemented in the model code to increase the accuracy in describing the exposure to PFAS in tap water and groundwater.

ANALYSIS OF THE CONTRIBUTION TO THE TOTAL UPTAKE

The predicted contributions of PFAS coming from groundwater, tap water and food to the predicted PFAS serum concentration was investigated using the most reliable model (according to the results of the testing procedure). The contribution of each route of exposure was predicted modifying the model code in order to consider the contribution of that route only. The contribution was predicted for the combined scenario: MLS+MS and for the highest PFAS serum concentration reached over time, so it was valid for that time only. However, considering the exposure scenario MLS, it is likely similar for each time before that point.

Evaluating how much of the highest PFAS serum concentration reached over time is attributable to the different routes of exposure was useful also to predict the values of PFAS serum concentration (and so the error) if a route of exposure had not been taken into account.

RESULTS

Looking at the results of several tests, the value of the predicted PFAS concentrations turned out to be heavily affected by the PFAS concentration in water, the water intake and the body weight of the subjects and this was valid for all the PBPK models investigated. This fact increases the importance of the analysis of the population characteristics and of the accuracy in the exposure assessment. Keeping in mind these considerations is important when looking at the results obtained by the analysis performed (reported in this section).

When not specified otherwise, all the analysis presented in this section are referred to the MLS+MS, that was assumed to be the most reliable scenario.

ANALYSIS AT INDIVIDUAL LEVEL

The standard output of the simulation with the Berkeley-Madonna program language for the Loccisano model was a table where were reported the predicted PFAS concentrations for every compartment corresponding to each unit of time (hour), as shown in the following figure:

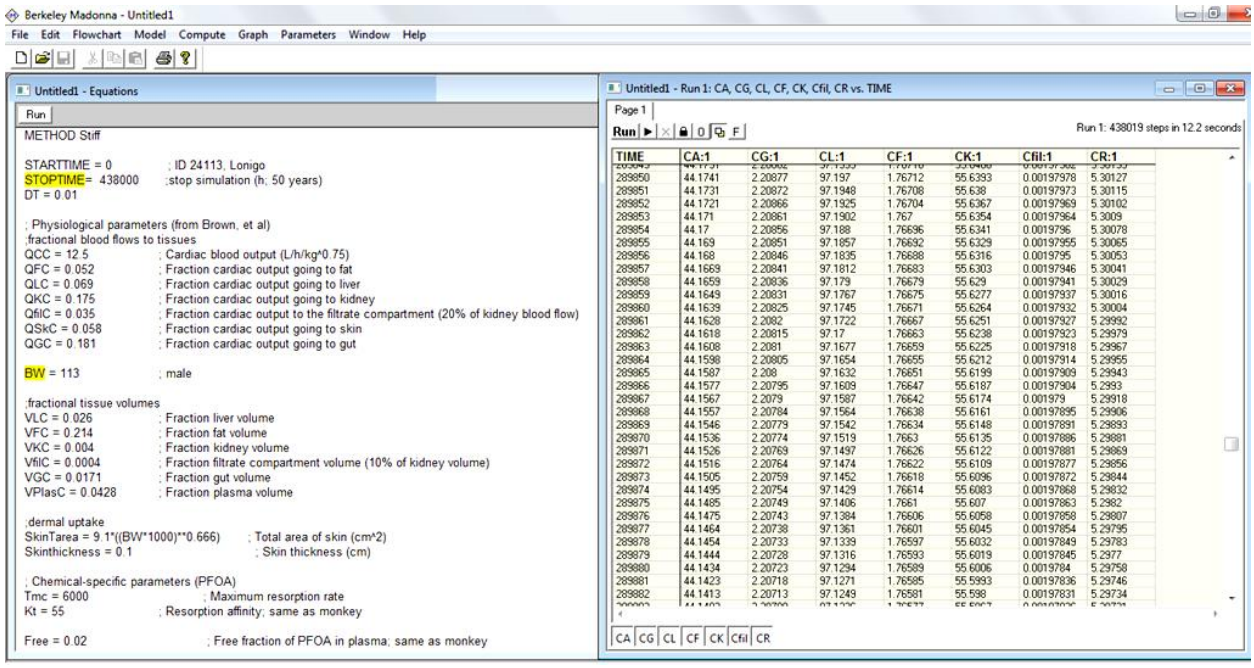


Figure 29 Loccisano model output for PFOA, for the subject 24113 (man), Lonigo, MLS, AS.

Looking at this table, PFOA concentrations predicted by the Loccisano model for the plasma compartment (i.e. PFOA serum concentrations) at the time of the blood sampling (T4) were compared to the observed PFOA serum concentrations for all the subjects. At the time T4 also were collected all the predicted PFOA concentrations for the other compartments.

The standard graphic output (chart) of the simulation with the Berkeley-Madonna program language for the Loccisano model is shown in the following figure:

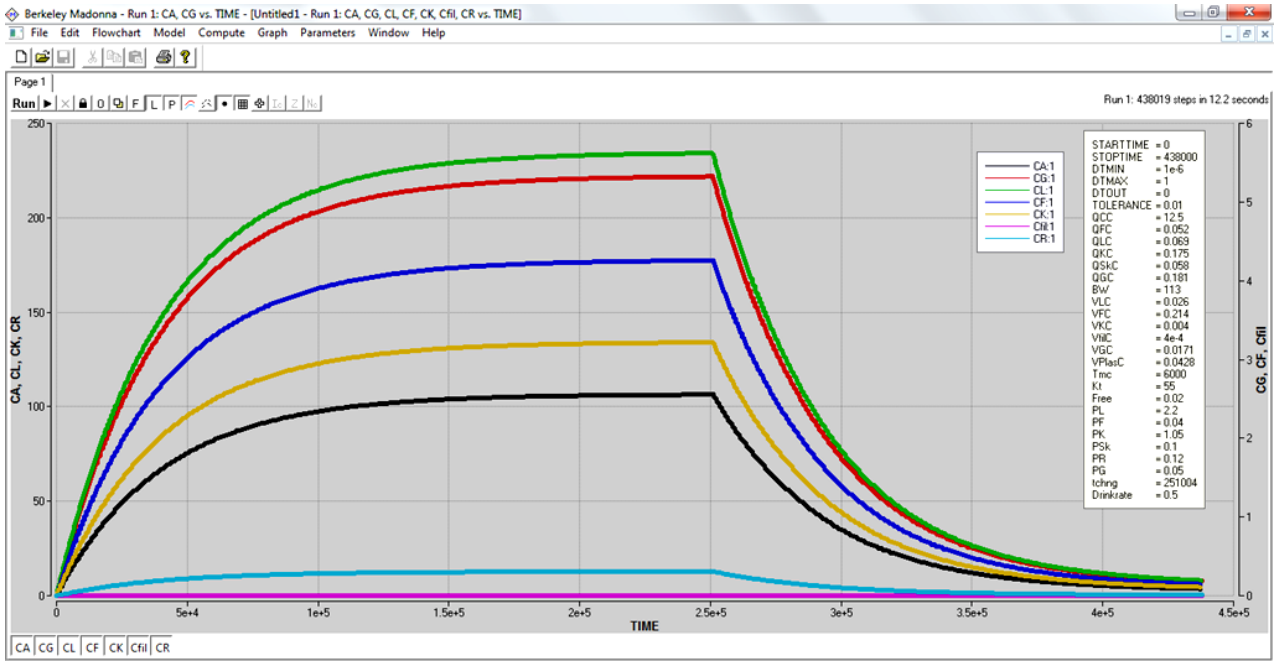


Figure 30 Loccisano model graphic output for PFOA, for the subject 24113, (man), Lonigo, MLS, AS.

Looking at the figure, all the PFOA concentration curves are in the same chart but two y-axes with two different scales are displayed since concentrations in different compartments have very different values. The unit of time is hours and the unit of concentrations is ng/mL.

PREDICTED VS OBSERVED PFOA SERUM CONCENTRATION

The predicted average PFOA serum concentration for the combined scenario MLS+AS was found lower respect to the observed average PFOA concentration for all the municipalities located in the B-red area (the most reliable results were predicted for this area) while it was found slightly higher for the municipality of Sarego (observed: 96.7 ng/mL, predicted: 138.9 ng/mL) and very similar for the municipality of Lonigo (observed: 69.7 ng/mL, predicted: 71.0 ng/mL). The standard deviations associated to the distributions of data were found higher for the observed values. The predicted and the observed average PFOA serum concentrations for the total population were found very similar (observed: 57.9 ng/mL, predicted: 46.5 ng/mL) but the observed value was underestimated for men (observed: 78.4 ng/mL, predicted: 45.1 ng/mL) and overestimated for women (observed: 38.5 ng/mL, predicted: 47.8 ng/mL).

Municipality		**Tot. Pop. [ng/mL]**		**Men [ng/mL]**		**Women [ng/mL]**	
		HBM	**Loccisano IL**	**HBM**	**Loccisano IL**	**HBM**	**Loccisano IL**
Sarego	**A.V.**	96.7	138.9	124.5	140.2	78.2	138.0
	St. Dev.	93.5	59.4	111.8	73.5	77.7	50.7
Lonigo	**A.V.**	69.7	71.0	103.7	80.6	39.3	62.4
	St. Dev.	54.0	39.7	53.1	32.3	32.9	44.1
Veronella	**A.V.**	71.6	16.4	129.0	17.1	42.9	16.0
	St. Dev.	78.2	4.2	118.9	6.5	22.3	2.8

Albaredo	**A.V.**	60.8	23.4	72.2	24.8	39.8	20.8
	St. Dev.	40.7	8.9	43.6	8.8	25.9	9.3
Legnago	**A.V.**	32.2	8.2	47.7	7.3	18.1	9.2
	St. Dev.	42.9	3.3	54.4	2.9	24.3	3.4
Tot. pop.	**A.V.**	57.9	46.5	78.4	45.1	38.5	47.8
	St. Dev.	62.3	54.9	71.9	54.7	44.4	55.7

Table 37 Observed (HBM) vs predicted (Loccisano IL) PFOA serum concentration: average value (A.V.) and standard deviation (St. Dev.). Combined scenario: MLS+AS. Comparison for total population (tot pop), for the group of men (men) and for the group of women (women).

The same considerations expressed previously for the average value are valid also for the median, even if the deviation from the observed value decreased for the subjects living in the B-red area and increased for those living in the A-red area. The deviation, when considering the median, was found higher for men and lower for the women in the B-red area, while in the A-red area was found higher for women and lower for men. Indeed, the median of the predicted PFOA serum concentrations was found slightly higher than the observed for the group of women.

Municipality	**Tot. Pop. [ng/mL]**		**Men [ng/mL]**		**Women [ng/mL]**	
	HBM	**Loccisano IL**	**HBM**	**Loccisano IL**	**HBM**	**Loccisano IL**
Sarego	73.3	141.0	90.3	139.6	59.1	141.0
Lonigo	56.3	70.3	100.6	82.2	24.5	54.1
Veronella	54.6	15.0	111.0	15.4	41.6	14.8
Albaredo	63.5	21.1	68.1	24.8	42.1	17.0
Legnago	20.3	8.2	26.3	6.6	7.7	9.0

Table 38 Observed (HBM) vs predicted (Loccisano IL) PFOA serum concentration for each municipality: median. Combined scenario: most likely scenario + average scenario (MLS+AS).

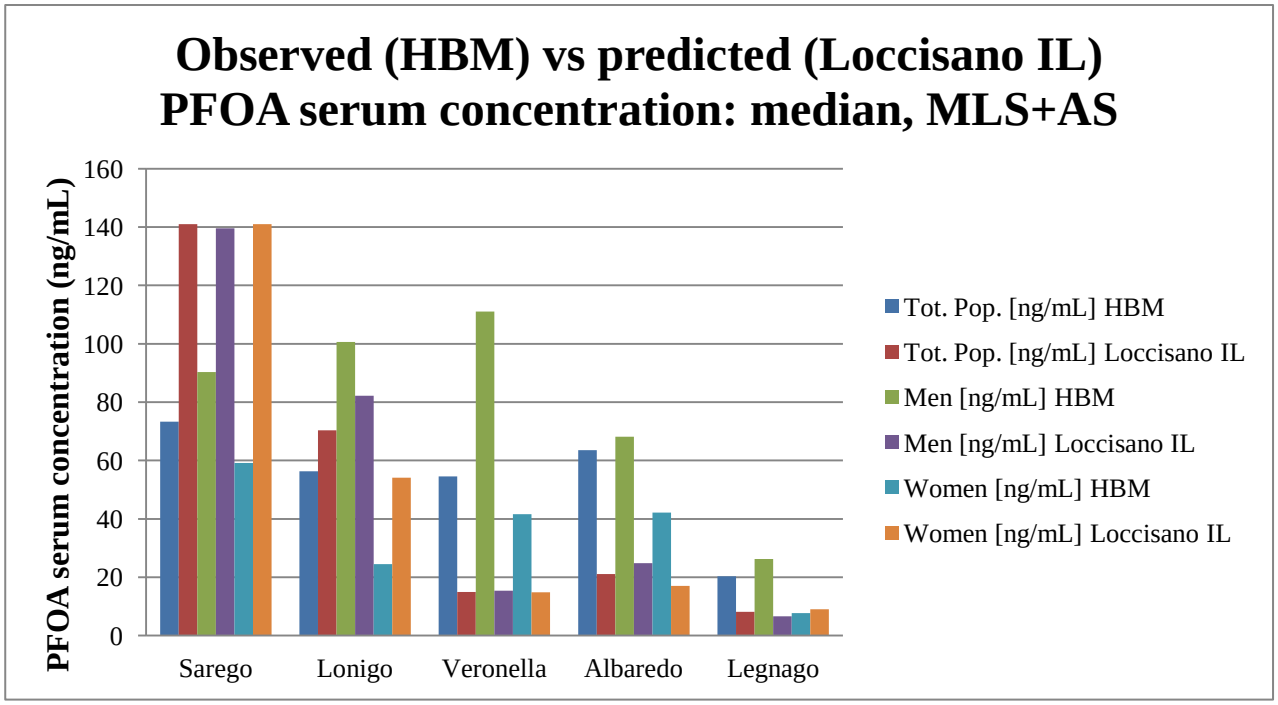


Figura 31 Observed (HBM) vs predicted (Loccisano IL) PFOA serum concentration for each municipality: median. Combined scenario: most likely scenario + average scenario (MLS+AS).

Municipality	Average value			median		
	Tot. Pop. [ng/mL]	Men [ng/mL]	Women [ng/mL]	Tot. Pop. [ng/mL]	Men [ng/mL]	Women [ng/mL]
Sarego	43.6	12.6	76.5	92.4	54.6	138.6
Lonigo	1.9	-22.3	58.8	24.9	-18.3	120.8
Veronella	-77.1	-86.7	-62.7	-72.5	-86.1	-64.4
Albaredo	-61.5	-65.7	-47.7	-66.8	-63.6	-59.6
Legnago	-74.5	-84.7	-49.2	-59.6	-74.9	16.9

Table 39 Observed (HBM) vs predicted (Loccisano IL) PFOA serum concentration for each municipality; deviation from the observed value, error: (%).

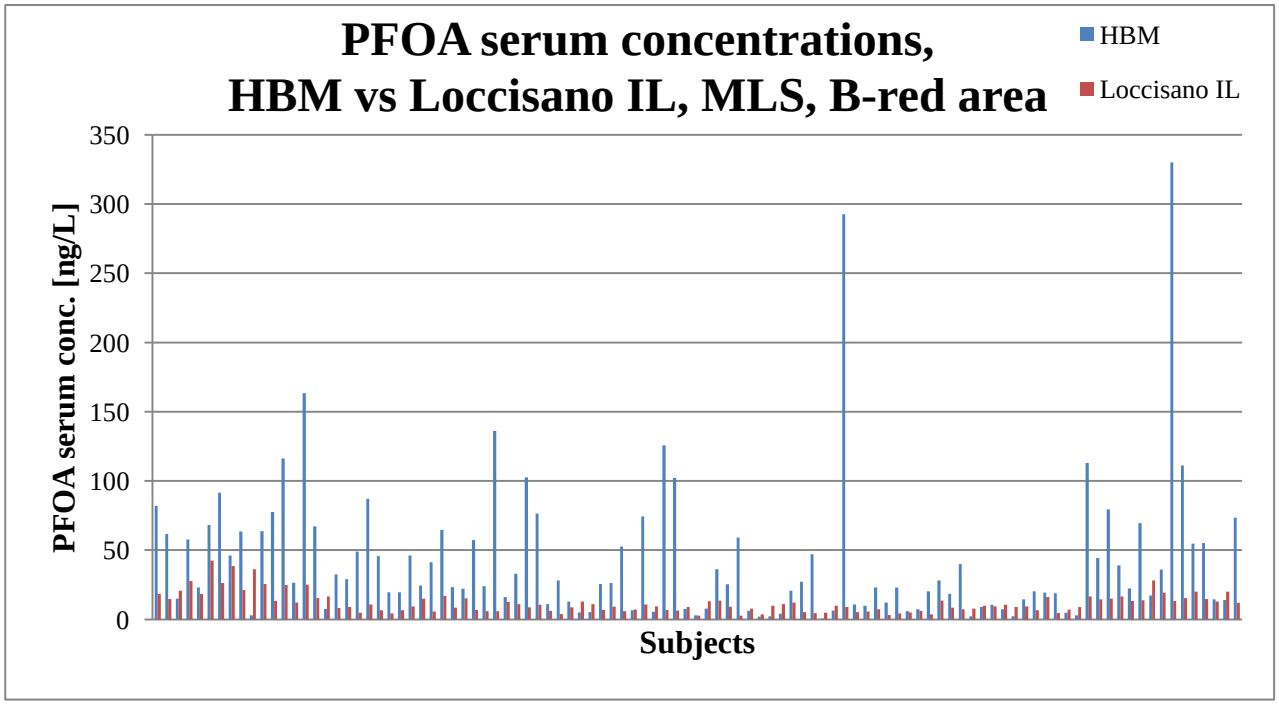


Figure 32 PFOA serum concentrations (ng/mL) observed (HBM) and predicted with the Loccisano model at individual level (Loccisano IL) for the B-red area. most likely scenario (MLS).

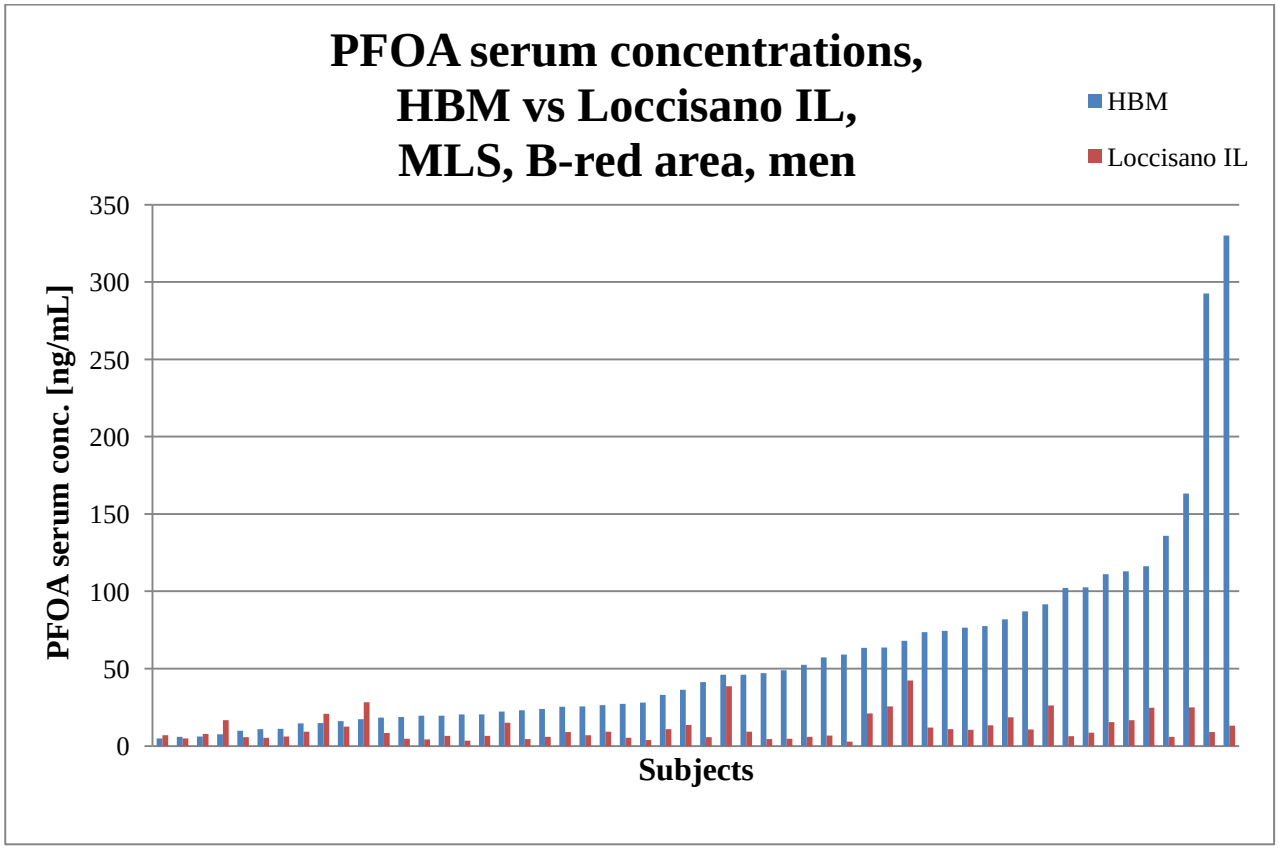


Figure 33 PFOA serum concentrations (ng/mL) observed (HBM) and predicted with the Loccisano model at individual level (Loccisano IL) for the B-red area, men. most likely scenario (MLS).

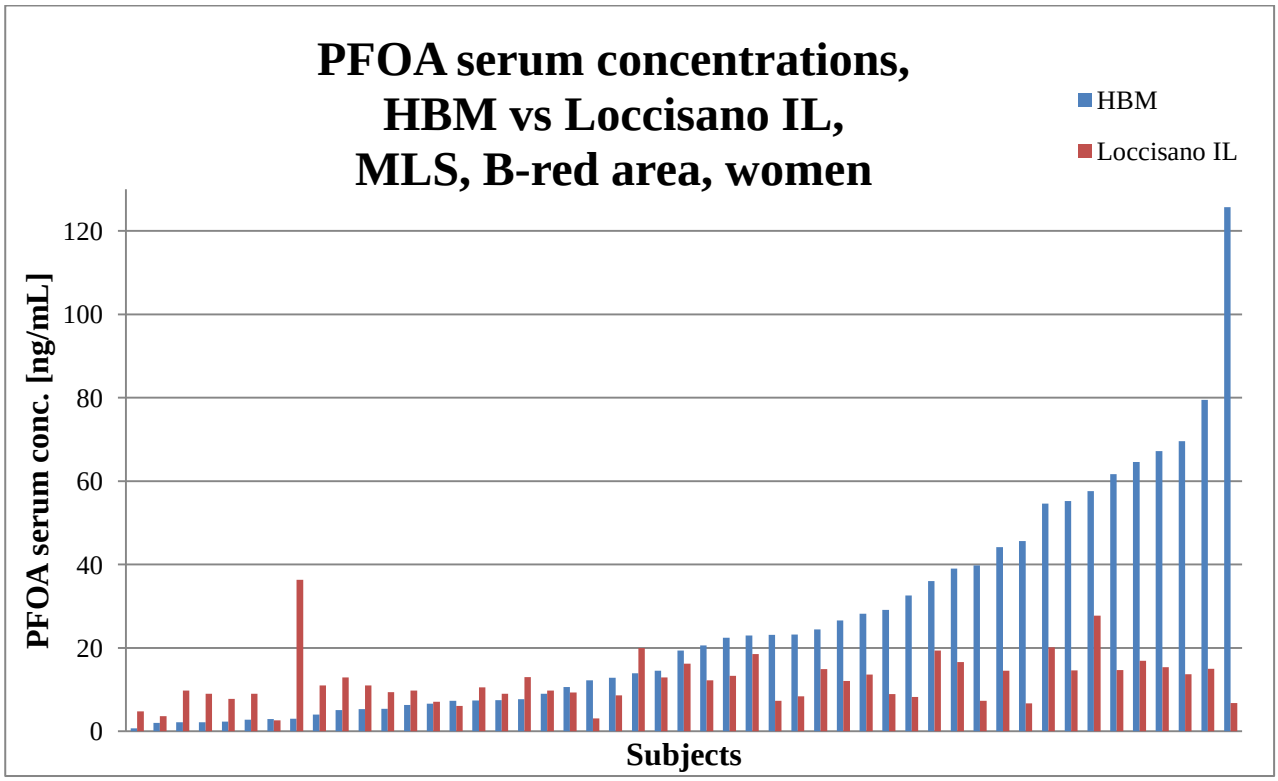


Figure 34 PFOA serum concentrations (ng/mL) observed (HBM) and predicted with the Loccisano model at individual level (Loccisano IL) for the B-red area, women. most likely scenario (MLS).

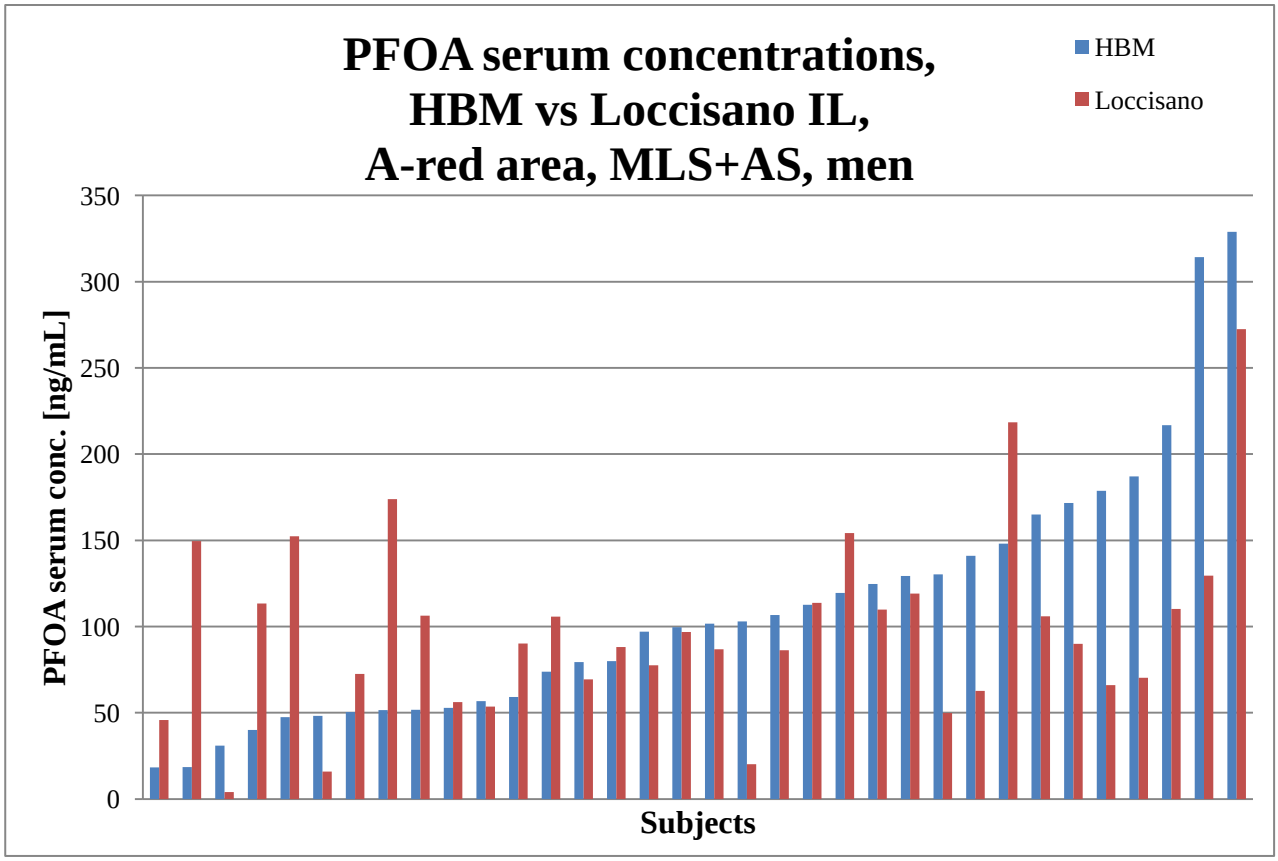


Figura 35 PFOA serum concentrations (ng/mL) observed (HBM) and predicted with the Loccisano model at individual level (Loccisano IL) for the A-red area, most likely scenario + average scenario (MLS+AS), men.

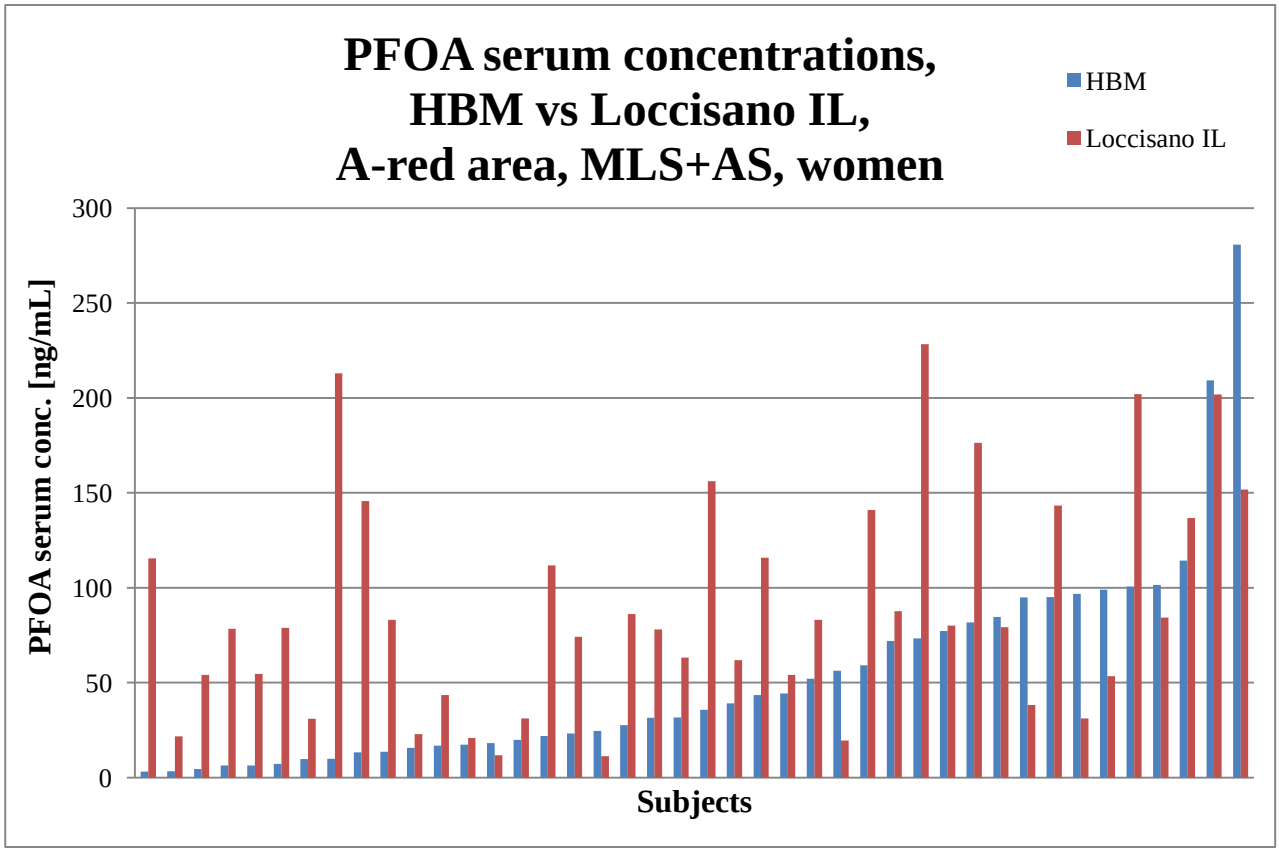


Figure 36 PFOA serum concentrations (ng/mL) observed (HBM) and predicted with the Loccisano model at individual level (Loccisano IL) for the A-red area, most likely scenario and average scenario (MLS+AS), women.

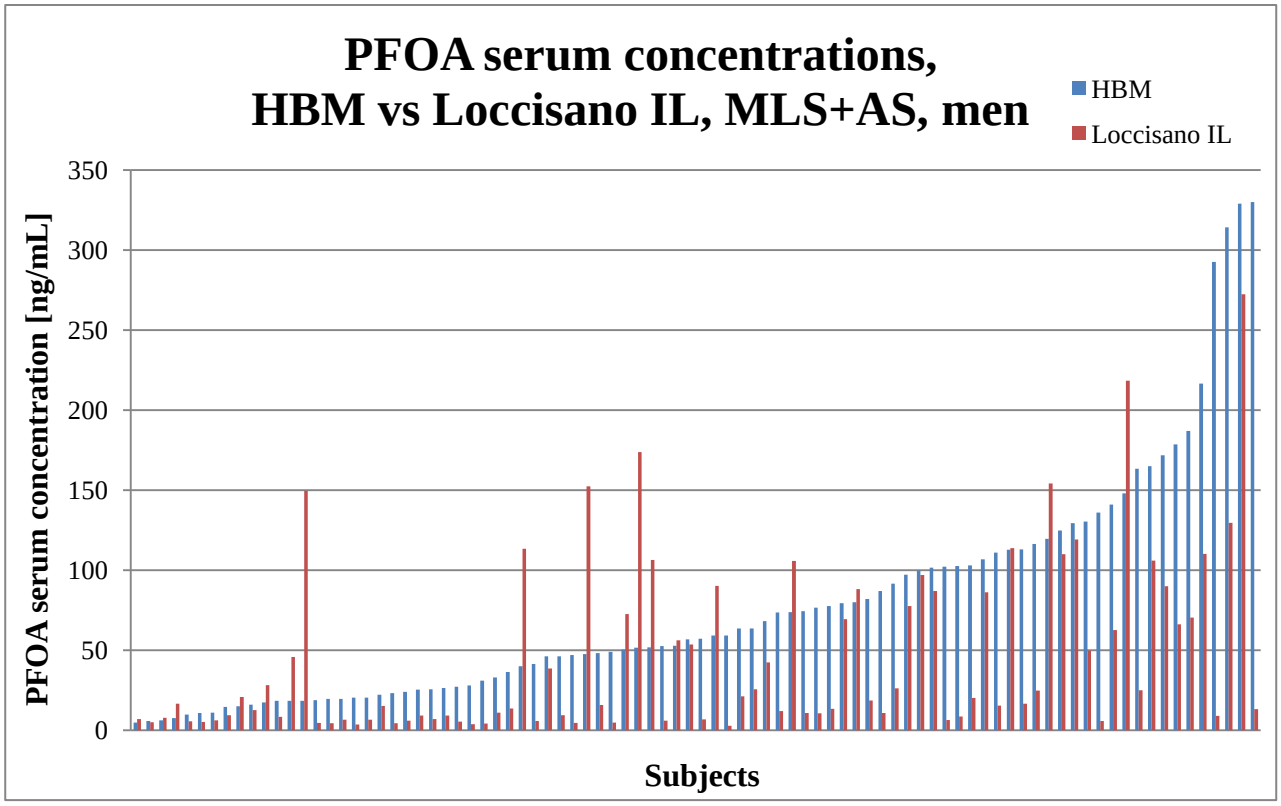


Figure 37 PFOA serum concentrations (ng/mL) observed (HBM) and predicted with the Loccisano model at individual level (Loccisano IL) for the total population, most likely scenario and average scenario (MLS+AS), men.

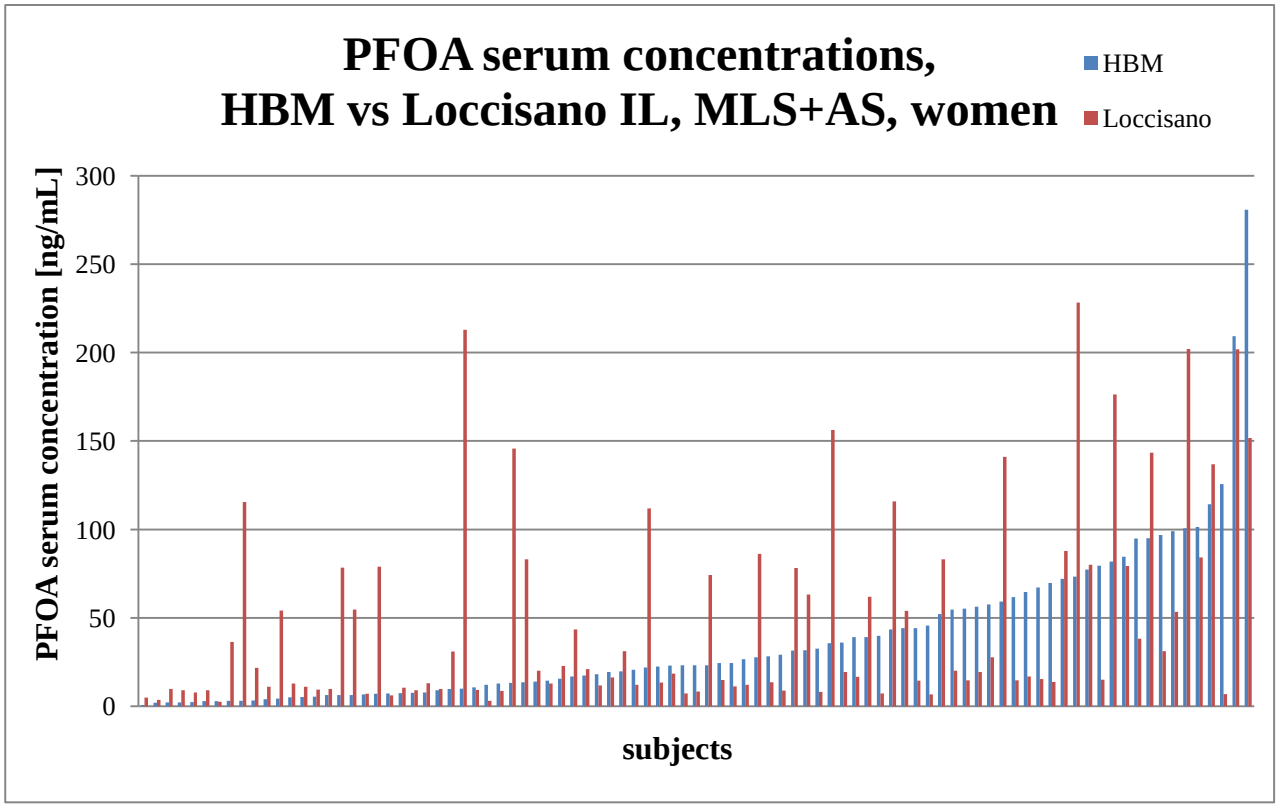


Figure 38 PFOA serum concentrations (ng/mL) observed (HBM) and predicted with the Loccisano model at individual level (Loccisano IL) for the total population, most likely scenario and average scenario (MLS+AS), women.

All the PFOA serum concentration data analyzed were found not normally distributed according to the outputs of the Shapiro-White test. So, the Wilcoxon rank sum test with continuity correction was applied to compare the distributions of data.

The null hypothesis of the Wilcoxon rank sum test with continuity correction (= samples from the same population) was rejected at significance level of 0.05 for the total population (W = 18766, p-value = 5.1E-03), for men (W = 5419.5, p-value = 4.7E-06), for the A-red area (W = 2263, p-value = 2.1E-02) and for the B-red area (W = 7959.5, p-value = 5.5E-10) but not for women (W = 3890.5, p-value = 0.4826).

So, the comparison showed a predicted distribution of PFOA concentration data different from the one observed for all the distributions except for women.

Wilcoxon rank sum test with continuity correction

	W	p-value	H_0
Men	5419.5	4.7E-06	rejected
Women	3890.5	0.4826	Not rejected
A-red area	2263	2.1E-02	rejected
B-red area	7959.5	5.5E-10	rejected
Tot pop	18766	5.1E-03	rejected

Table 40 results of the Wilcoxon rank sum test with continuity correction. W = statistic, p-value, H_0 = null hypothesis.

Also the statistic analysis that compared predicted (Loccisano IL) and measured (HBM) PFOA serum concentration at individual level and MLS showed a medium-low Pearson correlation coefficient both for men (0.46) and women (0.47). In particular the Pearson coefficient was low for the subjects living in the municipalities located in the B-red area, where the exposure assessment was very accurate. The reason for the low correlation between the two data set could be found in the PFAS high individual variability in human and in few cases maybe also in a not high accuracy in the value of the water intake parameter taken from the administered questionnaire.

These facts led to the following consideration: if the model cannot predict well the exposure of the single subject, the simulation at individual level become a huge waste of time only.

The Pearson correlation indexes for the MS were found equal to those calculated for the AS.

	Pearson, tot pop	Pearson, men	Pearson, women
Sarego	0.47	0.51	0.44
Lonigo	0.26	0.26	0.08
Veronella	-0.32	-0.55	-0.12
Albaredo	0.04	0.07	-0.48
Legnago	0.01	0.13	0.10

A-red area	0.41	0.43	0.41
B-red area	0.18	0.19	0.23
Tot pop	0.42	0.46	0.47

Table 41 Pearson correlation indexes (Pearson) for the municipalities in the red area, HMB vs Loccisano IL (MLS+AS) for the total population, men , women, the A-red area and the B-red area.

COMPARISON OF THE CREATED SCENARIOS

The values were extremely different also between the WCS+AS, the MLS+AS and the BCS+AS for the municipalities in the A-red area, as expected. The smallest deviation from the observed average PFOA serum concentration in each municipality in the A-red area was found for the MLS+AS (+44% for Sarego and +2% for Lonigo), while the largest difference was found for the WCS+AS (+227% for Sarego and +118% for Lonigo). On the contrary, for the municipality of Legnago, the average concentrations predicted for the combined scenarios: WCS+AS, MLS+AS and BCS+AS were found all similar (9.6, 8.2 and 8.0 ng/mL, respectively) and they all underestimated the observed average values (-70%, -75%, -75% ,respectively).

In some cases the observed PFOA serum concentrations were higher than the predicted one for the WCS+AS or lower than the BCS+AS, confirming a huge variability in the observed data, greater than the variability in the predicted values between the different scenarios. This fact confirmed again the necessity to adopt a comparison at aggregate level instead of one at individual level. To further support this theory, the observed average PFOA serum concentration was found always in the range of values between the average PFOA serum concentration predicted for the BCS+AS and for the WCS+AS for every municipality, for total population, for men and for women.

Average PFOA serum concentration						
	HBM [ng/mL]	**WCS+AS [ng/mL]**	**MLS+AS [ng/mL]**	**MLS+AS (%)**	**BCS+AS [ng/mL]**	**BCS+AS (%)**
Sarego	96.7	316.3	138.9	43	11.7	4
Lonigo	69.7	151.6	71.0	46	19.4	13
Legnago	32.2	9.6	8.2	88	8.0	82

Table 42 Average observed PFOA serum concentration (HBM), average PFOA serum concentration predicted for the worst case scenario + the average scenario (WCS+AS), for the most likely scenario + the average scenario (MLS+AS) and for the best case scenario + the average scenario (BCS+AS), and the values of MLS+AS and BCS+AS respect to the WCS+AS (percentages).

	HBM	Average PFOA serum concentration (error, %)		
	[ng/mL]	WCS+AS	MLS+AS	BCS+AS
Sarego	96.7	227	44	-88
Lonigo	69.7	118	2	-72
Legnago	32.2	-70	-75	-75

Table 43 deviation from the average observed PFOA serum concentration (HBM): (error,%) for average PFOA serum concentration predicted for the worst case scenario + the average scenario (WCS+AS), for the most likely scenario + the average scenario (MLS+AS) and for the best case scenario + the average scenario (BCS+AS).

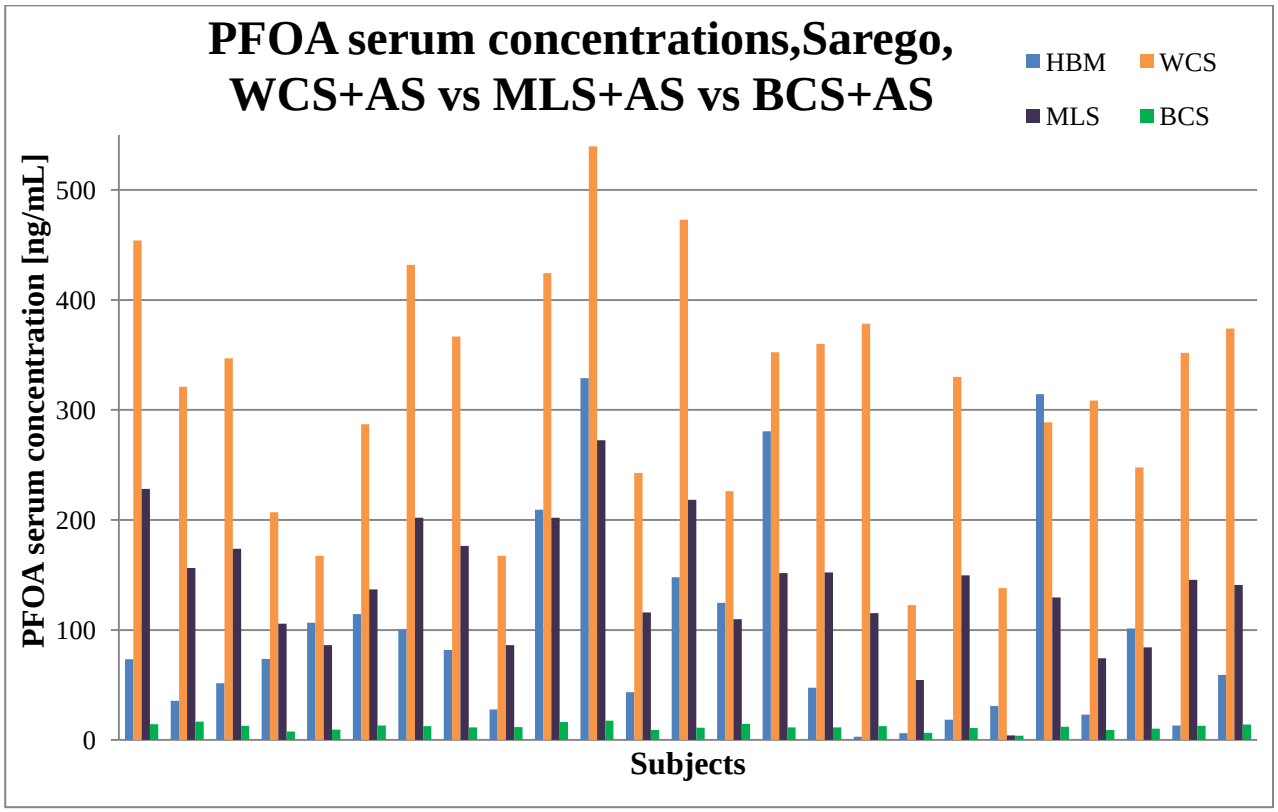


Figure 39 comparison of PFOA serum concentrations observed in the human biomonitoring study (HBM)and predicted for the combined scenarios (worst case scenario + the average scenario (WCS+AS), most likely scenario + the average scenario (MLS+AS) and best case scenario + the average scenario (BCS+AS)) for the subjects living in the municipality of Sarego.

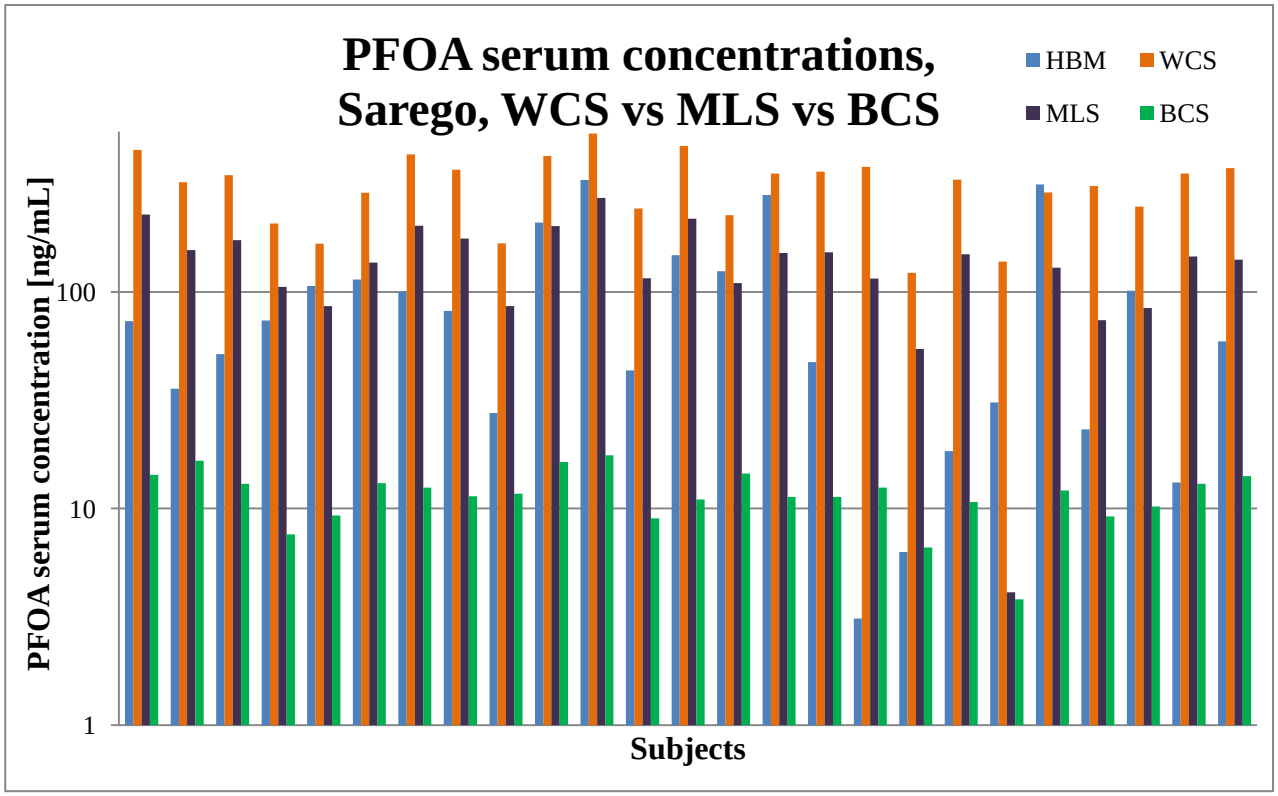


Figure 40 comparison of PFOA serum concentrations observed in the human biomonitoring study (HBM)and predicted for the combined scenarios (worst case scenario + the average scenario (WCS+AS), most likely scenario + the average scenario (MLS+AS) and best case scenario + the average scenario (BCS+AS)) for the subjects living in the municipality of Saregο. Logarithmic scale for y-axis.

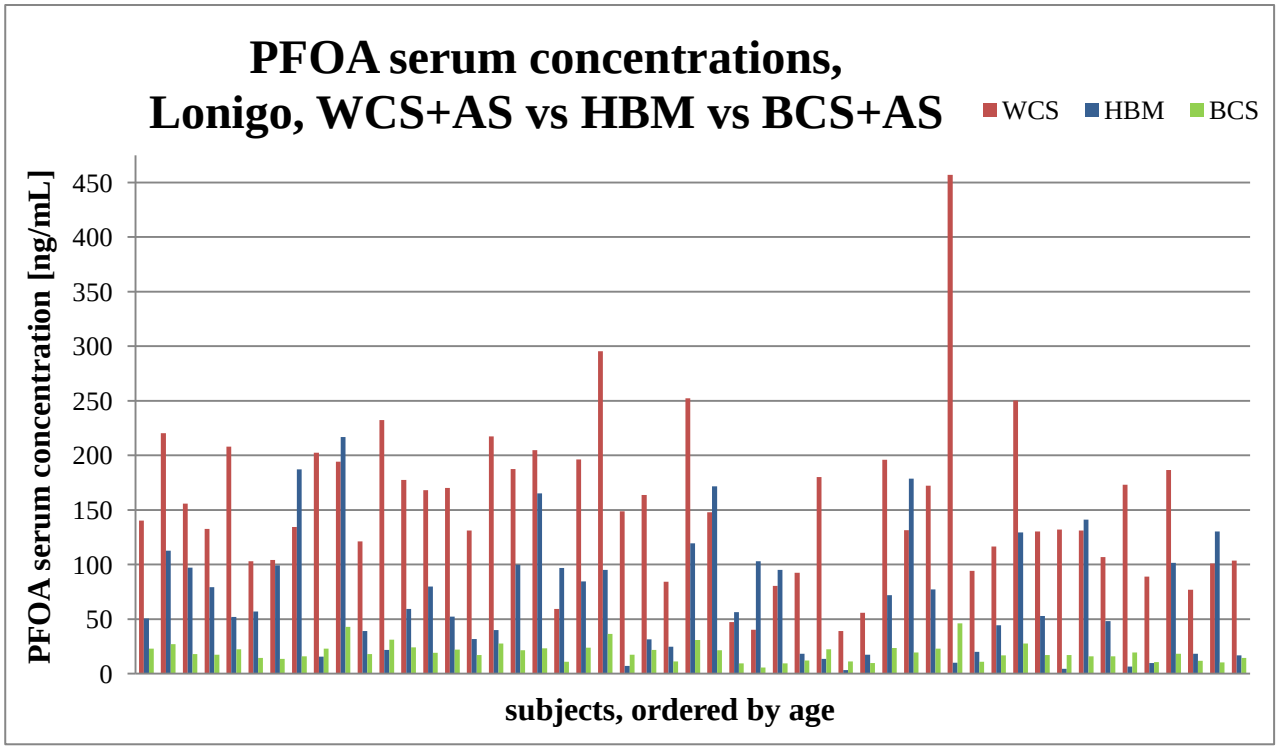


Figure 41 Comparison of PFOA serum concentrations observed in the human bio-monitoring study (HBM) and predicted for the combined scenarios (worst case scenario + the average scenario (WCS+AS) and best case scenario + the average scenario (BCS+AS)) for the subjects living in the municipality of Lonigo.

The results using MLS+MS were totally different from those obtained for the MLS+AS as shown by the comparison for the municipality of Sarego. This result was expected since in the A-red area the average value was really higher than the median for the distribution of PFOA concentrations in groundwater over time. The Pearson correlation index between the predicted and the observed PFOA serum concentrations was equal to 0.47 for both the combined scenarios MLS+MS and MLS+AS.

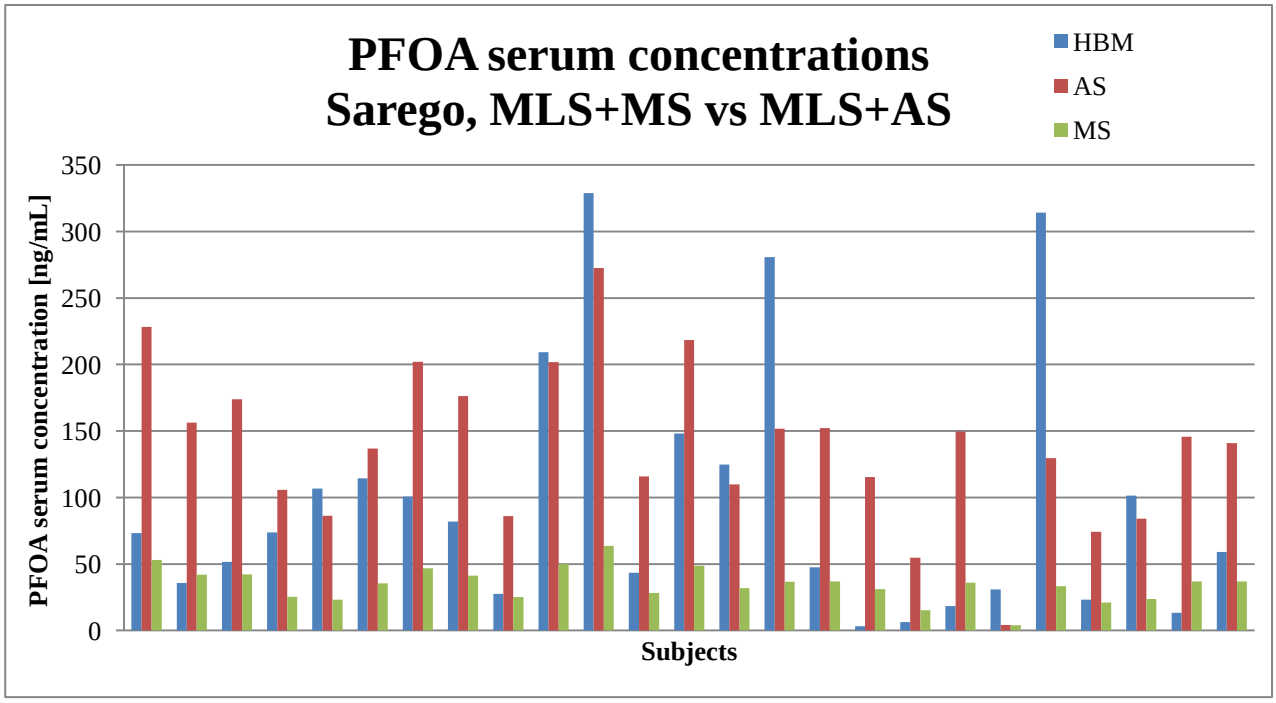


Figure 42 Comparison of PFOA serum concentrations for the subjects living in the municipality of Sarego at individual level between the observed values (HBM), the predicted values for the Most Likely Scenario + the Median Scenario (MLS+MS) and the Most Likely Scenario + the Average Scenario (MLS+AS).

PFOA CONCENTRATIONS IN OTHER TISSUES

PFOA concentrations for the other compartments of the Loccisano model were predicted at the individual level and followed the PFOA serum concentration trend. The predicted PFOA concentration for the filtrate compartment (Cfil) was found extremely low (<1.0E-02 ng/mL) for all the municipalities and scenarios, so it was not reported. The highest values were predicted for the liver compartment, then for kidney. The PFOA concentrations predicted in these two compartments were higher than those predicted for the plasma compartment. On the contrary, PFOA concentrations in gut, fat and rest of the body were lower than PFOA serum concentrations. Of course, PFOA predicted concentrations in these tissues reflected the value of the partition coefficients adopted.

Municipality	CA [ng/mL]	CG [ng/mL]	CL [ng/mL]	CF [ng/mL]	CK [ng/mL]	CR [ng/mL]
Sarego	138.9	6.9	305.6	5.6	175.0	16.7
Lonigo	71.0	3.5	156.2	2.8	89.4	8.5
Veronella	16.4	0.8	36.0	0.7	20.6	2.0
Albaredo	23.4	1.2	51.4	0.9	29.5	2.8
Legnago	8.2	0.4	18.1	0.3	10.3	1.0
Tot pop	46.5	2.3	102.2	1.9	58.6	5.6

Table 44 average PFOA concentrations predicted in the following compartments of the Loccisano model: plasma (CA), gut (CG), liver (CL), fat (CF), kidney (CK) and rest of the body (CR).

Predicted PFAS concentrations for other tissues can be obtained not only with multi-compartment PBPK models (e.g. the Loccisano model, the ML1 and the ML2) but also with one-compartment models (e.g. the Thompson model and the Bartell model), if values for partition coefficients are available. In fact, if the output of the one-compartment model is the PFAS serum concentration, multiplying the predicted value for the serum:tissue partition coefficients found in literature, it is possible to obtain the PFAS concentration in each tissue.

ANALYSIS OF THE POPULATION SUBSAMPLE

Very small differences were found in considering Average PFOA serum concentration with the analysis on the total population and not with the analysis on the population subsample only. This was true for both men and women and both the A-red area and the B-red area. The "errors" committed, calculated for the MLS+AS, ranged from 1.8% to 9.6% for the observed data and from the 5.6% to the 7.8% for the predicted values. Errors were higher for men and for the HBM study.

	Tot pop	men	women
HBM	7.6%	9.6%	1.8%
Loccisano IL	6.7%	7.8%	5.6%

Table 45 Differences in considering Average PFOA serum concentration (%) predicted with the analysis involving the total population (T. P.) and not with the analysis involving only the population subsample (P.S.), which included only the subjects with age (A) > 20 years and exposure time (E) > 10 years. Values for the Most likely scenario plus the average scenario (MLS+AS). HBM = Human bio-monitoring study. IL = Individual Level. Scenario: MLS+AS.

The Loccisano model predicted PFOA serum concentrations for the total population and for the population subsample committing approximately the same relative errors, as shown in the following table:

	Tot pop		men		women	
	T.P.	**P.S. (A > 20y, E > 10y)**	**T.P.**	**P.S. (A > 20y, E > 10y)**	**T.P.**	**P.S. (A > 20y, E > 10y)**
error	-19.7%	-20.4%	-42.5%	-43.4%	24.2 %	28.8%
difference	-0.7%		-0.9%		4.7%	

Table 46 Relative errors (difference from the observed data) in predicted average PFOA serum concentrations (%) for the total population (T.P.) and for the population subsample (P.S.) and errors difference: (error associated to the simulation of the total population – error associated to the simulation of the population subsample). Scenario: MLS+AS.

	T.P.	P.S. A > 20, E > 10	B-red area	B-red area, A > 20, E > 10
HBM	57.9	62.3	42.7	44.1

Loccisano IL	46.5	49.6	11.9	12.9

Table 47 Difference in Average PFOA serum concentration (ng/mL) between the analysis involving the entire total population (T. P.) and the analysis involving only the population subsample (P.S.) which included only the subjects with age (A) > 20 years and exposure time (E) > 10 years. Values for the Most likely scenario plus the average scenario (MLS+AS). HBM = Human bio-monitoring study. IL = Individual Level.

	men		**women**	
	T.P.	**P.S. A > 20, E > 10**	**T.P.**	**P.S. A > 20, E > 10**
HBM	78.4	85.9	38.5	39.2
Loccisano IL	45.1	48.6	47.8	50.5

Table 48 Difference in Average PFOA serum concentration (ng/mL) between the analysis involving the entire total population (T. P.) and the analysis involving only the population subsample (P.S.), which included only the subjects with age (A) > 20 years and exposure time (E) > 10 years. Values for the Most likely scenario plus the average scenario (MLS+AS). HBM = Human bio-monitoring study. IL = Individual Level.

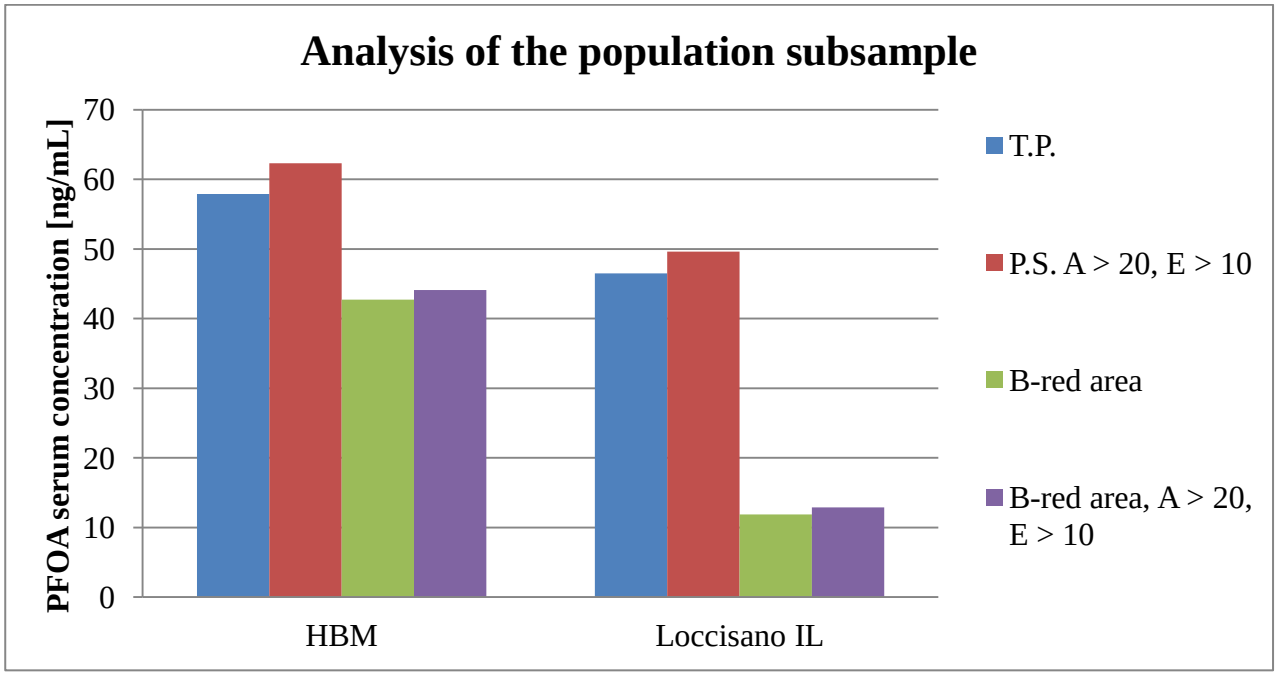


Figure 43 Difference in Average PFOA serum concentration (ng/mL) between the analysis involving the entire total population (T. P.) and the analysis involving only the population subsample (P.S.), which included only the subjects with age (A) > 20 years and exposure time (E) > 10 years. Values for the Most likely scenario plus the average scenario (MLS+S). HBM = Human bio-monitoring study. IL = Individual Level.

The significance of these results seemed to be that no substantial differences (<10%) existed in observed and predicted PFOA serum concentration between adults and adolescents and between subjects exposed for a long time and subjects exposed for less than 10 years when the two variables are investigated together.

The analysis investigating the influence of age and the time of exposure separately showed slightly different results found for the analysis investigating the effect of the two variables combined. However this analysis was affected by substantial differences in the number of men (30 out of 128 subjects in the analysis on age and 81 out of 151 subjects in the analysis of the time of exposure) respect to women and, in the analysis on age only, in the number of subjects living in the A-red area (55 out of 128 subjects) respect to those living in the B-red area that compromised the validity of the results found.

ANALYSIS AT AGGREGATE LEVEL

THOMPSON MODEL OUTPUTS

In the tables below are reported the outputs of the Thompson model at aggregate level for each municipality. The output for this model are presented also alone and not in comparison with the other PBPK models because it includes not only the predicted PFAS serum concentration (CA), but also the predicted PFAS daily absorbed dose (D) and the predicted PFAS daily exposure for kg of body weight (E). The CA found with the Thompson model was compared with the CA found using the other models and the results are reported in the next paragraph "model comparison at aggregate level".

PFOA	CA [ng/mL]	D [ng/(kg (BW)*day)]	E [ng/(kg(BW)*day)]
Sarego	46.9 (MS) 213.3 (AS)	29.0	31.9
Lonigo	39.7 (MS) 107.1 (AS)	14.6	16.0
Legnago	6.2	0.8	0.9
Veronella	13.8	1.9	2.1
Albaredo	20.0	2.7	3.0

Table 49 outputs of the Thompson model for each municipality. Predicted PFOA serum concentration (CA), PFOA daily absorbed dose (D), PFOA daily exposure for kg of body weight (E).

PFOA, men	CA [ng/mL]	D [ng/(kg (BW)*day)]	E [ng/(kg(BW)*day)]
Sarego	43.8 (MS) 200.2 (AS)	27.2	29.9
Lonigo	38.3 (MS) 103.7 (AS)	14.1	15.5

Legnago	5.4	0.7	0.8
Veronella	14.5	2.0	2.2
Albaredo	20.5	2.8	3.1

Table 50 outputs of the Thompson model for men and for each municipality. Predicted PFOA serum concentration (CA), PFOA daily absorbed dose (D), PFOA daily exposure for kg of body weight (E).

PFOA, women	**CA [ng/mL]**	**D [ng/(kg (BW)*day)]**	**E [ng/(kg(BW)*day)]**
Sarego	49.5 (MS) 224.5 (AS)	30.5	33.6
Lonigo	41.1 (MS) 110.4 (AS)	15.0	16.5
Legnago	7.3	1.0	1.1
Veronella	13.4	1.8	2.0
Albaredo	18.8	2.6	2.8

Table 51 outputs of the Thompson model for women and for each municipality. Predicted PFOA serum concentration (CA), PFOA daily absorbed dose (D), PFOA daily exposure for kg of body weight (E).

PFOS	**CA [ng/mL]**	**D [ng/(kg (BW)*day)]**	**E [ng/(kg(BW)*day)]**
Sarego	15.3	1.1	1.2
Lonigo	13.6	0.9	1.0
Legnago	7.3	0.5	0.6
Veronella	8.5	0.6	0.6
Albaredo	9.2	0.6	0.7

Table 52 outputs of the Thompson model for each municipality. Predicted PFOS serum concentration (CA), PFOS daily absorbed dose (D), PFOS daily exposure for kg of body weight (E).

PFOS, men	**CA [ng/mL]**	**D [ng/(kg (BW)*day)]**	**E [ng/(kg(BW)*day)]**
Sarego	13.8	1.0	1.0
Lonigo	12.7	0.9	1.0

Legnago	6.0	0.4	0.5
Veronella	7.2	0.5	0.5
Albaredo	9.0	0.6	0.7

Table 53 outputs of the Thompson model for men and for each municipality. Predicted PFOS serum concentration (CA), PFOS daily absorbed dose (D), PFOS daily exposure for kg of body weight (E).

PFOS, women	CA [ng/mL]	D [ng/(kg (BW)*day)]	E [ng/(kg(BW)*day)]
Sarego	16.4	1.1	1.2
Lonigo	14.6	1.0	1.1
Legnago	9.3	0.6	0.7
Veronella	9.5	0.7	0.7
Albaredo	9.7	0.7	0.7

Table 54 outputs of the Thompson model for men and for each municipality. Predicted PFOS serum concentration (CA), PFOS daily absorbed dose (D), PFOS daily exposure for kg of body weight (E).

MODEL COMPARISON AT AGGREGATE LEVEL, PFOA

All the models tested in this study underestimated the average PFOA serum concentrations measured in the HBM study. The ML2 turned out to be the model that provided the most accurate predicted values (-38%) while Bartell provided the less accurate result (-68%), as expected. However, the errors were very similar for all the models except for ML2 (ranging from -61% to -68%). The error was higher for the subjects living in the B-red area (A-red area: -41% ; B-red area: -72%) even if the accuracy in the exposure assessment was higher. The higher accuracy in the exposure assessment for the B-red area implies that the output obtained for that area provided more reliable information on the model testing procedure.

The Thompson model and the Bartell model predicted slightly better than the Loccisano AL model the subjects living in the A-red area. Predicted PFOA concentrations from the ML2 were incredibly close to the measured values (-12%). All the models predicted higher PFAS serum concentrations for the subjects living in the A-red area, as the observed values and, looking at the concentrations

predicted for the municipalities, the trend was very similar to the one observed in the values from the HBM study, except for the municipality of Veronella. In this municipality in fact, the most relevant underestimation occurred for all the models. The cause of this anomaly observed in Veronella is likely not to search in the model equations but in the exposure assessment. In fact, the PFOA concentrations given as input in the models were higher for the subjects living in Loccisano respect to those for the subjects living in Veronella but the observed data were slightly higher for the subjects living in Veronella (average value: 71.6 ng/mL) than for the subjects living in Lonigo (average value: 69.7 ng/mL). The presence of a higher number of women respect to men in Veronella increased the importance of the error committed since women showed an average concentration lower than men in every municipality. In fact, the relative error committed was higher for men in every municipalities.

	PFOA serum concentration comparison at aggregate level [ng/mL]					
Municipality	**HBM**	**ML2**	**ML1**	**Loccisano AL**	**Thompson**	**Bartell**
Sarego	96.7	78.0	46.2	40.6	46.9	46.4
Lonigo	69.7	63.4	38.2	34	39.7	38.5
Veronella	71.6	27.5	17.5	17.0	13.8	12.2
Albaredo	60.8	39.3	24.5	23.2	20.0	18.9
Legnago	32.2	13.0	8.6	8.6	6.2	4.3
Tot pop	57.8	40.0	24.5	22.3	23.3	21.8
A-red area	78.6	68.2	40.8	36.2	42.1	41.1
B-red area	42.6	19.4	12.5	12.2	9.5	7.8

Table 55 Average PFOA concentration comparison at aggregate level [ng/mL]. HBM = Human Bio-monitoring study, ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

	Error (%)				
Municipality	**ML2**	**ML1**	**Loccisano AL**	**Thompson**	**Bartell**

Sarego	-19	-52	-58	-51	-52
Lonigo	-9	-45	-51	-43	-45
Veronella	-62	-76	-76	-81	-83
Albaredo	-35	-60	-62	-67	-69
Legnago	-60	-73	-73	-81	-87
total pop	-38	-61	-64	-65	-68
A-red area	-12	-48	-53	-46	-47
B-red area	-56	-71	-72	-79	-83

Table 56 Average PFOA concentration comparison at aggregate level, deviation from the measured value: error (%).ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

Keeping in mind that the predicted concentrations using both Thompson and Bartell models were referred to a worst case scenario (since the exposure to PFAS had to be constant in time), the actual errors in using the two models should be higher than those presented in this study. So, using Thompson and Bartell models for PFOA for the Veneto population produced errors higher than 65%, while using Loccisano AL the error was the 64%. However this little difference cannot justify the higher use of time in using Loccisano AL due to the accuracy in the exposure assessment (and this is more true for Loccisano IL). On the other hand, the original Loccisano model should not require a different waste of time and gives also an idea of the concentrations in other tissues.

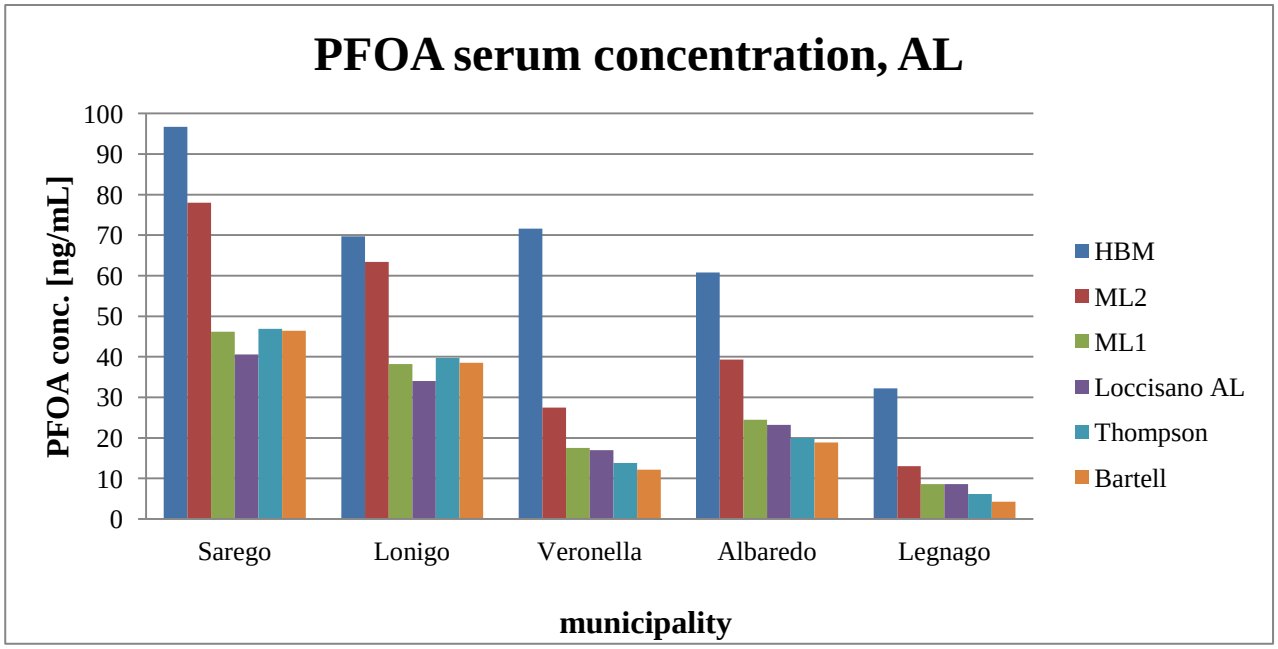


Figure 44 Average PFOA concentration comparison at aggregate level [ng/mL]. HBM = Human Bio-monitoring study, ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

The underestimation for men was higher than for total population but similar considerations were made. The ML2 was still the model that provided the most accurate predicted values (error: -53%), with the best results for the A-red area (error: -40%) and for the B-red area (error: -68%), while all the other models provided less accurate result (average error: -73%), as expected. However, in the B-red area, the error was higher using Bartell (error: -86%) and slightly lower using Loccisano AL (error: -80%). On the contrary, Thompson and Bartell predicted slightly better than Loccisano AL the subjects living in the A-red area. All the models provided better results for the subjects living in the A-red area, as for the total population.

men	**PFOA serum concentration comparison at aggregate level [ng/mL]**				
Municipality	**HBM**	**ML2**	**Loccisano AL**	**Thompson**	**Bartell**
Sarego	124.5	74.5	39.4	43.8	45.5
Lonigo	103.7	62.7	34.2	38.3	39.1
Veronella	129	29.4	18.1	14.5	14.3

Albaredo	72.2	41	24.3	20.5	20.4
Legnago	47.7	11.4	7.5	5.4	4.3
Men (tot)	79.4	37.3	21.1	21.1	21.1
A-red area	109.8	66.2	35.7	39.9	41.0
B-red area	60.2	19.1	11.9	9.3	8.5

Table 57 Average PFOA concentration comparison at aggregate level for the group of men [ng/mL]. HBM = Human Bio-monitoring study, ML2 = Modified Loccisano Version 2, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

men	**Error (%)**			
Municipality	**ML2**	**Loccisano AL**	**Thompson**	**Bartell**
Sarego	-40	-68	-65	-63
Lonigo	-40	-67	-63	-62
Veronella	-77	-86	-89	-89
Albaredo	-43	-66	-72	-72
Legnago	-76	-84	-89	-91
Men (tot)	-53	-73	-73	-73
A-red area	-40	-67	-64	-63
B-red area	-68	-80	-85	-86

Table 58 Average PFOA concentration comparison at aggregate level for the group of men; deviation from the measured value: error (%).ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

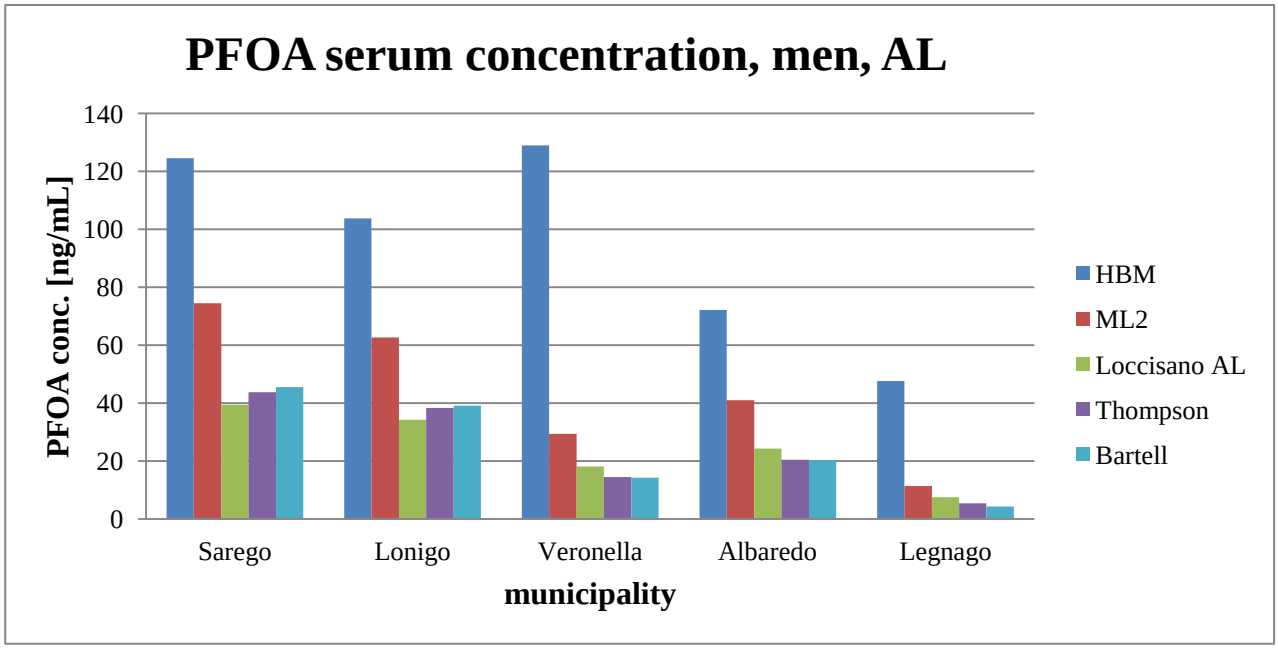


Figure 45 Average PFOA concentration comparison at aggregate level for the group of men [ng/mL]. HBM = Human Bio-monitoring study, ML2 = Modified Loccisano Version 2, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

All the models except the ML2 predicted PFOA concentrations lower than the measured values but the underestimation was lower respect to the total population. The ML2 slightly overestimated the measured values (error: +11%) but this result derived from an higher overestimation for the subjects living in the A-red area (error: +30%) and an underestimation for those living in the B-red area (error: -22%). The ML18 provided the most accurate output for women (error: -9%) but again, this value derived from an overestimation for the subjects living in the A-red area (error: +7%) and an underestimation for those living in the B-red area (error: -36%). Keeping in mind the higher accuracy for the outputs concerning the B-red area, providing values for a coefficient N did not seemed useful from a physiological point of view. In fact, adding a coefficient N in the equation for the plasma compartment to consider menstruation losses, would have resulted in a decrease of the average PFOA serum concentrations predicted by the ML2 model also for the women living in the B-red area. However, that value was lower respect to the observed value, so, this adjustment would have produced a decreasing in the accuracy of the model output. In conclusion, that would not have been an optimization of the model.

For the same reason the ML2 seemed to be the most reliable model for PFOA, since it provided the lowest underestimation for the subjects living in the B-red area.

In conclusion, a slight correction of the ML2 parameters should be implemented to increase the average predicted PFOA concentration in the B-red area. In implementing this operation an additional correction of the ML2 parameter should be considered to take into account the PFOA lost with menstruation. So, the parameters should be corrected starting from the values obtained with ML18 and not from those obtained with the ML2. The Bartell model produced the less accurate result (error: -46%), especially for the subjects living in the B-red area (error: -75%). The Loccisano AL and the Thompson model produced very similar results (errors: -39% for Loccisano AL; -33% for Thompson), with less accuracy for the subjects living in the B-red area (errors: -51% for Loccisano AL; -61% for Thompson).

women	**PFOA serum concentration comparison at aggregate level [ng/mL]**					
Municipality	**HBM**	**ML 2**	**ML18**	**Loccisano AL**	**Thompson**	**Bartell**
Sarego	78.2	78.0	64.0	40.6	49.5	42.7
Lonigo	39.3	64.1	52.6	33.8	41.1	34.6
Veronella	42.9	26.4	21.6	16.3	13.4	9.9
Albaredo	39.8	36.0	29.5	21.0	18.8	15.1
Legnago	18.1	15.2	12.5	10.0	7.3	4.0
Women (tot)	38.5	42.7	35.0	23.5	25.7	20.8
A-red area	53.2	69.1	56.7	36.2	44.1	37.5
B-red area	25.8	20.0	16.4	12.6	10.0	6.6

Table 59 Average PFOA serum concentration comparison at aggregate level for the group of women [ng/mL]. HBM = Human Bio-monitoring study, ML2 = Modified Loccisano Version 2, ML18% = Modified Loccisano minus 18%, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

women	**Error (%)**

Municipality	ML2	ML18	Loccisano AL	Thompson	Bartell
Sarego	0	-18	-48	-37	-45
Lonigo	63	34	-14	5	-12
Veronella	-38	-50	-62	-69	-77
Albaredo	-10	-26	-47	-53	-62
Legnago	-16	-31	-45	-60	-78
Women (tot)	11	-9	-39	-33	-46
A-red area	30	7	-32	-17	-30
B-red area	-22	-36	-51	-61	-75

Table 60 Average PFOA serum concentration comparison at aggregate level for the group of women; deviation from the measured value: error (%).ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

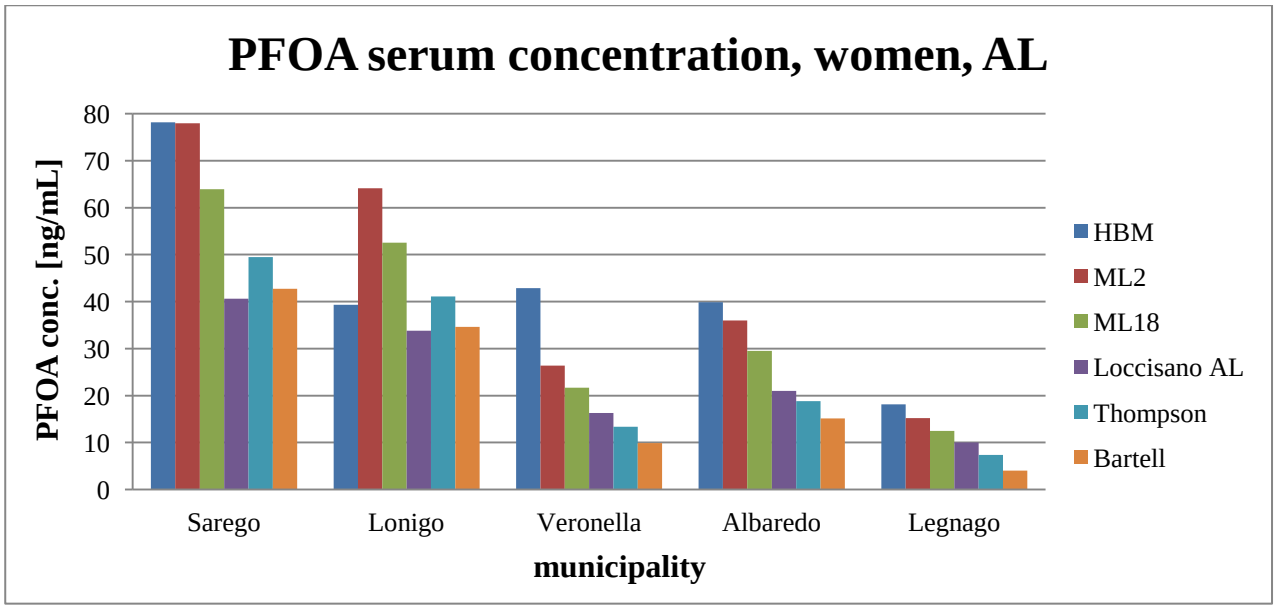


Figure 46 Average PFOA concentration comparison at aggregate level for the group of women [ng/mL]. HBM = Human Bio-monitoring study, ML2 = Modified Loccisano Version 2, ML18% = Modified Loccisano minus 18%, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

In conclusion all the models tested in this study underestimated the measured average PFOA serum concentration. However, taking into account the uncertainties in the exposure assessment and the literature on PBPK models, all the models can be considered quite reliable in predicting PFOA serum concentration when measured

concentrations ranges from medium to very high. The output of the ML2 was the most accurate while the Loccisano AL, the Thompson and the Bartell models provided similar results, even if the Loccisano AL turned out to be a little more accurate. All the models predicted better women than men and the subjects in the A-red area than those in the B-red area.

	PFOA serum concentration comparison at aggregate level [ng/mL]					
	HBM	ML2	Loccisano AL	Thompson	Bartell	ML18
total pop	57.8	40.0	22.3	23.3	21.8	-
Men	79.4	37.3	21.1	21.1	21.1	-
women	38.5	42.7	23.5	25.7	20.8	35.0

Table 61 Average PFOA serum concentration comparison at aggregate level [ng/mL] for the total population, for the group of men and for the group of women. HBM = Human Bio-monitoring study, ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

	Errors (%)				
	ML2	**Loccisano AL**	**Thompson**	**Bartell**	**ML18**
total pop	-38	-64	-65	-68	-
Men	-53	-73	-73	-73	-
women	11	-9	-39	-33	-46

Table 62 Average PFOA serum concentration comparison at aggregate level for the total population, for the group of men and for the group of women; deviation from the measured value: error (%).ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

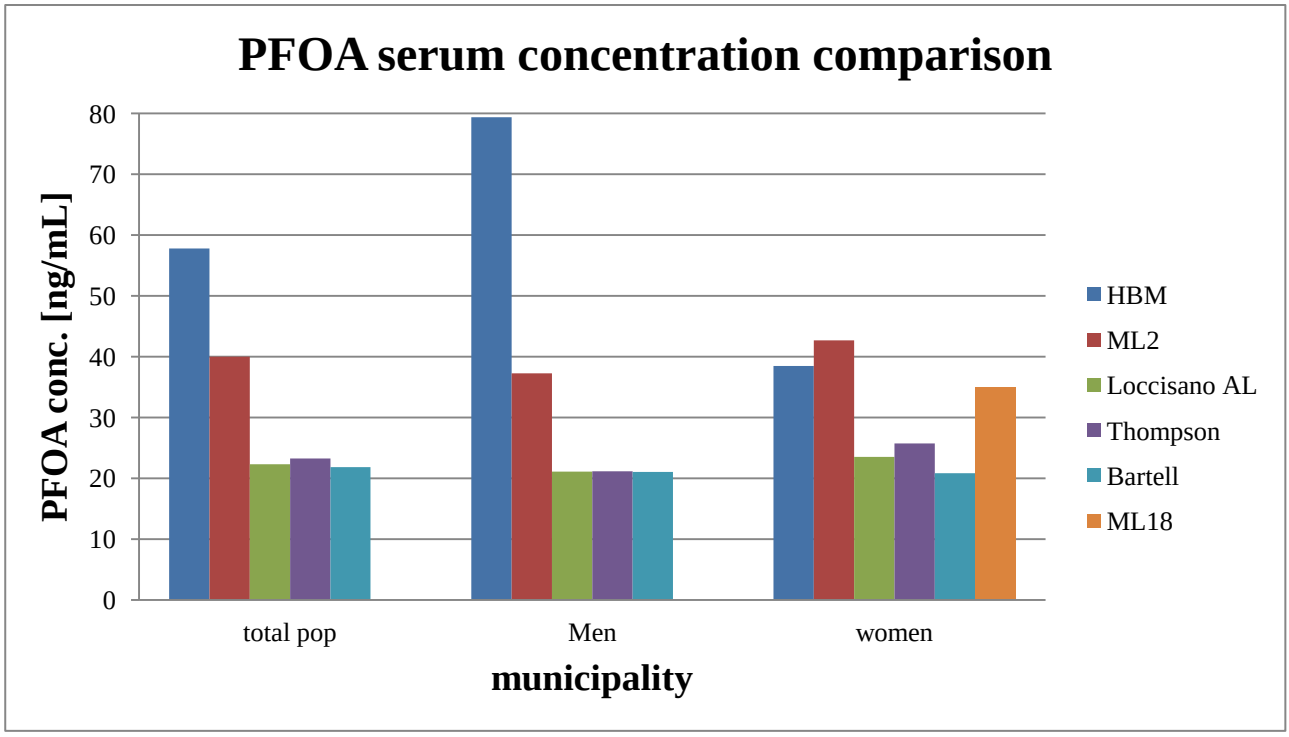


Figure 47 Average PFOA serum concentration comparison at aggregate level [ng/mL] for the total population, for the group of men and for the group of women. HBM = Human Bio-monitoring study, ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

B-red area	PFOA serum concentration comparison at aggregate level [ng/mL]					
	HBM	ML2	Loccisano AL	Thompson	Bartell	ML18
total pop	42.6	19.4	12.2	9.5	7.8	-
Men	60.2	19.1	11.9	9.3	8.5	-
women	25.8	20.0	12.6	10.0	6.6	16.4

Table 63 Average PFOA serum concentration comparison at aggregate level [ng/mL] in the B-red area for the total population, for the group of men and for the group of women. HBM = Human Bio-monitoring study, ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

B-red area	Errors (%)				
	ML2	Loccisano AL	Thompson	Bartell	ML18
total pop	-56	-72	-79	-83	-
Men	-68	-80	-85	-86	-

women	-22	-36	-51	-61	-75

Table 64 Average PFOA serum concentration comparison at aggregate level in the B-red area for the total population, for the group of men and for the group of women; deviation from the measured value: error (%). ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

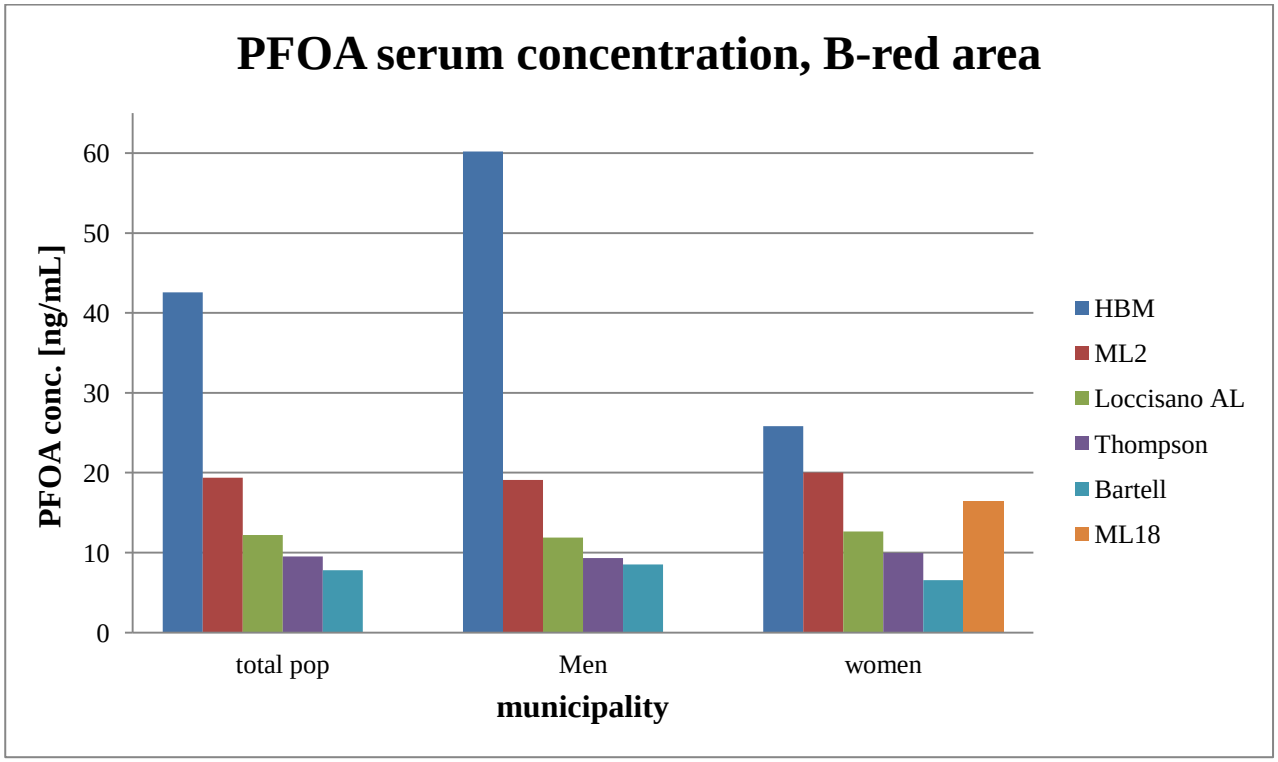


Figure 48 Average PFOA serum concentration comparison at aggregate level [ng/mL] in the B-red area for the total population, for the group of men and for the group of women. HBM = Human Bio-monitoring study, ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

MODEL COMPARISON AT AGGREGATE LEVEL, PFOS

All the models tested in this study overestimated the average PFOS serum concentrations observed in the HBM study. The ML2 turned out to be the model that provided the most accurate predicted values (+50%) while the Thompson model provided the less accurate result (+134%), as expected. The ML1 and the Loccisano model produced similar results (ML1: +82%; Loccisano: +85%). The Bartell model predicted surprisingly well the observed concentrations (+ 63%). The error was slightly higher for the subjects living in the A-red area except for Bartell (A-red area: + 88% ; B-red area: +79%).

	average PFOS serum concentrations (ng/mL)

Municipality	HBM	ML2	ML1	Loccisano AL	Thompson	Bartell
Sarego	5.7	8.9	11.1	11.2	15.3	9.3
Lonigo	5.3	7.8	9.6	9.7	13.6	8.2
Veronella	4.6	5.7	6.9	7.1	8.5	6.3
Albaredo	3.8	6.9	8.4	8.5	9.2	6.9
Legnago	3.4	5	6	6.1	7.3	5.8
total pop	4.4	6.6	8.0	8.1	10.5	7.1
A-red area	5.4	8.2	10.1	10.2	14.2	8.6
B-red area	3.6	5.4	6.5	6.6	7.8	6.1

Table 65 Average PFOS serum concentration comparison at aggregate level [ng/mL]. HBM = Human Bio-monitoring study, ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

	Error (%)				
Municipality	**ML2**	**ML1**	**Loccisano AL**	**Thompson**	**Bartell**
Sarego	56	95	96	168	63
Lonigo	47	81	83	157	55
Veronella	24	50	54	85	37
Albaredo	82	121	124	142	82
Legnago	47	76	79	115	71
total pop	50	82	85	134	63
A-red area	50	86	87	160	57
B-red area	49	80	83	115	68

Table 66 Average PFOS serum concentration comparison at aggregate level for the total population, for each municipality, for the subjects living in the A-red area and for the subjects living in the B-red area; deviation from the measured value: error (%). ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

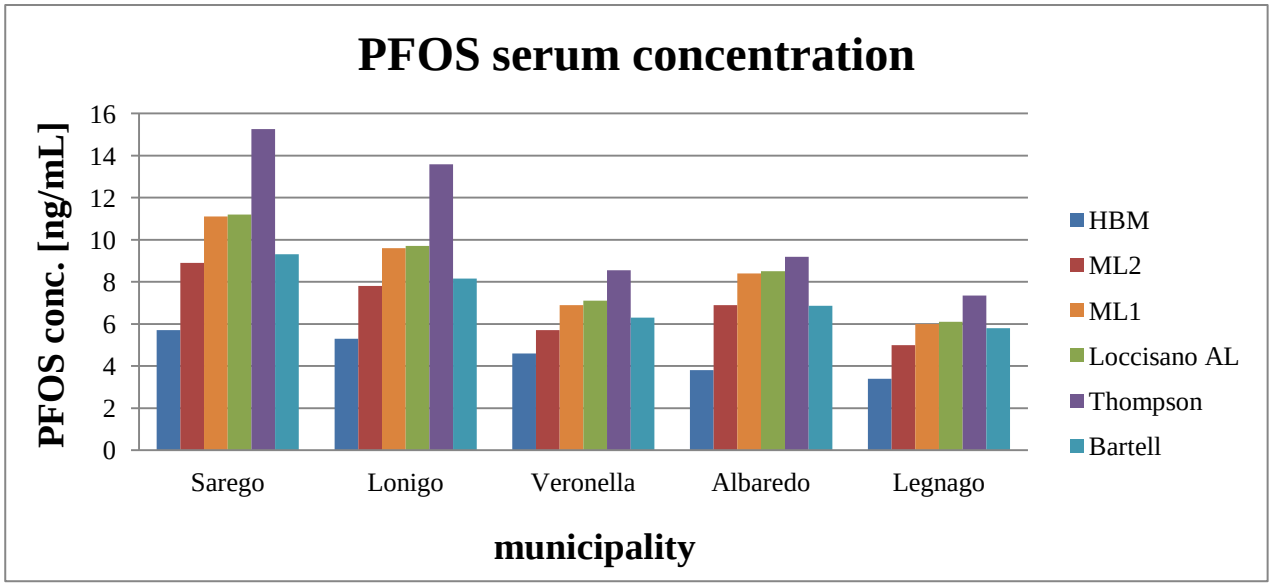


Figure 49 Average PFOS serum concentration comparison at aggregate level [ng/mL]. HBM = Human Bio-monitoring study, ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

The overestimation for men was lower than for total population but similar considerations can be made. The ML2 was still the model that provided the most accurate predicted values (error: +12%), with the most accurate results for the A-red area (error: +15%) and for the B-red area (error: +10%). All the models provided better results for the subjects living in the B-red area except for Bartell, as for the total population. The ML1, Loccisano model and Bartell model provided similar results (error: +35%, +38%, +39% respectively) while the Thompson model predicted higher concentrations (error: +96%), especially in the A-red area.

men	**average PFOS serum concentrations (ng/mL)**					
Municipality	**HBM**	**ML2**	**ML1**	**Loccisano**	**Thompson**	**Bartell**
Sarego	6.7	7.9	9.7	9.9	15.3	9.3
Lonigo	6.6	7.5	9.2	9.4	13.6	8.2
Veronella	4.3	4.9	5.8	5.9	8.5	6.5
Albaredo	4.2	6.0	7.4	7.5	9.2	7.0
Legnago	4.2	4.2	5.0	5.1	7.3	5.8
Men (tot)	5.1	5.8	7.0	7.1	10.2	7.0
A-red area	6.6	7.6	9.3	9.5	14.1	8.5

B-red area	4.2	4.6	5.6	5.7	7.8	6.1

Table 67 Average PFOS serum concentration comparison at aggregate level [ng/mL]. HBM = Human Bio-monitoring study, ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

men	**Error (%)**				
Municipality	**ML2**	**ML1**	**Loccisano AL**	**Thompson**	**Bartell**
Sarego	18	45	48	128	39
Lonigo	14	39	42	106	24
Veronella	14	35	37	98	51
Albaredo	43	76	79	119	67
Legnago	0	19	21	74	38
Men (tot)	12	35	38	96	39
A-red area	15	41	44	113	29
B-red area	10	32	34	85	45

Table 68 Average PFOS serum concentration comparison at aggregate level for men, for men in each municipality, for the group of men living in the A-red area and for the group of men living in the B-red area; deviation from the measured value: error (%). ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

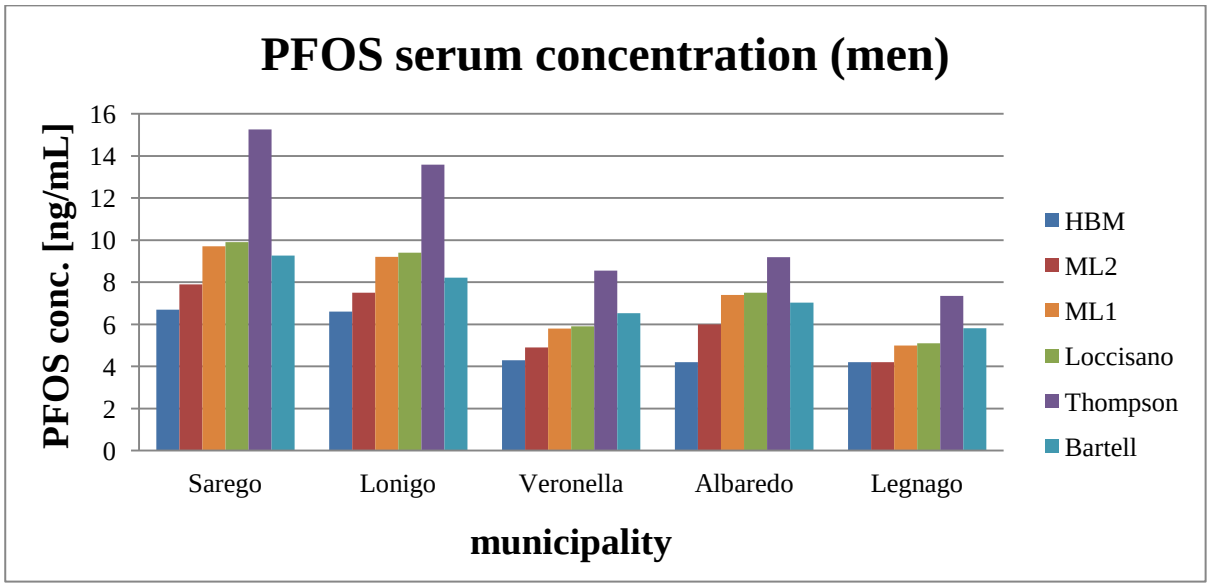


Figure 50 Average PFOS serum concentration comparison at aggregate level for men [ng/mL]. HBM = Human Bio-monitoring study, ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

The overestimation for women was higher than for total population for all the models. The ML29 provided the best results (error: +47%), as expected. The MLV was slightly less precise than the ML29 (error: +63%). The only underestimation of the observed values was registered for the municipality of Veronella using the ML29 (error: -4%). The biggest error was made using the Thompson model (error: +242%), in particular for the municipality of Legnago (error: +288%).

women	**average PFOS serum concentrations (ng/mL)**							
Municipality	**HBM**	**ML29**	**MLV**	**ML2**	**ML1**	**Loccisano**	**Thompson**	**Bartell**
Sarego	5.1	6.4	6.9	9	11.2	11.3	16.4	9.1
Lonigo	4.1	5.8	6.3	8.2	10.0	10.1	14.6	7.9
Veronella	4.7	4.5	5.1	6.3	7.6	7.7	9.5	6.1
Albaredo	3.1	4.3	5.1	6	7.3	7.4	9.7	6.5
Legnago	2.4	4.3	4.8	6.1	7.3	7.5	9.3	5.8
Women (tot)	3.6	5.1	5.6	7.2	8.8	8.9	12.1	7.0
A-red area	4.5	6.0	6.5	8.5	10.4	10.5	15.2	8.3
B-red area	3.0	4.3	4.9	6.1	7.4	7.5	9.4	5.9

Table 69 Average PFOS concentration comparison at aggregate level for women [ng/mL]. HBM = Human Bio-monitoring study, ML29 = Modified Loccisano 29%, MLV = Modified Loccisano-Verner, ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

women	**Errors (%)**						
Municipality	**ML29**	**MLV**	**ML2**	**ML1**	**Loccisano**	**Thompson**	**Bartell**
Sarego	25	35	76	120	122	222	78
Lonigo	41	54	100	144	146	256	93
Veronella	-4	9	34	62	64	102	30
Albaredo	39	65	94	135	139	213	110
Legnago	79	100	154	204	213	288	142
total pop	47	63	108	152	157	242	102

A-red area	36	47	92	135	137	244	88
B-red area	57	77	122	167	173	241	115

Table 70 Average PFOS serum concentration comparison at aggregate level for the group of women; deviation from the measured value: error (%). ML29 = Modified Loccisano 29%, MLV = Modified Loccisano-Verner,ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

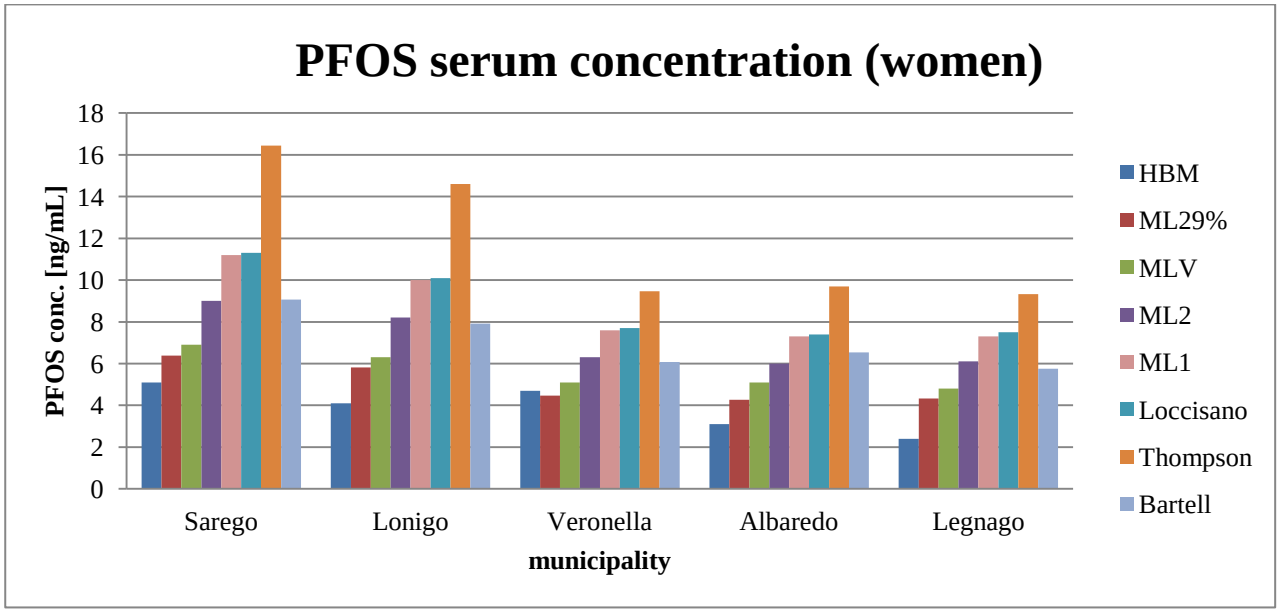


Figure 51 Average PFOS concentration comparison at aggregate level for women [ng/mL]. HBM = Human Bio-monitoring study, ML29 = Modified Loccisano 29%, MLV = Modified Loccisano-Verner, ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

The huge difference in PFOS serum predicted values between the Thompson model and the Bartell model was quite unexpected. Considering that both the two models, as for they are created, should slightly overestimate the observed PFOS serum concentrations, the Bartell model for PFOS was considered the most accurate of the two.

	average PFOS serum concentrations (ng/mL)							
	HBM	ML2	ML1	Locc (AL)	Thomp	Bartell	ML29	MLV
tot pop	4.5	6.7	8.2	8.3	10.8	7.2	-	-
Men	5.1	5.8	7.0	7.2	10.2	7.1	-	-
Women	3.6	7.2	8.8	8.9	12.1	7.0	5.1	5.6

Table 71 Average PFOS serum concentration comparison at aggregate level [ng/mL] for the total population, for the group of men and for the group of women. HBM = Human Bio-monitoring study, ML29 = Modified Loccisano 29%, MLV = Modified Loccisano-Verner, ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

	Errors (%)						
	ML2	ML1	Locc (AL)	Thomp	Bartell	ML29	MLV
tot pop	50	82	85	134	63	-	-
Men	12	35	38	96	39	-	-
Women	108	152	157	242	102	47	63

Table 72 Average PFOS serum concentration comparison at aggregate level for the total population, for the group of men and for the group of women; deviation from the measured value: error (%). HBM = Human Bio-monitoring study, ML29 = Modified Loccisano 29%, MLV = Modified Loccisano-Verner, ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

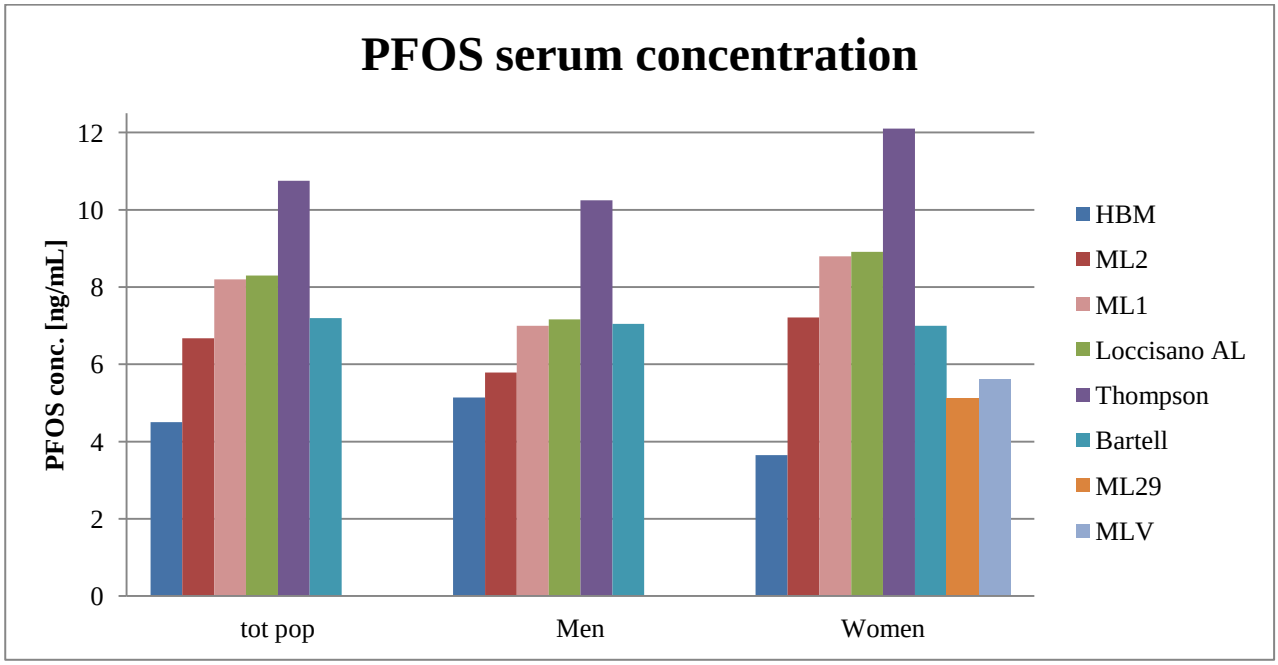


Figure 52 Average PFOS serum concentration comparison at aggregate level [ng/mL] for the total population, for the group of men and for the group of women. HBM = Human Bio-monitoring study, ML29 = Modified Loccisano 29%, MLV = Modified Loccisano-Verner, ML2 = Modified Loccisano Version 2, ML1 = Modified Loccisano Version 1, Loccisano AL = Loccisano model at aggregate level, Thompson = Thompson model, Bartell = Bartell model.

ANALYSIS OF THE CONCENTRATION TREND OVER TIME AND OF THE CONTRIBUTION TO THE TOTAL UPTAKE

The shape of the PFAS concentration curves in tissues over time changed for men and women, from one municipality to another and for the different scenarios. In this section are presented the considerations about the shape of PFAS concentration curve in tissues over time relying on the outputs of the ML2, that turned out to be the most reliable PBPK model for the Veneto population. All the considerations were referred to the PFAS average serum concentration (CA) trend over time obtained for a group of subjects living in the same municipality. So, the values reported are average values for a group of subjects but the considerations about the PFAS concentration trend over time (i.e. the shape of the PFAS concentration curve) are valid for all the subjects belonging to the group represented by the same curve. It is important to remember that only the exposure to PFAS in food continued after the sampling time for all the subjects and for every scenarios.

The probable value assumed by CA at the steady state (CA_{ss}) respect to the highest PFAS serum concentration reached over time (CA_{max}), was found relevant and very different for PFOA and PFOS (for total population: CA_{ss} = 49% of CA_{max} for PFOA and 84% of CA_{max} for PFOS), while the difference between CA_{max} and PFAS serum concentration at the date of the blood sampling (CA_{bs}) was found small (for total population: 13% for PFOA and 5% for PFOS).

In this section are also reported the contributions of PFAS coming from groundwater, tap water and food to the total PFAS serum concentration predicted by the ML2 model.

The entity of the contribution from each route of exposure was found very different for PFOA and PFOS and for the A-red area and B-red area while it was found similar for men and women. Food resulted to be the main route of exposure for both the PFAS in the analysis on the total population and on the B-red area while for

PFOA, the analysis on the A-red area attributed to groundwater the principal contribution to the highest PFOA serum concentration.

PFOA

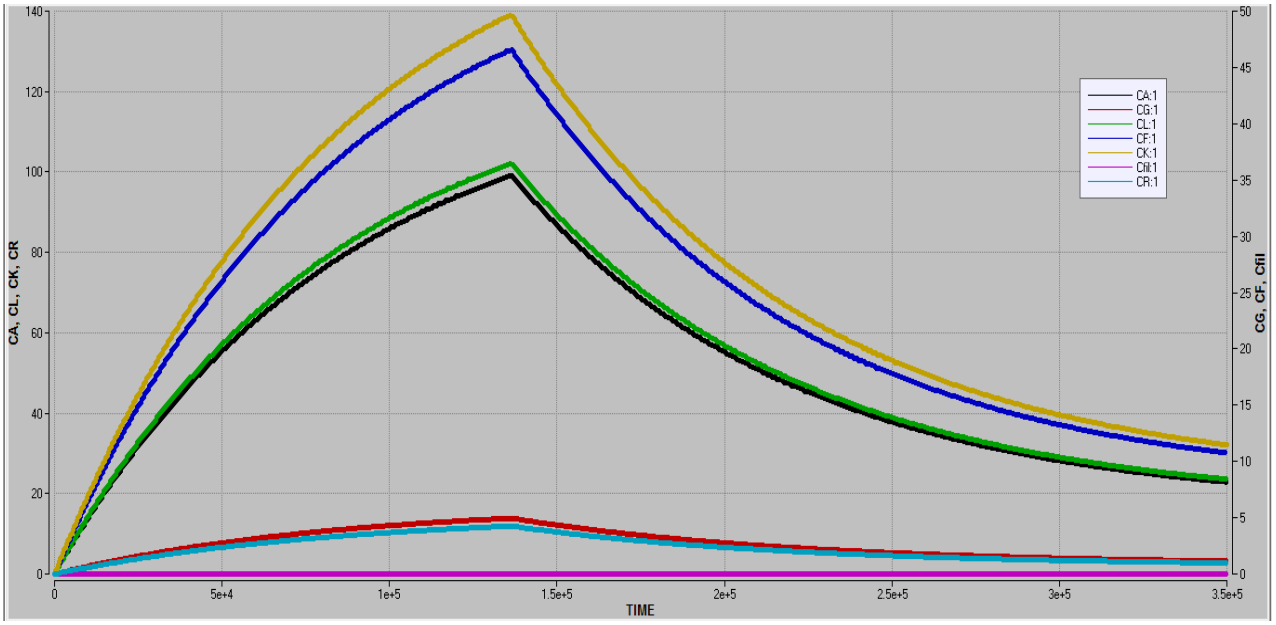


Figure 53 PFOA concentration in tissues (ng/mL) over time (hours). ML2, Sarego, MLS+MS, tot pop

Looking at the trend over time for the subjects living in the municipality of Sarego, MLS+MS, the predicted PFOA serum concentration (CA), like the concentrations in the other tissues (PFOA concentration in gut (CG), PFOA concentration in liver (CL), PFOA concentration in kidney (CK), PFOA concentration in fat (CF), PFOA concentration in the rest of the body (CR), PFOA concentration in the filtrate compartment (Cfil)), followed a logarithmic growth (40% of the maximum PFOA serum concentration (CA_{max}) after 4 years, 80% of the CA_{max} after 10 years). According to the ML2, the maximum of the predicted PFOA serum concentration over time (CA_{max}) was equal to 98 ng/mL and was reached in 2014 (after 17 years from the beginning of the simulation), just before the end of the exposure to groundwater and the rapid decrease of PFOA concentration in tap water. The predicted steady state (CA = 20 ng/mL) will be reached after 30 years from that date. CA will decrease at 70% of the CA_{max} after 4 years from the peak (= point at

which CA=CA_{max}) and at 40% of the CA_{max} after 11.5 years from the peak. PFOA serum concentration at blood sampling (CAbs) was equal to the 75% of CA_{max}.

In 2014, in correspondence of the peak, the contribution to CA_{max} coming from groundwater was equal to the 80%. Without considering the exposure to groundwater the curve, after achieving the peak, did not decrease sharply, achieving the steady state at a value that was the 77% of CA_{max}.

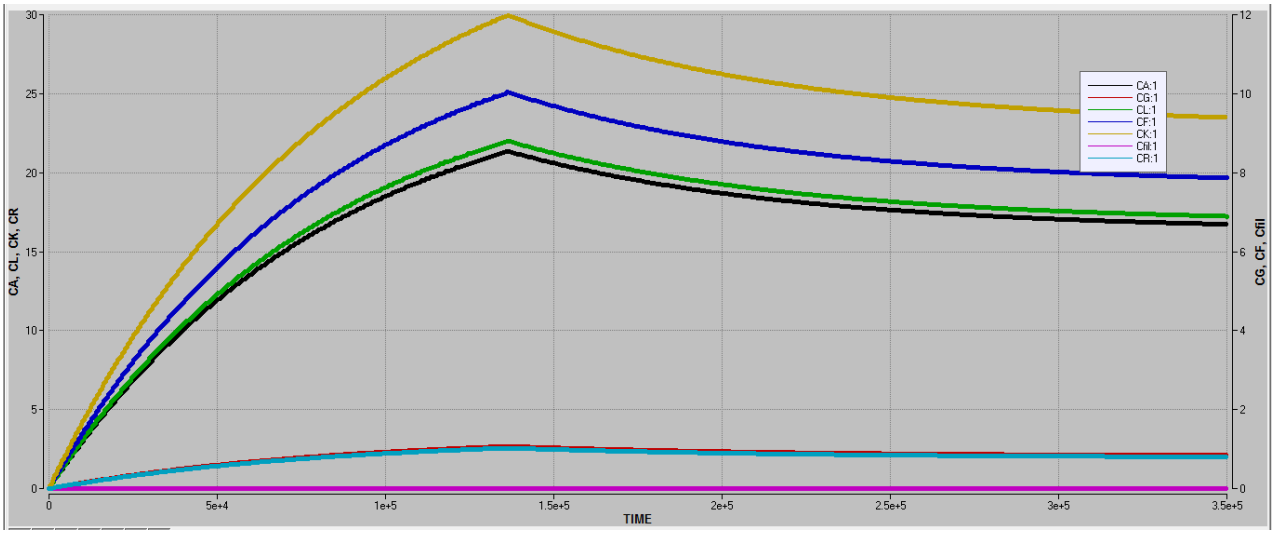


Figure 54 PFOA concentration in tissues (ng/mL) over time (hours). ML2, Sarego, MLS+MS, tot pop. No exposure to groundwater.

At the peak (2014, when the exposure to groundwater stopped) the predicted contribution of food to CA_{max} was the 10% of the total. The same contribution was predicted for tap water. The shape of the curves considering the exposure to groundwater only was very similar to the one that considered all the exposure sources (70% of the maximum value after 4 years from the peak and at 40% of the maximum value after 11.5 years from the peak). The predicted curve for men was very similar to the curve for women but a little more flat, with a maximum lower of 5%.

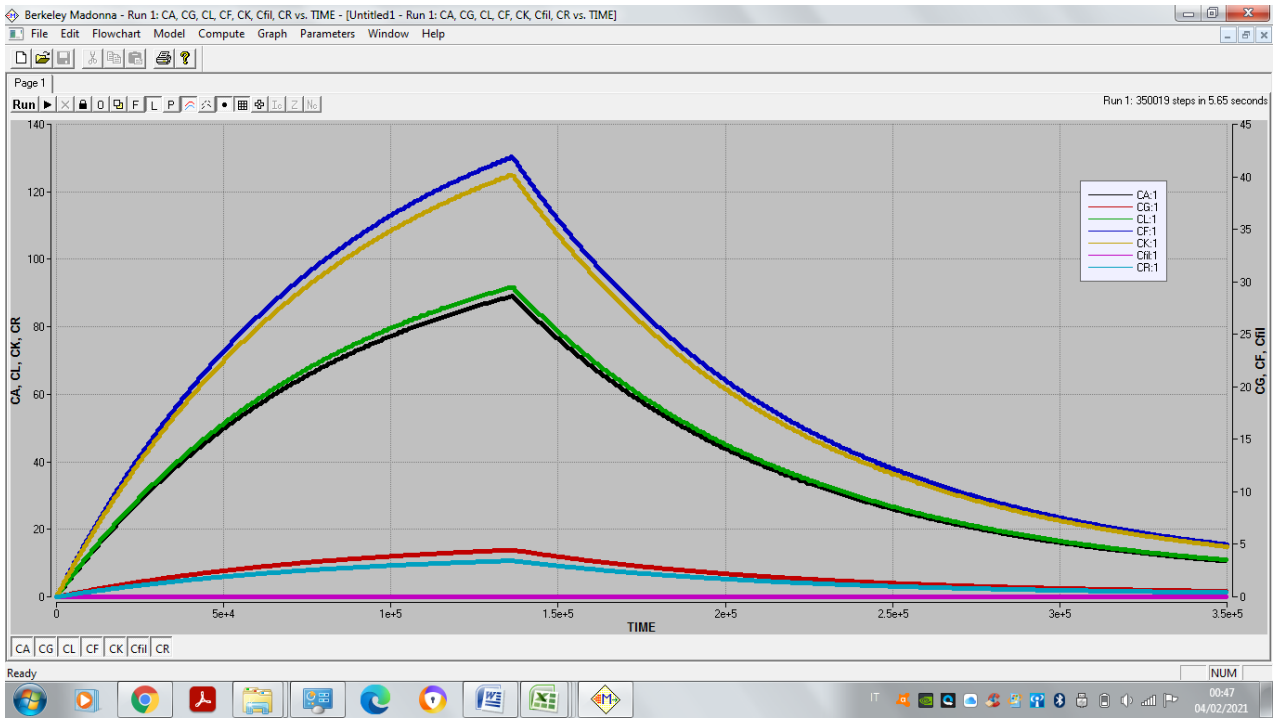


Figure 55 PFOA concentration in tissues (ng/mL) over time (hours). ML2, Sarego, MLS+MS, tot pop. No exposure to food

The PFOA serum concentration curve for the subjects living in the municipality of Lonigo was similar to the one for the subjects living in the municipality of Sarego: after 6 years from the peak CA was equal to the 58% of the CA_{max} (CA_{max} = 80 ng/mL). After 35 years from the end of the principal exposure (groundwater) and after 32 years from the end of the exposure to tap water, with only the food exposure that continues, the predicted value of serum PFOA concentration will reach the steady state at a value equal to the 20% of CA_{max} (16 ng/mL). CA at the sampling time (CAbs) was equal to the 75% of the CA_{max}.

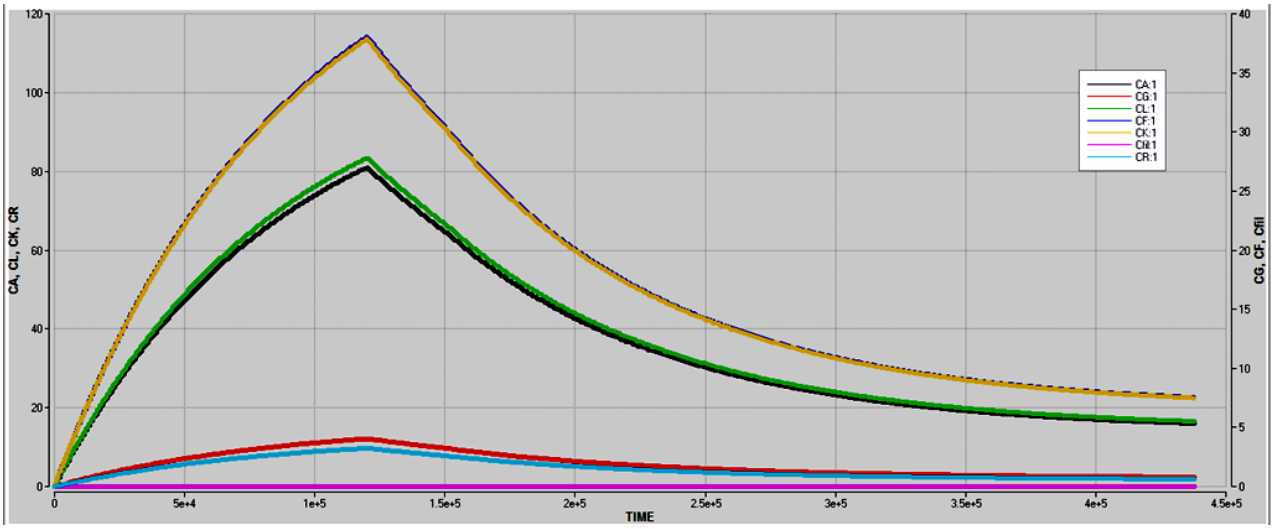


Figure 56 PFOA concentration in tissues (ng/mL) over time (hours). ML2, Lonigo, tot pop, MLS+MS

According to the ML2 outputs, the predicted contribution to CA_{max} derived for the 55% from groundwater, for the 32% from tap water and for the 13% from food.

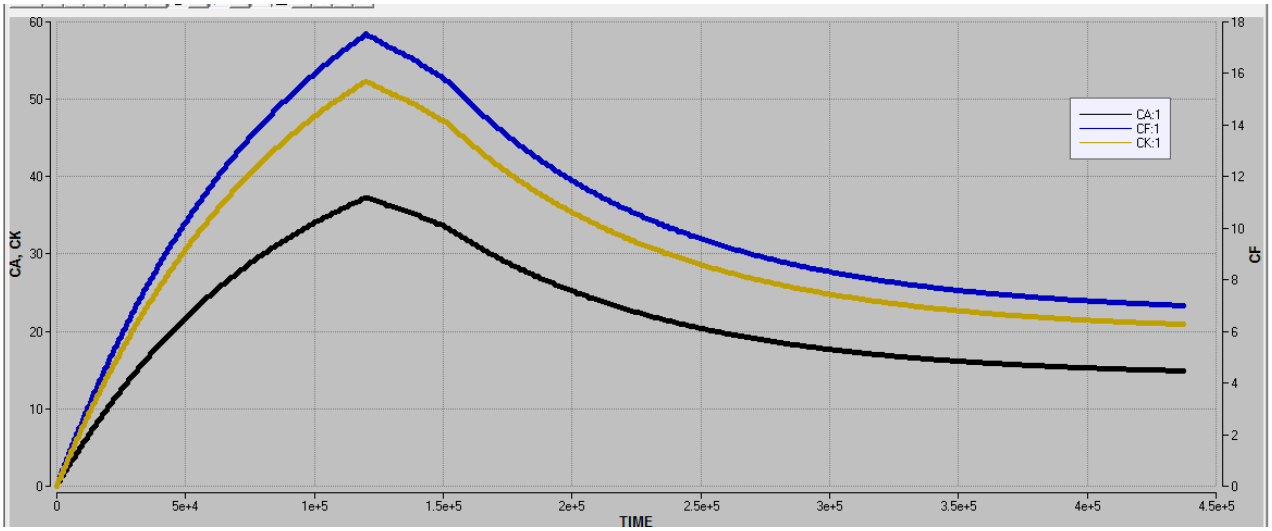


Figure 57 PFOA concentration in tissues (ng/mL) over time (hours). ML2, Lonigo, tot pop, MLS+MS, exposure to food and tap water only.

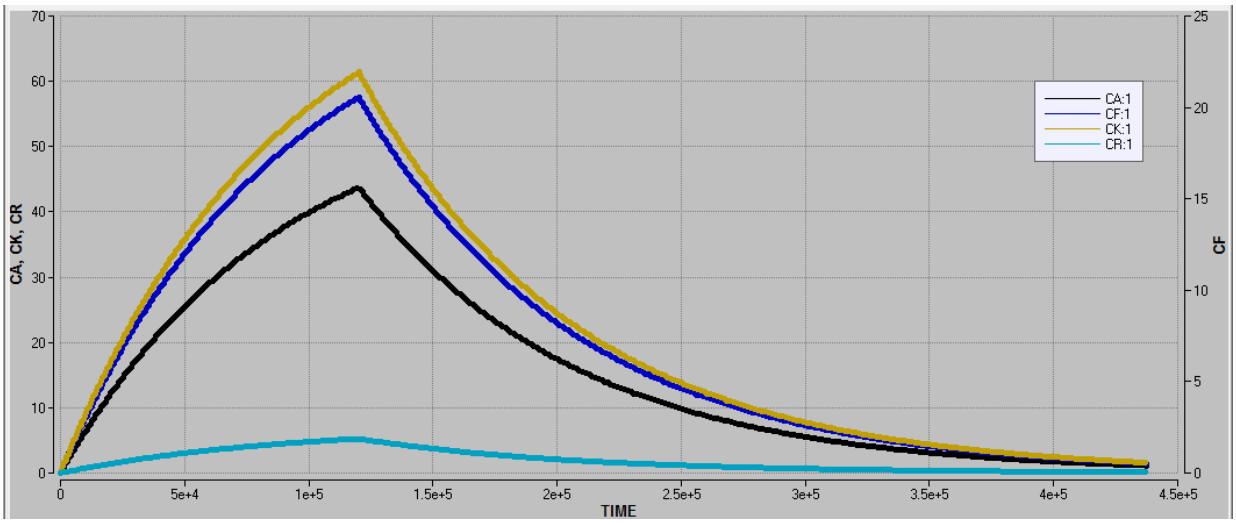


Figure 58 PFOA concentration in tissues (ng/mL) over time (hours). ML2, Lonigo, tot pop, MLS+MS, exposure to groundwater only.

For the municipality of Veronella the predicted shape of the CA curve was very different from the one obtained for Sarego and Lonigo. The curve was flatter and CA_{max} (29 ng/mL) was lower and followed by a very slower decrease. The lower CA_{max} was due to the absence of the exposure to groundwater and the slower decrease was caused by the slow decrease in the PFOA concentration in tap water for the municipality of Veronella over the period 2014-2017. The value of CA at the sampling time (CAbs) was equal to the 96% of the CA_{max}. The steady state was reached after 15 years from the peak and was equal to the 50% of CA_{max}.

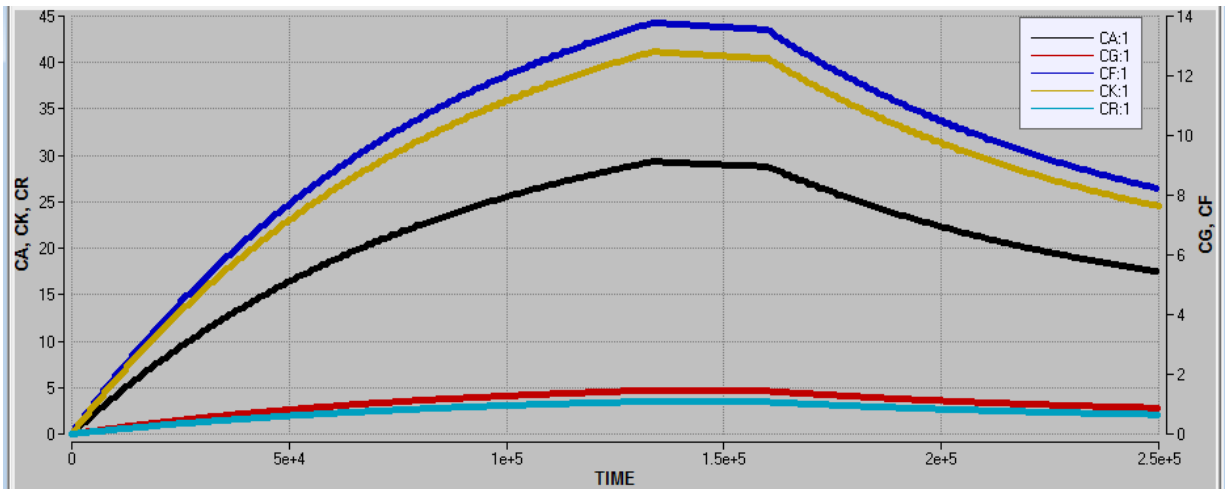


Figure 59 PFOA concentration in tissues (ng/mL) over time (hours). ML2, Veronella, tot pop.

The predicted contribution of PFOA to the CA_{max} derived for the 73% from tap water and for the 27% from food.

A very similar situation was found for the municipalities of Albaredo d'Adige, even if the concentration at the steady state was lower (27% of CA_{max} instead of 50%).

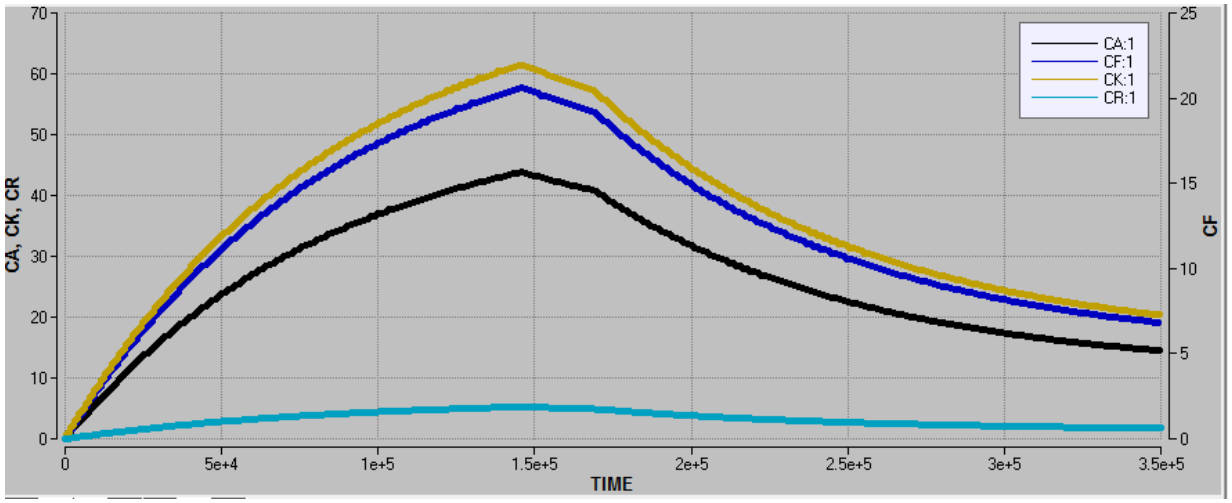


Figure 60 PFOA concentration in tissues (ng/mL) over time (hours). ML2, Albaredo, tot pop

A different shape of the CA curve was provided by the ML2 model for the subjects living in the municipality of Legnago, where the CA_{max} was in 2016, due to the relevant decrease in PFOA concentrations in tap water that happened in that year. In fact CAbs was equal to the 97% of the CA_{max} and CA at the steady state was equal to the 85% of the CA_{max}. The main contribution to CA_{max} in Legnago derived from the exposure to food (65%). The contributions derived from tap water (22%) and groundwater (13%) were similar.

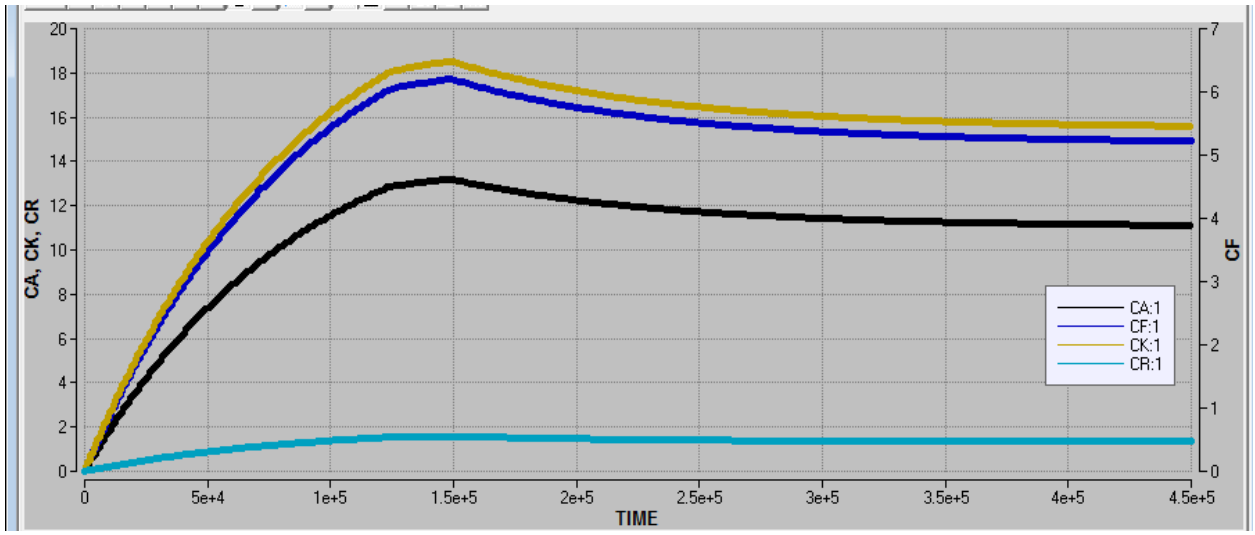


Figure 61 PFOA concentration in tissues (ng/mL) over time (hours). ML2, Legnago, tot pop

The principal route of exposure for PFOA was groundwater for the subjects living in the A-red area (80% in Sarego and 55% in Lonigo) and food for those living in the B-red area (but only for the results found in Lonigo, since in Albaredo and Veronella the principal route was tap water). For total population, the principal route of exposure was food (35%), followed by tap water (33%) and then groundwater (32%), but looking at the data divided for municipality, only in the municipality of Legnago food was found the principal route of exposure (65%), while for all the other municipalities it was the least significant.

Looking at the CA values over time predicted at time points preceding the time correspondent to CA_{max}, it was evident that the steady state was not achieved only for the subjects living in the municipalities in the A-red area.

	Characteristics of the PFOA serum concentration curve over time respect to CA_{max} (%)				
Municipality	CA_{bs}	CA_{ss}	$CA_{Groundwater}$	$CA_{Tapwater}$	CA_{Food}
Sarego	75	22	80	10	10
Lonigo	75	20	55	32	13
Veronella	96	50	0	73	27
Albaredo	93	27	0	82	18

Legnago	97	85	13	22	65
Total pop.	87	49	32	33	35
Men	88	51	30	34	37
women	86	48	34	33	33

Table 73 Percentages of the PFOA serum concentration maximum value reached over time (CAmax) for ML2, MLS+MS, total population. CAbs = PFOA serum concentration at blood sampling expressed as percentage of CAmax (%). CAss = PFOA serum concentration at steady state expressed as percentage of CAmax (%). $CA_{Groundwater}$ = contribution to the PFOA serum concentration maximum value derived from the exposure to groundwater expressed as percentage of CAmax (%). $CA_{Tapwater}$ = contribution to the PFOA serum concentration maximum value derived from the exposure to tap water expressed as percentage of CAmax (%). CA_{Food} = contribution to the PFOA serum concentration maximum value derived from the exposure to food expressed as percentage of CAmax (%).

PFOS

Since the PFOS concentration values in environmental matrixes were very lower respect to the values predicted for PFOA in every municipality, the shape of the predicted curve for PFOS serum concentration was flatter and not so different from one municipality to another. The contribution of groundwater, tap water and food to the highest PFOS serum concentration predicted by the ML2 model (CA_{max}) was also similar.

Looking at the group of subjects living in the municipality of Sarego, CA_{max} was found equal to10.2 ng/mL, CA_{bs} was the 88% of CA_{max} and CA_{ss} was the 64% of CA_{max}. The contribution to CA_{max} derived from the exposure to groundwater was equal to the 22% of the total, from the tap water was the 28% of the total and from the food was the 50% of the total.

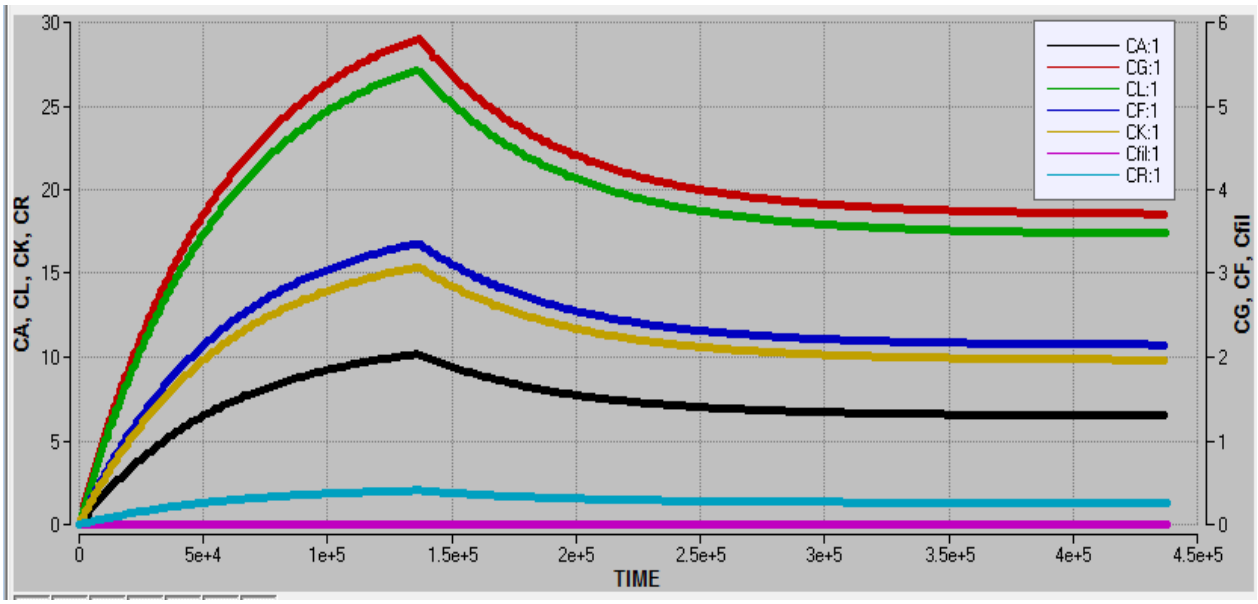


Figure 62 PFOS concentration in tissues (ng/mL) over time (hours). ML2, Sarego, MLS, tot pop

For the group of women CA_{max} (= 7.6 ng/mL) turned out to be about the 25% of the CA_{max} found for the total population in the municipality of Sarego but the trend of CA over time was very similar to the trend found for total population. In fact, CA_{bs} for the group of women was equal to the 84% of CA_{max} and CA_{ss} was equal to the 64% of CA_{max}.

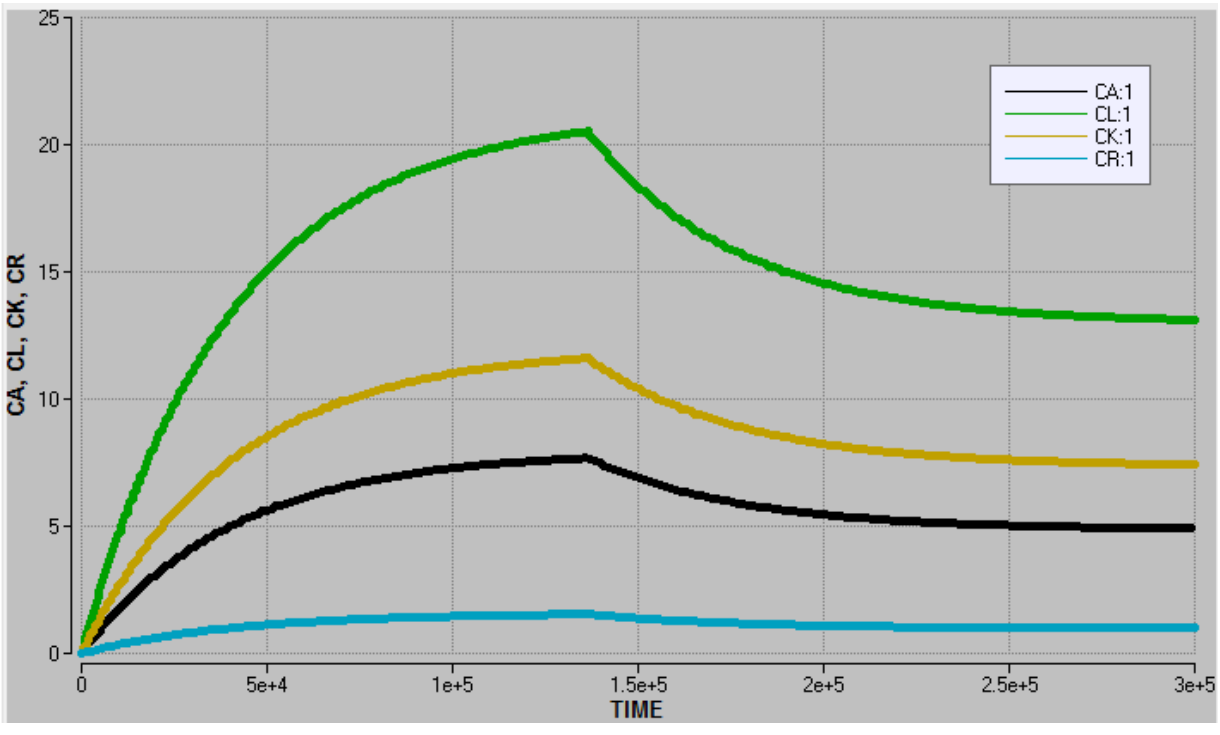


Figure 63 PFOS concentration in tissues (ng/mL) over time (hours). ML2, Sarego, MLS, women

In the municipality of Lonigo CA_{max} found for total population was equal to 8.4 ng/mL, CA_{bs} was equal to the 93% of CA_{max} and CA_{ss} was equal to the 76% of CA_{max}. The contribution to CA_{max} derived from the exposure to groundwater was the 28% of CA_{max}, from the tap water was the 10% of CA_{max} and from the food was the 62% of CA_{max}.

In the municipality of Veronella CA_{bs} was found equal to the 95% of CA_{max} and CA_{ss} was equal to the 90% of CA_{max}. The contribution to CA_{max} derived from the exposure to groundwater was the 6% of CA_{max}, from the tap water was the 16% of CAmax and from the food was the 78% of CA_{max}.

Also in the municipality of Albaredo CA_{bs} was found equal to the 95% CA_{max} and Ca_{ss} equal to the 90% of CA_{max}. The contribution to the PFOA serum concentration maximum value derived from the exposure to groundwater was the 6% of CA_{max}, from the tap water was the 22% of CA_{max} and from the food was the 72% of CA_{max}.

In the municipality of Legnago CA_{bs} was the 98% of CA_{max} and CA_{ss} was the 95% of CA_{max}. The contribution to the PFOA serum concentration maximum value derived from the exposure to groundwater was the 6% of CA_{max}, from the tap water was the 6% of CA_{max} and from the food was the 88% of CA_{max}.

For PFOS, the principal route of exposure for total population was food (73%), followed by groundwater (15%) and then tap water (13%).

	PFOS serum concentration trend over time. Concentrations respect to CA_{max} (%)				
Municipality	**CA_{bs}**	**CA_{ss}**	**$CA_{Groundwater}$**	**$CA_{Tapwater}$**	**CA_{Food}**
Sarego	88	64	22	28	50
Lonigo	93	76	28	10	62
Veronella	95	90	6	16	78
Albaredo	95	90	6	22	72
Legnago	98	95	6	6	88

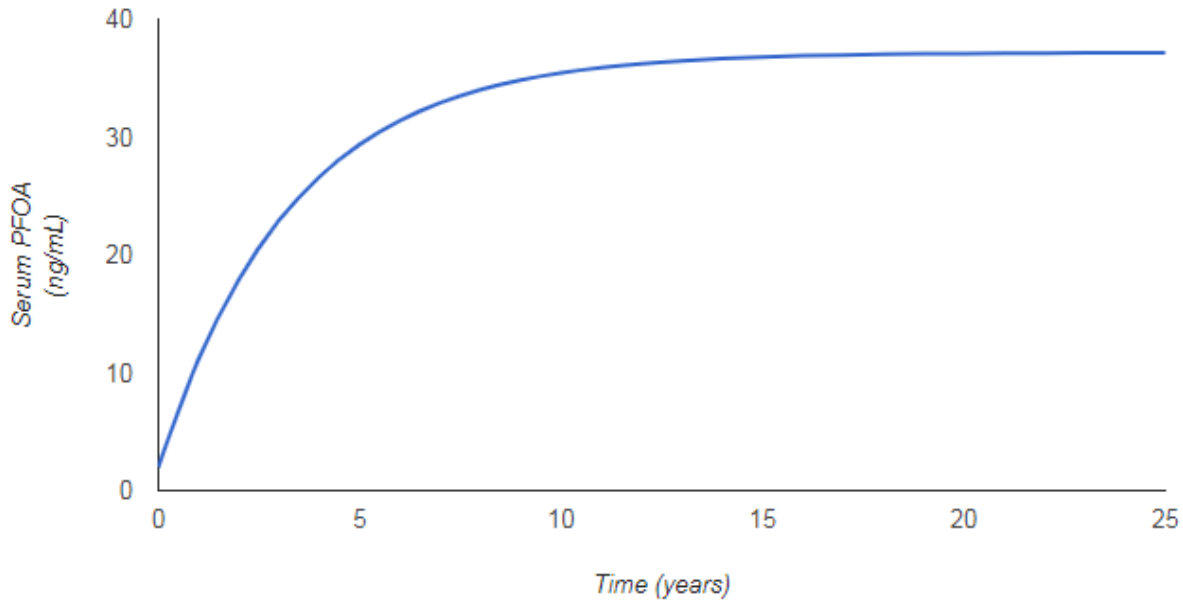
Total pop.	95	84	15	13	73
Men	95	85	14	12	74
women	94	83	15	13	72

Figure 64 Percentages of the PFOS serum concentration maximum value reached over time (CAmax) for ML2, MLS+MS, total population. CAbs = PFOS serum concentration at blood sampling expressed as percentage of CAmax (%). CAss = PFOS serum concentration at steady state expressed as percentage of CAmax (%). $CA_{Groundwater}$ = contribution to the PFOS serum concentration maximum value derived from the exposure to groundwater expressed as percentage of CAmax (%). $CA_{Tapwater}$ = contribution to the PFOS serum concentration maximum value derived from the exposure to tap water expressed as percentage of CAmax (%). CA_{Food} = contribution to the PFOS serum concentration maximum value derived from the exposure to food expressed as percentage of CAmax (%).

For PFOS the shape of the CA curve given as output of ML2 for women was found very similar to the one found for men, since the N coefficient produced a uniform decrease in CA levels over time. On the contrary, the CA curve produced by the model with the N_{fert} coefficient was totally different, since it showed an initial high peak, due to the high initial PFOS concentrations in tissues and an immediate decrease, as expected.

Bartell model

The shape of the predicted CA curve provided as output of the Bartell model was very similar to the one obtained whit the ML2 until the peak, then, the steady state was achieved in the CA curve in Bartell while it started to decrease according to the ML2 (and the Loccisano) model output. This difference was likely entirely due to the different exposure to PFAS given as input in the models. Therefore, a comparison of the predicted concentration curves had no significance if not until the time in which CA_{max} is achieved. When the concentration raised the two curves were found similar, with an analogous logarithmic growth.



Starting serum PFOA concentration: 2 ng/mL
Water PFOA concentration: 300 ppt
Serum PFOA contribution from other ongoing exposures: 1.67 ng/mL
Water ingestion rate: 16.6 ml/kg/d
Volume of distribution: 0.17 L/kg
Half-life of PFOA in serum: 2.3 years
Steady-state ratio for serum:water concentrations: 118.35
Predicted steady-state serum PFOA concentration: 37.17 ng/mL

Figure 65 example of the Bartell model output implemented in the online serum calculator (Lu S, Bartell SM. Serum PFAS Calculator for Adults, Version 1.2, 2020, www.ics.uci.edu/~sbartell/pfascalc.html).

LOCCISANO IL VS LOCCISANO AL

Comparing the predicted serum PFOA average concentrations at individual level (Loccisano IL) and at aggregate level (Loccisano AL) for the MLS+AS, very similar results were obtained (46.5 ng/mL vs 52.4 ng/mL, respectively). So, the analysis at aggregate level was faster but not less reliable than the analysis at individual level. The error committed using the Loccisano model at aggregate level over the total population studied was even less than that committed using the Loccisano model at individual level for the MLS+AS (AL: -9.6% vs IL: -19.8%). Similar results were found for the combined scenario: MLS+MS, since the model construction was the

same and PFOA concentration values in the groundwater for the municipality in the A-red area were less but always very higher respect to those in the B-red area.

Tot pop	serum PFOA average concentrations (ng/mL)						
	Sarego	**Lonigo**	**Veronella**	**Albaredo**	**Legnago**	**Tot pop**	**B-red area**
HBM	96.7	69.7	71.6	60.8	32.2	57.9	42.7
Loccisano IL, MLS+AS	138.9	71.0	16.4	23.4	8.2	46.5	11.9
Loccisano AL, MLS+AS	166.8	77.3	17.0	23.2	8.6	52.4	12.2

Table 74 serum PFOA average concentrations (ng/mL) measured (HBM) and predicted with Loccisano model at individual level (Loccisano IL) and at aggregate level (Loccisano AL) in the five municipalities and the total population (tot pop).

Tot pop	Errors in predicted PFOA average concentrations (%)						
	Sarego	**Lonigo**	**Veronella**	**Albaredo**	**Legnago**	**Tot pop**	**B-red area**
Loccisano IL, MLS+AS	43.6	1.9	-74.5	-61.5	-77.2	-19.8	-72.1
Loccisano AL, MLS+AS	72.5	10.9	-73.3	-61.8	-76.3	-9.6	-71.3

Table 75 Deviation from the observed value: errors (%) in average PFOA serum concentration predicted with Loccisano model at individual level (Loccisano IL) and at aggregate level (Loccisano AL) respect to the observed serum PFOA average concentration (HBM).

Men	serum PFOA average concentrations (ng/mL)						
	Sarego	Lonigo	Veronella	Albaredo	Legnago	Tot pop	**B-red**

							area
Loccisano IL, MLS+AS	140.2	80.6	17.1	24.8	7.3	44.7	11.7
Loccisano AL, MLS+AS	163.7	78.6	18.1	24.3	7.5	46.9	11.8

Table 76 serum PFOA average concentrations measured (HBM) and predicted with Loccisano model at individual level (Loccisano IL) and at aggregate level (Loccisano AL) in men.

Men	**Errors in predicted PFOA average concentrations (%)**						
	Sarego	**Lonigo**	**Veronella**	**Albaredo**	**Legnago**	**Tot pop**	**B-red area**
Loccisano IL, MLS+AS	12.6	-22.3	-86.7	-65.7	-84.6	-43.4	-80.5
Loccisano AL, MLS+AS	31.5	-24.2	-86.0	-66.3	-84.3	-40.7	-80.3

Table 77 Deviation from the observed value: errors (%) in average PFOA serum concentration predicted with Loccisano model at individual level (Loccisano IL) and at aggregate level (Loccisano AL) respect to the observed average PFOA serum concentration (HBM).

Women	**serum PFOA average concentrations (ng/mL)**						
	Sarego	**Lonigo**	**Veronella**	**Albaredo**	**Legnago**	**Tot pop**	**B-red area**
Loccisano IL, MLS+AS	138.0	62.4	16	20.8	9.2	47.7	12.0
Loccisano AL, MLS+AS	166.8	75.8	16.3	21.0	10.0	56.8	12.6

Table 78 comparison between the average PFOA serum concentrations observed (HBM) and predicted with Loccisano model at individual level (Loccisano IL) and at aggregate level (Loccisano AL) in women.

Women	Errors in predicted PFOA average concentrations (%)						
	Sarego	Lonigo	Veronella	Albaredo	Legnago	Tot pop	B-red area
Loccisano IL, MLS+AS	76.5	58.7	-62.7	-47.8	-49.1	24.1	-53.5
Loccisano AL, MLS+AS	113.3	92.7	-62.0	-47.3	-44.9	47.6	-51.1

Table 79 Deviation from the observed value: errors (%) in average PFOA serum concentration predicted with Loccisano model at individual level (Loccisano IL) and at aggregate level (Loccisano AL) respect to the observed average PFOA serum concentration (HBM).

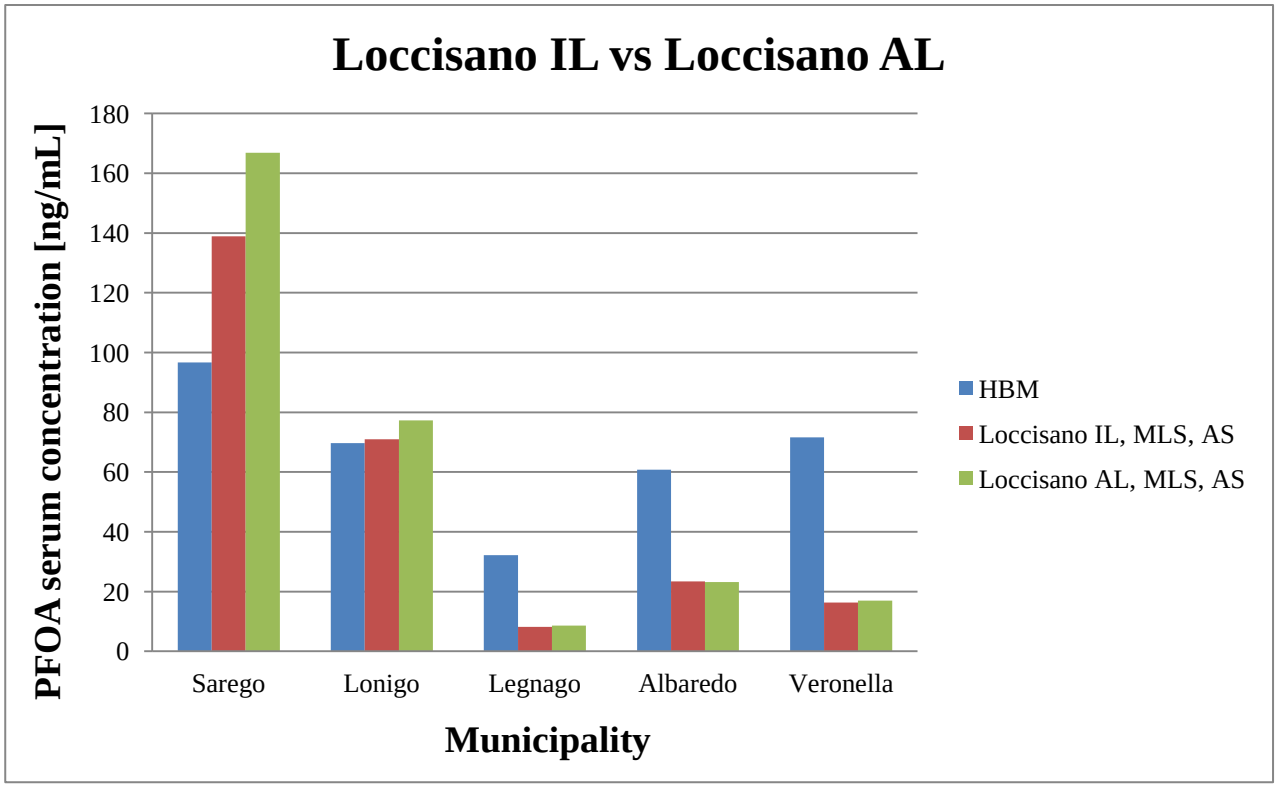


Figure 66 comparison between the average PFOA serum concentrations observed (HBM) and predicted with Loccisano model at individual level (Loccisano IL) and at aggregate level (Loccisano AL).

The average PFOA concentration predicted for the municipality of Sarego resulted to be slightly farer from the observed value when the analysis was carried out at individual level (34.8 ng/L) respect to the analysis at aggregate level (40.0 ng/L), MLS+MS used. This fact confirmed that a less time expensive simulation at aggregate level should be preferred, and this is even more true when the accuracy of the exposure assessment at individual level is not high.

DISCUSSION

In this study three well-known PBPK models specifically created to assess PFAS concentrations in humans and one new model derived from one of these PBPK models were tested. The testing procedure consisted in comparing the PFAS serum concentrations predicted by the PBPK models with the observed PFAS serum concentrations obtained in the context of a HBM study. The population under study lived in the Veneto Region and presented an high Average PFOA serum concentration and a medium-low PFOS average serum concentration. The results found applying this testing procedure were so valid especially for the conditions previously described.

Various aims were pursued by the work activity described in this study. First of all, as previously claimed, the most important purpose was testing all the analyzed PBPK models for their ability to predict observed data at aggregate level and, for one of the model tested, also at individual level. This analysis aimed at identifying the best of the tested models in predicting PFAS serum concentrations, taking into account also the time necessary to set up the simulations and the skills of the operator required to obtain the prediction, as well as the difficulties in collecting accurate data for the exposure assessment (e.g. PFAS concentrations in water at individual level). The analysis involved the total study population but comparisons were made also between the group of men and the group of women and between the municipalities located in a higher exposed area (A-red area) and municipalities located in a lower exposed area (B-red area). The analysis also provided a comparison between several exposure scenarios created to provide an indication of the level of uncertainty associated to different parameters in the final result. Secondly, looking at the results of the comparison between predicted and observed PFAS serum concentrations, some parameters (i.e. T_m, Free, P.C.) and a differential equation (for plasma compartment) of a complex multi-compartment PBPK model were modified to obtain a new model that is able to make better predictions for the

subjects living in the Veneto region. This model optimization aimed also at providing information about the best value (among those found in the literature) for several PBPK parameters for the subjects analyzed in this case-study. So, the optimization process was essential to provide useful information about PBPK models developed for PFAS other than to implement a PBPK model that is able to predict PFAS serum concentrations in the subjects living in the Veneto region better than the other models tested. Moreover, using a comparative analysis, the differences in the results obtained using the same PBPK model at individual level and at aggregate level were shown. Furthermore, differences in the final result running the model for a subsample of the total population were investigated, in order to assure the reliability of the results for all the performed analysis. Also the predicted PFAS serum concentration trend over time was investigated, to provide an indication of the PFAS serum concentrations before and after the time of the blood sampling. The contribution of each route of exposure to the predicted serum concentration was also predicted using the model that showed the best results. Finally, a prediction of the PFOA concentration in several tissues of the human body was made in case observed data for other organs will be collected in the next future.

Before focusing on the main discussion about the principal results obtained with this study, few general considerations have to be underlined.

First of all, when the level of uncertainty in the exposure assessment is quite high and the inter-individual variability in pharmacodynamics and toxicokinetics of a compound is large, like in the present case-study, comparing different models, with different structure and level of complexity seems to be very useful. In fact, it is essential to found the most appropriate model for a compound in a specific situation. However, an effective comparative analysis must keep into account several parameters in order to suggest the best model for a specific case-study. The parameters evaluated in this study are: complexity of the model (i.e. level of experience required), model reliability, type of data requested in input, accuracy of

the predicted results, precision of the predicted results, time required to run the simulations, economic cost and degree of the invasiveness.

Secondly, a great advantage of using the Loccisano model or the ML1 and the ML2 models respect to the Thompson model is that many information are available in the model output, like the PFAS serum concentration trend over time. Also using the online PFASs serum calculator (i.e. the Bartell model) there's the possibility to have an idea of the PFAS serum concentration trend over time, but only if the exposure does not vary in time. So, when the purpose of the analysis is to have a prediction of the PFAS concentration over time (and not only a prediction for one point in time), only the Loccisano model, the ML1 and the ML2, implemented in the Berkeley-Madonna software, are recommended. This is true especially when the exposure vary over time and it is important to have a prediction value in the future.

Finally, as already stated above, using a multi-compartment PBPK model (e.g. the models derived by the original Loccisano model) is not the only way to obtain predicted PFAS concentrations for other tissues. In fact, also a one-compartment model can be used if the values for partition coefficients are available. So, when the purpose of the analysis is to predict PFAS concentrations in several tissues, all the PBPK models tested in the present study can be used.

We also underline the fact that only the PFASs serum calculator (i.e. the Bartell model) provides the possibility to predict serum concentrations for other PFASs, not considered in the present study (PFHxS and PFNA).

PFOA

All the PBPK models tested for the most reliable scenario underestimated the observed PFOA serum concentrations but all the predicted values were of the same order of magnitude. Average PFOA serum concentration was predicted better for the group of women and for the A-red area. The ML2 model slightly overestimated the average observed PFOA concentration for the group of women, but slightly underestimated for the women in the B-red area (for whom an higher accuracy of

the results was assessed), so the ML18 was not implemented (further adjustments of the model parameters are needed). The Bartell model produced the least accurate results but running the Loccisano model and the Thompson model very similar values were predicted. A slightly improvement was observed in using the ML1, while the error significantly decreased in using the ML2. The Loccisano IL model predicted results very similar to those found using the Loccisano AL model. Moreover, observed PFOA serum concentrations were found higher than the predicted values for the WCS (both WCS+AS and WCS+MS) and lower than the predicted values for the BCS (both BCS+AS and BCS+MS) for few subjects.

In conclusion, the ML2 model at aggregate level was the best model to predict PFOA serum concentration for the subjects in the Veneto region. The errors in the results predicted by the other models at aggregate level were similar but the Bartell model and the Thompson model required less time and efforts to set up the simulations. Moreover, the level of expertise required by the Bartell model to run the simulations is very low, thanks to the implementation of the online serum calculator for PFASs.

So, keeping in mind that the actual value will likely be slightly underestimated (especially for men) when the observed average PFOA serum concentration is high, the ML2 at aggregate level is the model suggested to obtain the most accurate results and the Bartell model at the aggregate level is the model suggested to obtain a quick but quite accurate analysis, when the exposure end is not distant in time. Moreover, performing the analysis at the individual level is not recommended, especially when the exposure at individual level is not very accurate. In fact, the results between the Lossisano IL model and the Loccisano AL model were similar but too much time was spent and too much efforts were required to set up the model parameters and the simulations for the first.

In a post-ban situation, when PFOA concentration in water is high and decreases significantly over time, the use of a model that allows to vary the exposure over

time (e.g. the Loccisano model) seemed not essential to obtain a consistent improvement in the result accuracy if the prediction refers to a time not so distant from the end of the exposure (one-two years). On the contrary, after few years from the end of the exposure the use of a PBPK model that allows to vary the exposure seemed necessary to avoid huge errors.

In conclusion, the Bartell model can be considered a good model to predict PFOA serum concentrations in a post-ban situation when the exposure is mainly from diet (especially from drinking water) if the exposure is quite constant in time and the prediction is for a time near the end of the exposure, since it is very user friendly, time saving and quite accurate. It is important to underline that the model underestimated the observed values, especially for men.

PFOS

All the PBPK models overestimated the observed PFOS serum concentrations. The highest errors occurred using the Thompson model, both for men and women (the average predicted concentration was more than double of the observed one). All the models predicted better PFOS concentrations in men. Excellent results for the group of women were obtained using the ML29 model and the MLV model.

In conclusion, the ML2 model at aggregate level was the best model also to predict PFOS serum concentration for the subjects in the Veneto region. The errors in the results predicted by the Loccisano AL and the Bartell models were similar, but the Bartell model required less time, less efforts and a lower level of expertise to set up the simulations, for the reason previously cited.

So, when the observed average PFOS serum concentration is not high, the ML2 at aggregate level is the model suggested to obtain the most accurate results for men, while the ML29 is the model suggested for women. The Bartell model at the aggregate level is the model suggested to obtain a quick but quite accurate analysis when the exposure is mainly from diet (water and food).

MENSTRUATION LOSSES

In the Gomis study the differences in concentrations between men and women could not be fully explained by menstruation. Other studies (Wong et al., 2014) came to the same conclusion for PFOS in USA. Moreover "additional gender-specific processes such as breastfeeding, maternal serum:cord transfer and postpartum haemorrhage had a negligible impact on the modelled concentration in women's serum" (Gomis et al., 2017), so the cause could perhaps be found in some processes linked to the sex hormones. These causes were not investigated in the present study. However, assuming that the percentages associated to menstruation losses found in Gomis study are valid also for the women of the Veneto region, even in this study the differences in observed PFOA concentrations between men and women was not fully explained by menstruation. On the contrary, the 29% less observed in PFOS concentration for women was here entirely explained by the percentage found for menstruation losses in the Gomis study (Gomis et al., 2017). This could suggest that the difference between men and women attributed to menstruation losses is lower.

CONCLUSIONS

The main aim of this work was testing several PBPK models with the observed data obtained by the HBM study on the Veneto population exposed to PFOA and PFOS and proposing a new model to better predict the observed data. The new model was created changing some parameters and equation terms in the Loccisano model. All the values for the parameters and terms changed were found or derived by the literature on PBPK modelling for PFAS. Moreover this work showed the differences between the Loccisano multi-compartment model, the Thompson one-compartment model and the Online serum calculator, developed by Bartell. This work also showed the importance of the exposure assessment step in model validation.

All the tested models underestimated the observed PFOA serum concentrations and overestimated the observed PFOS serum concentrations. However, the predicted and the observed values were of the same order of magnitude for all the municipalities. So, keeping in mind the uncertainties associated to a PBPK model testing procedure and the results obtained by the PBPK models until now, we can assert that using the PBPK models presented in this study, very satisfactory results were obtained for the subjects living in the Veneto region. Of course, the best results were obtained using the ML2 model both for PFOA and PFOS, since the ML2 model derived from an optimization of the Loccisano model that aimed at reducing the deviation of the predicted values from the observed data.

The choice of the analysis at individual level is necessarily related to the availability of good quality input data, that is an accurate individual exposure assessment. If it's not the case, like in our study, this approach seems to be an unnecessary waste of energy and time only, when compared to the analysis at aggregate level.

The analysis of a population subsample showed no difference in predicting PFOA serum concentrations including or excluding young subjects (14-20 years of age)

with a not long time of exposure (< 10 years). This result was confirmed by the observed data analysis.

This work also showed that using a one compartment PBPK model (Bartell, 2017) could provide good results in case of a PFOS contamination at low concentrations in the environment. So, even if the ML2 at aggregate level is the model suggested to obtain the most accurate results, the Bartell model at the aggregate level is the model suggested to obtain a quick but quite accurate analysis, when the exposure end is not distant in time.

Thanks to the collaboration with ARPAE, the Veneto Region and ARPAV, in the next future more accurate data on subjects location and on the PFAS concentrations in groundwater will be likely available. As a consequence, the accuracy of the exposure assessment will increase and an in-depth analysis will be performed with a more reliable comparison of different approaches.

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ANNEX A

FEW EXAMPLES OF BERKELEY-MADONNA SCRIPTS FOR THE ADAPTED LOCCISANO MODEL

Below, an example of a script created for the Berkeley Madonna software to develop a simulation using the adapted Loccisano model for the subject 24113 (man), PFOA, MLS, AS.

“

METHOD Stiff

STARTTIME = 0 ; PFOA, ID 24113, Lonigo

STOPTIME= 438000 ;stop simulation (h; 50 years)

DT = 0.01

; Physiological parameters (from Brown, et al)

;fractional blood flows to tissues

QCC = 12.5 ; Cardiac blood output (L/h/kg^0.75)

QFC = 0.052 ; Fraction cardiac output going to fat

QLC = 0.069 ; Fraction cardiac output going to liver

QKC = 0.175 ; Fraction cardiac output going to kidney

QfilC = 0.035 ; Fraction cardiac output to the filtrate compartment (20% of kidney blood flow)

QSkC = 0.058 ; Fraction cardiac output going to skin

QGC = 0.181 ; Fraction cardiac output going to gut

BW = 113 ; male

;fractional tissue volumes

VLC = 0.026 ; Fraction liver volume

VFC = 0.214 ; Fraction fat volume

VKC = 0.004 ; Fraction kidney volume

VfilC = 0.0004 ; Fraction filtrate compartment volume (10% of kidney volume)

VGC = 0.0171 ; Fraction gut volume

VPlasC = 0.0428 ; Fraction plasma volume

;dermal uptake

SkinTarea = 9.1*((BW*1000)**0.666) ; Total area of skin (cm^2)

Skinthickness = 0.1 ; Skin thickness (cm)

; Chemical-specific parameters (PFOA)

Tmc = 6000 ; Maximum resorption rate

Kt = 55 ; Resorption affinity; same as monkey

Free = 0.02 ; Free fraction of PFOA in plasma; same as monkey

PL = 2.2 ; Liver/plasma partition coefficient

PF = 0.04 ; Fat/plasma partition coefficient

PK = 1.05 ; Kidney/plasma partition coefficient

PSk = 0.1 ; Skin/plasma partition coefficient

PR = 0.12 ; Rest of the body/plasma partition coefficient

PG = 0.05 ;Gut/blood plasma coeff.

kurinec = 0.0003 ; Elimination rate (1/h); estimated from data of Harada, et al 2005

kurine = kurinec*BW**(-0.25)

; Free fraction of chemical in tissues

FreeL = Free/PL ;liver

FreeF = Free/PF ;fat

FreeK = Free/PK ;kidney

FreeSk = Free/PSk ;skin

FreeR = Free/PR ;rest of tissues

FreeG = Free/PG ;gut

; Exposure parameters

tchng = 251004 ; number of hours from birth to the end of P1 (February 2014)

T2 = 269400 ; number of hours from birth to the end of P2 (from Feb 2014 to Apr 2016)

T3 = 282540 ; number of hours from birth to the end of P3 (from Apr 2016 to Ott 2017, from Ott 2017 the ;concentration in tap water is 5

T4 = 284700 ; number of hours from birth to the end of the sampling time, 2018

;turn dose on/off

DoseOn20142016 = IF time>tchng THEN 1.0 ELSE 0.0

DoseOn20162017 = IF time>T2 THEN 1.0 ELSE 0.0

DoseOn2017 = IF time>T3 THEN 1.0 ELSE 0.0

DoseOnpozzo = IF time<tchng THEN 1.0 ELSE 0.0

;direct input to plasma (IV dose)

;IVconc = 0.0 ;iv uptake (ug/kg/day)

;IVdose = IVconc*BW ;(ug/day)

; Dermal exposure

Dermconc = 0.0 ; Dermal concentration (mg/mL)

Dermvol = 0.001 ; Dermal exposure volume (mL)

Dermdose = Dermconc*Dermvol*1000 ; (ug)

Skinarea = 972 ; Exposed area on skin (cm^2)

; Oral exposure

; Oral uptake (ug/kg/day), MB average values (average of LB and UB in the ISS document)

Oraldose = 0.021 ; (ug/day), PFAS total daily intake through food estimated for the n-subject in µg/day (= ;PTIFn /1000)

Drinkconcpozzo = 1.657 ; PFAS concentration in groundwater in the municipality of Lonigo, AS, (µg/L)

Drinkconcreteal2014 = 0.249 ; (ug/L) PFAS concentration in tap water in the municipality of Lonigo (= ;PCTWm /1000) for all the time period P1

Drinkconcrete20142016 = 0.160 ; difference in PFAS concentration in tap water between P1 and P2 (ug/L)

Drinkconcrete20162017 = 0.028 ; difference in PFAS concentration in tap water between P2 and P3 (ug/L)

Drinkconcretedal2017 = 0.056 ; difference in PFAS concentration in tap water between P3 and P4 (ug/L)

Drinkrate = 1.5/3 ; WIR24113 = water daily intake rate for the subject 24113 (L/day)

Drinkdosepozzo = Drinkconcpozzo*Drinkrate ; PFAS daily intake through groundwater (ug/day)

Drinkdoserete2014 = Drinkconcreteal2014*Drinkrate ; PFAS daily intake through tap water during the time ;period P1 (ug/day)

Drinkdoserete20142016 = Drinkconcrete20142016*Drinkrate ; difference in PFAS daily intake through tap ;water between P1 and P2 (ug/day)

Drinkdoserete20162017 = Drinkconcrete20162017*Drinkrate ; difference in PFAS daily intake through tap ;water between P2 and P3 (ug/day)

Drinkdoserete2017 = Drinkconcretedal2017*Drinkrate ; difference in PFAS daily intake through tap water ;between P3 and P4 (ug/day)

Tinput = 24 ; to spread the exposure over a period of 24 hours

;oral

Inputcibo = IF MOD(time,24) <=Tinput THEN Oraldose/Tinput ELSE 0.0 ; input created to describe the ;PFAS exposure through food (µg/h)

;drinking water

Inputpozzo = IF MOD(time,24) <= Tinput THEN Drinkdosepozzo/Tinput ELSE 0.0 ; input created to describe ;the exposure to PFAS in groundwater (µg/h)

Inputrete1 = IF MOD(time,24) <= Tinput THEN Drinkdoserete2014/Tinput ELSE 0.0 ; input created to describe the exposure to PFAS in tap water during the time period P1 (µg/h)

Inputrete2 = IF MOD(time,24) <= Tinput THEN Drinkdoserete20142016/Tinput ELSE 0.0 ; input created to ;describe the difference in PFAS exposure in tap water between the time periods P1 and P2 (µg/h)

Inputrete3 = IF MOD(time,24) <= Tinput THEN Drinkdoserete20162017/Tinput ELSE 0.0 ; input created to ;describe the difference in PFAS exposure in tap water between the time periods P2 and P3 (µg/h)

Inputrete4 = IF MOD(time,24) <= Tinput THEN Drinkdoserete2017/Tinput ELSE 0.0 ; input created to ;describe the difference in PFAS exposure in tap water between the time periods P3 and P4 (µg/h)

; Scaling parameters

QC = QCC*BW**0.75 ;Cardiac output (L/h)

Htc = 0.44 ;hematocrit (dimensionless)

QCP = QC*(1-Htc) ; Plasma flow (L/h)

QL = QLC*QCP ; Plasma flow to liver (L/h)

QF = QFC*QCP ; Plasma flow to fat (L/h)

QK = QKC*QCP ; Plasma flow to kidney (L/h)

Qfil = 0.2*QK ; Plasma flow to filtrate compartment (L/h); 20% of QK

QG = QGC*QCP ; Plasma flow to gut (L/h)

QSk = IF Dermconc >0.0 THEN QSkC*QCP*(Skinarea/SkinTarea) else 0.0 ;plasma flow to skin

QR = QCP - QL - QF - QK - Qfil - QG -QSk ; Plasma flow to rest of the body (L/h)

Qbal = QCP - (QL+QF+QK+QFil+QG+QSk) ; balance check

VL = VLC*BW ; Liver volume (L)

VF = VFC*BW ; Fat volume (L)

VK = VKC*BW ; Kidney volume (L)

Vfil = VfilC*BW ; Fitrate compartment volume (L)

VG = VGC*BW ; Gut volume (L)

VPlas = VPlasC*BW ; Plasma volume (L)

VSk = (Skinarea*Skinthickness)/1000 ; Skin volume (L)

VR = 0.84*BW - VL - VF - VK - Vfil - VG - VPlas - VSk ; Rest of the body volume (L)

Vbal = (0.84*BW)-(VL+VF+VK+VFil+VG+Vplas+VSk) ; Balance check

Tm = Tmc*BW**0.75 ;transporter maximum

;>>>>>>>>>>>>>>>Model equations<<<<<<<<<<<<<<<<<

;Plasma compartment

APlas' = QF*CF*FreeF+(QL+QG)*CL*FreeL+QR*CR*FreeR+QSk*CSk*FreeSk+QK*CK*FreeK-QCP*CA*Free

init APlas = 0.0

CAFree = APlas/VPlas ;free concentration of chemical in plasma; ug/L (ng/mL)

CA = CAFree/Free ;total concentration of chemical in plasma

; Gut compartment

AG' = QG*(CA*Free-CG*FreeG) + Inputcibo + Inputpozzo*DoseOnpozzo + Inputrete1 - Inputrete2*DoseOn20142016 - Inputrete3*DoseOn20162017 - Inputrete4*DoseOn2017

init AG = 0.0

CG = AG/VG ; Concentration in gut (ug/L)

CVG = CG/PG ; Concentration leaving gut (ug/L)

; Liver compartment

AL' = (QL*(CA*Free))+(QG*CG*FreeG) - ((QL+QG)*CL*FreeL) ; Rate of change in liver (ug/h)

init AL = 0.0

CL = AL/VL ; Concentration in liver (ug/L)

CVL = CL/PL ; Concentration leaving liver (ug/L)

; Fat compartment

AF' = QF*(CA*Free-CF*FreeF) ; Rate of change in fat (ug/h)

```
init AF = 0.0

CF = AF/VF                                  ; Concentration in fat (ug/L)

CVF = CF/PF                                 ; Concentration leaving fat (ug/L)

; Kidney compartment

AK' = QK*(CA*Free-CK*FreeK) + (Tm*Cfil)/(Kt+Cfil)        ; Rate of change in
kidneys (ug/h)

init AK = 0.0

CK = AK/VK                                  ; Concentration in kidneys (ug/L)

CVK = CK/PK                                 ; Concentration leaving kidneys (ug/L)

; Filtrate compartment

Afil' = Qfil*(CA*Free-Cfil) - (Tm*Cfil)/(Kt+Cfil); Rate of change in filtrate
compartment (ug/h)

init Afil = 0.0

Cfil = Afil/Vfil                            ; Concentration in filtrate compartment
(ug/L)


; Storage compartment for urine

Adelay' = Qfil*Cfil-kurine*Adelay

init Adelay = 0.0

; Urine

Aurine' = kurine*Adelay

init Aurine = 0.0
```

; Skin compartment

ASk' = QSk*(CA*Free-CSk*FreeSk); Rate of change in skin(ug/h)

init ASk = 0.0

CSk = ASk/VSk ; Concentration in skin compartment (ug/L)

CVSk = CSk/PSk ; Concentration leaving skin compartment (ug/L)

; Rest of the body

AR' = QR*(CA*Free-CR*FreeR) ; Rate of change in rest of the body (ug/h)

init AR = 0.0

CR = AR/VR ; Concentration in rest of the body (ug/L)

CVR = CR/PR ; Concentration leaving rest of the body (ug/L)

Display TmC,Kt,Free,PL,PK,PF,PR,PSK,PG,tchng,BW,QCC,QFC,QLC,QKC,QGC,QSkC, VFC,VLC,VKC,VGC,VFilC,VPlasC,Drinkrate

Display CG, CL, CF,CK,CA,Cfil,CR ; plot

”.

Below, an example of a script created for the Berkeley Madonna software to develop a simulation using the adapted Loccisano model for all the women living in the municipality of Lonigo, PFOS:

“

METHOD Stiff

STARTTIME = 0 ; PFOS, ID all women, Lonigo,

STOPTIME=438000 ;end of simulation (h); 50 years

DT = 0.01

; Physiological parameters (from Brown, et al 1997)

;fractional blood flows

QCC = 12.5 ; Cardiac blood output (L/h/kg^0.75)

QFC = 0.052 ; Fraction cardiac output going to fat

QLC = 0.069 ; Fraction cardiac output going to liver

QKC = 0.175 ; Fraction cardiac output going to kidney

;QfilC = 0.035 ; Fraction cardiac output to the filtrate compartment (20% of kidney blood flow)

QSkC = 0.058 ; Fraction cardiac output going to skin

QGC = 0.181 ; Fraction cardiac output going to gut

BW = 60.70 ; Average body weight (kg), woman (Lonigo)

;fractional tissue volumes

VLC = 0.026 ; Fraction liver volume

VFC = 0.214 ; Fraction fat volume

VKC = 0.004 ; Fraction kidney volume

VfilC = 0.0004 ; Fraction filtrate compartment volume (10% of kidney volume)

VGC = 0.0171 ; Fraction gut volume

VPlasC = 0.0428 ; Fraction plasma volume (58% of blood)

Htc = 0.44 ; hematocrit

;for dermal exposure

SkinTarea = 9.1*((BW*1000)**0.666) ; Total area of skin (cm^2)

Skinthickness = 0.1 ; Skin thickness (cm)

; Chemical-specific parameters (PFOS)

Tmc = 3500 ; Maximum resorption rate

Kt = 23.0 ; Resorption affinity

Free = 0.025 ; Free fraction of PFOS in plasma

PL = 3.72 ; Liver/blood partition coefficient

PF = 0.14 ; Fat/blood partition coefficient

PK = 0.8 ; Kidney/blood partition coefficient

PSk = 0.29 ; Skin/blood partition coefficient

PR = 0.2 ; Rest of the body/blood partition coefficient

PG = 0.57 ; Gut/blood partition coeff.

kurinec = 0.001 ; urinary elimination rate constant (/h/kg^-0.25); estimated from Harada, et al 2005

kurine = kurinec*BW**(-0.25)

; Free fraction of chemical in tissues

```
FreeL = Free/PL                 ;liver

FreeF = Free/PF                 ;fat

FreeK = Free/PK                 ;kidney

FreeSk = Free/PSk               ;skin

FreeR = Free/PR                 ;rest of tissues

FreeG = Free/PG                 ;gut

; Exposure parameters

tchng = 114374     ; fino al febbraio 2014;

T3 = 145910 ; questo è il tempo fino a ottobre 2017

T4 = 146642 ; tempo al prelievo, 2018;

;turn dose on/off

DoseOnpozzo = IF time<tchng THEN 1.0 ELSE 0.0

DoseOn2017 = IF time>T3 THEN 1.0 ELSE 0.0

; Dermal exposure

Dermconc = 0.0                                  ; Dermal concentration
(mg/mL)

Dermvol = 0.0                                   ; Dermal exposure volume
(mL)

Dermdose = Dermconc*Dermvol*1000                ; (ug)

Skinarea = 5                                    ; Exposed area on skin (cm^2)

; Oral exposure
```

; Oral uptake (ug/kg/day), MB average values (average of LB and UB in the ISS document)

Oraldose = 0.0427 ; PFAS average total daily intake through food estimated for the women living in Lonigo, ;µg/day (= PTIFn ;/1000)

Drinkconcpozzo = 0.037 ; (ug/L) PFAS concentration in groundwater in the municipality of Lonigo, (µg/L)

Drinkconcrete= 0.012 ; (ug/L) PFAS concentration in tap water in the municipality of Lonigo (= PCTWm ;/1000) for all the time period P1

Drinkconcretedal2017 = 0.007 ; difference in PFAS concentration in tap water between P1 and P2 (ug/L)

Drinkrate = 1.50/3 ; $CWIR_{womenLonigo}$ = average contaminated water daily intake rate for the women in Lonigo ;(L/day)

Drinkdosepozzo = Drinkconcpozzo*Drinkrate ; PFAS daily intake through groundwater (ug/day)

Drinkdoserete = Drinkconcrete*Drinkrate ; PFAS daily intake through tap water during the time period P1 ;(ug/day)

Drinkdoserete2017 = Drinkconcretedal2017*Drinkrate ; difference in PFAS daily intake through tap water ;between P1 and P2 (ug/day)

Tinput = 24 ; duration of dose (h), to spread the exposure over a period of 24 hours

;oral

Inputcibo = IF MOD(time,24) <=Tinput THEN Oraldose/Tinput ELSE 0.0 ; input created to describe the ;PFAS exposure through food (µg/h)

;drinking water

Inputpozzo = IF MOD(time,24) <= Tinput THEN Drinkdosepozzo/Tinput ELSE 0.0 ; input created to describe ;the exposure to PFAS in groundwater (µg/h)

Inputrete1 = IF MOD(time,24) <= Tinput THEN Drinkdoserete/Tinput ELSE 0.0 ; input created to describe the ;exposure to PFAS in tap water during the time period P1 (µg/h)

Inputrete4 = IF MOD(time,24) <= Tinput THEN Drinkdoserete2017/Tinput ELSE 0.0 ; input created to ;describe the difference in PFAS exposure in tap water between the time periods P1 and P2 (µg/h)

; Scaling parameters

QC = QCC*BW**0.75 ; Cardiac output (L/h)

QCP = QC*(1-Htc) ; adjust for plasma flow

QL = QLC*QCP ; Plasma flow to liver (L/h)

QF = QFC*QCP ; Plasma flow to fat (L/h)

QK = QKC*QCP ; Plasma flow to kidney (L/h)

Qfil = 0.2*QK ; Plasma flow to filtrate compartment (L/h); 20% of QK

QG = QGC*QCP ; Plasma flow to gut (L/h)

QSk = IF Dermconc >0.0 THEN QSkC*QCP*(Skinarea/SkinTarea) else 0.0 ;plasma flow to skin

QR = QCP - QL - QF - QK - Qfil - QG -QSk ; Plasma flow to rest of the body (L/h)

Qbal = QCP - (QL+QF+QK+QFil+QG+QSk) ; balance check--better be 0

VL = VLC*BW ; Liver volume (L)

```
VF = VFC*BW                    ; Fat volume (L)

VK = VKC*BW                    ; Kidney volume (L)

Vfil = VfilC*BW                ; Fitrate compartment volume (L)

VG = VGC*BW                    ; Gut volume (L)

VPlas = VPlasC*BW              ; Plasma volume (L)

VSk = (Skinarea*Skinthickness)/1000                    ; Skin volume (L)

VR = 0.84*BW - VL - VF - VK - Vfil - VG - VPlas - VSk          ; Rest of the
body volume (L)

Vbal = (0.84*BW)-(VL+VF+VK+VFil+VG+VPlas+VSk)          ; Balance check-
-better be 0

Tm = Tmc*BW**0.75              ;transporter maximum


;>>>>>>>>>>>>>>>>>>>> Model equations
<<<<<<<<<<<<<<<<<<<<<<<<<<<<

; Plasma compartment

APlas' =
QF*CF*FreeF+(QL+QG)*CL*FreeL+QR*CR*FreeR+QSk*CSk*FreeSk+QK*CK
*FreeK-QCP*CA*Free

init APlas = 0.0

CAFree = APlas/VPlas                   ; free concentration of PFOS in plasma in
ug/L (ng/mL)

CA = CAfree/Free                       ; total concentration in plasma

; Gut compartment
```

AG' = QG*(CA*Free-CG*FreeG) + Inputcibo + Inputpozzo*DoseOnpozzo + Inputrete1- Inputrete4*DoseOn2017

init AG = 0.0

CG = AG/VG ; Concentration in gut (ug/L)

CVG = CG/PG ; Concentration leaving gut (ug/L)

; Liver compartment

AL' = (QL*(CA*Free))+(QG*CG*Freeg) - ((QL+QG)*CL*FreeL) ;Rate of change in liver (ug/h)

init AL = 0.0

CL = AL/VL ; Concentration in liver (ug/L)

CVL = CL/PL ; Concentration leaving liver (ug/L)

; Fat compartment

AF' = QF*(CA*Free-CF*FreeF) ; Rate of change in fat (ug/h)

init AF = 0.0

CF = AF/VF ; Concentration in fat (ug/L)

CVF = CF/PF ; Concentration leaving fat (ug/L)

; Kidney compartment

AK' = QK*(CA*Free-CK*FreeK) + Tm*Cfil/(Kt+Cfil) ; Rate of change in kidneys (ug/h)

init AK = 0.0

CK = AK/VK ; Concentration in kidneys (ug/L)

CVK = CK/PK ; Concentration leaving kidneys (ug/L)

; Filtrate compartment

Afil' = Qfil*(CA*Free-Cfil) - Tm*Cfil/(Kt+Cfil) ; Rate of change in filtrate compartment (ug/h)

init Afil = 0.0

Cfil = Afil/Vfil ; Concentration in filtrate compartment (ug/L)

; Storage compartment for urine

;Adelay' = Qfil*Cfil-kurine*Adelay

;init Adelay = 0.0

; Urine

;Aurine' = kurine*Adelay

Aurine' = Qfil*Cfil - kurine*Aurine

init Aurine = 0.0

; Skin compartment

ASk' = QSk*(CA*Free-CSk*FreeSk) ; Rate of change in skin (ug/h)

init ASk = DermDose

CSk = ASk/VSk ; Concentration in skin compartment (ug/L)

CVSk = CSk/PSk ; Concentration leaving skin compartment (ug/L)

; Rest of the body

AR' = QR*(CA*Free-CR*FreeR) ; Rate of change in rest of the body (ug/h)

init AR = 0.0

CR = AR/VR ; Concentration in rest of the body (ug/L)

CVR = CR/PR ; Concentration leaving rest of the body (ug/L)

Display TmC,Kt,Free,PL,PK,PF,PR,PSK,PG,tchng,BW,QCC,QFC,QLC,QKC,QGC,QSkC, VFC,VLC,VKC,VGC,VFilC,VPlasC,Drinkrate

Display CG, CL, CF,CK,CA,Cfil,CR ;for plotting

".

ANNEX B

R SCRIPTS FOR SHAPIRO AND WILCOXON TESTS

“

```
rm(list=ls())
cartella<-"C:\\Users\\sgiannini\\Documents\\tesi dottorato lorenzo\\"
file_180_obs<-read.csv2(paste0(cartella,"completo.csv"))
file_sarego<-read.csv2(paste0(cartella,"Sarego.csv"))
hist(subset(file_180_obs,Area=="A")$misurati)
hist(subset(file_180_obs,Area=="A")$stimati)
hist(subset(file_180_obs,Area=="B")$misurati)
hist(subset(file_180_obs,Area=="B")$stimati)
# ####### data seem not normally distributed


shapiro.test(subset(file_180_obs, Area == "A")$misurati)
shapiro.test(subset(file_180_obs, Area == "A")$stimati)

shapiro.test(subset(file_180_obs, Area == "B")$misurati)
shapiro.test(subset(file_180_obs, Area == "B")$stimati)
# ####### Shapiro-Wilk null hypothesis (normality) rejected.


# ##################################################
# #######       Observed VS Predicted       #######
# ##################################################
```

```
selezione<-file_180_obs

tutti<-
data.frame(cbind(dato=c(rep("misurato",dim(selezione)[1]),rep("stimato",dim(selezi
one)[1])),

                    valore=c(selezione$misurati,selezione$stimati)))

tutti$valore<-as.numeric(tutti$valore)


test_MS <- wilcox.test(tutti$valore ~ tutti$dato)

test_MS


# Wilcoxon rank sum test with continuity correction

#

# data:  tutti$valore by tutti$dato

# W = 18766, p-value = 0.00506

# alternative hypothesis: true location shift is not equal to 0


# ####### null hypothesis rejected: data are significantly different between the two
groups


# ##################################################

# #######          [Area] A VS B          #######

# ##################################################


selezione<-file_180_obs[file_180_obs$Area=="A",]

area_A<-
data.frame(cbind(dato=c(rep("misurato",dim(selezione)[1]),rep("stimato",dim(selezi
one)[1])),

                    valore=c(selezione$misurati,selezione$stimati)))
```

```
area_A$valore<-as.numeric(area_A$valore)


test_MS_A <- wilcox.test(area_A$valore ~ area_A$dato)
test_MS_A


# Wilcoxon rank sum test with continuity correction
#
# data:  area_A$valore by area_A$dato
# W = 2263, p-value = 0.02138
# alternative hypothesis: true location shift is not equal to 0



selezione<-file_180_obs[file_180_obs$Area=="B",]
area_B<-
data.frame(cbind(dato=c(rep("misurato",dim(selezione)[1]),rep("stimato",dim(selezi
one)[1])),
                 valore=c(selezione$misurati,selezione$stimati)))
area_B$valore<-as.numeric(area_B$valore)


test_MS_B <- wilcox.test(area_B$valore ~ area_B$dato)
test_MS_B


# Wilcoxon rank sum test with continuity correction
#
# data:  area_B$valore by area_B$dato
# W = 7959.5, p-value = 5.459e-10
# alternative hypothesis: true location shift is not equal to 0
```

```
# ####### null hypothesis rejected for A-red area and B-red area
# ####### Greater differences are evident for the B-red area


# ##################################################
# #######           sex: [F - M]           #######
# ##################################################

selezione<-file_180_obs[file_180_obs$Sesso=="F",]
femmine<-
data.frame(cbind(dato=c(rep("misurato",dim(selezione)[1]),rep("stimato",dim(selezi
one)[1])),
                 valore=c(selezione$misurati,selezione$stimati)))
femmine$valore<-as.numeric(femmine$valore)

test_MS_F <- wilcox.test(femmine$valore ~ femmine$dato)
test_MS_F

# Wilcoxon rank sum test with continuity correction
#
# data:  femmine$valore by femmine$dato
# W = 3890.5, p-value = 0.4826
# alternative hypothesis: true location shift is not equal to 0


selezione<-file_180_obs[file_180_obs$Sesso=="M",]
maschi<-
data.frame(cbind(dato=c(rep("misurato",dim(selezione)[1]),rep("stimato",dim(selezi
one)[1])),
```

```
                valore=c(selezione$misurati,selezione$stimati)))
maschi$valore<-as.numeric(maschi$valore)

test_MS_M <- wilcox.test(maschi$valore ~ maschi$dato)
test_MS_M

# Wilcoxon rank sum test with continuity correction
#
# data:  maschi$valore by maschi$dato
# W = 5419.5, p-value = 4.709e-06
# alternative hypothesis: true location shift is not equal to 0

# ####### For men: null hypothesis rejected, data are significantly different between the two groups
# ####### For women: null hypothesis is not rejected, data are not significantly different between the two groups

# ######################################
# #######       Sarego, MS       #######
# ######################################

selezione<-file_sarego
sarego<-
data.frame(cbind(dato=c(rep("misurato",dim(selezione)[1]),rep("stimato",dim(selezione)[1])),
                valore=c(selezione$misurati,selezione$stimati)))
sarego$valore<-as.numeric(sarego$valore)

test_MS_sarego <- wilcox.test(sarego$valore ~ sarego$dato)
```

```
test_MS_sarego

# Wilcoxon rank sum test with continuity correction
#
# data:  sarego$valore by sarego$dato
# W = 447, p-value = 0.009321
# alternative hypothesis: true location shift is not equal to 0

# ####### null hypothesis rejected.

# ##################################################
# #######          Observed data          #######
# ##################################################

misurati_sesso<-file_180_obs[,2:3]

test_MS_sesso_misurati <- wilcox.test(misurati_sesso$misurati ~
misurati_sesso$Sesso)
test_MS_sesso_misurati

# Wilcoxon rank sum test with continuity correction
#
# data:  misurati_sesso$misurati by misurati_sesso$Sesso
# W = 2269, p-value = 5.598e-07
# alternative hypothesis: true location shift is not equal to 0

misurati_area<-file_180_obs[,c(1,3)]
```

```
test_MS_area_misurati <- wilcox.test(misurati_area$misurati ~ misurati_area$Area)
test_MS_area_misurati

# Wilcoxon rank sum test with continuity correction
#
# data:  misurati_area$misurati by misurati_area$Area
# W = 5360, p-value = 2.46e-05
# alternative hypothesis: true location shift is not equal to 0

# ####### null hypothesis rejected both for sex and area in the observed data.

”

rm(list=ls())

cartella<-"C:\\Users\\sgiannini\\Documents\\tesi dottorato lorenzo\\"

sesso<-read.csv2(paste0(cartella,"confronto_sesso.csv"))

area<-read.csv2(paste0(cartella,"confronto_area.csv"))

hist(subset(area,area=="A")$pfos)
hist(subset(area,area=="B")$pfos)

hist(subset(sesso,sesso=="maschi")$pfos)
hist(subset(sesso,sesso=="femmine")$pfos)
```

```
# ####### data don’t seem normally distributed

shapiro.test(subset(area,area=="A")$pfos)
#
# Shapiro-Wilk normality test
#
# data:  subset(area, area == "A")$pfos
# W = 0.8151, p-value = 2.386e-08
#
shapiro.test(subset(area,area=="B")$pfos)
#
# Shapiro-Wilk normality test
#
# data:  subset(area, area == "B")$pfos
# W = 0.7693, p-value = 2.017e-11
#

shapiro.test(subset(sesso,sesso=="maschi")$pfos)
#
# Shapiro-Wilk normality test
#
# data:  subset(sesso, sesso == "maschi")$pfos
# W = 0.85246, p-value = 6.713e-08
#
shapiro.test(subset(sesso,sesso=="femmine")$pfos)
#
# Shapiro-Wilk normality test
```

```
#
# data:  subset(sesso, sesso == "femmine")$pfos
# W = 0.65292, p-value = 2.383e-13

# ####### Shapiro-Wilk test: null hypothesis (= data normally distributed) rejected

# ####################################
# #######        Sex        #######
# ####################################

test_sesso <- wilcox.test(sesso$pfos ~ sesso$sesso)
test_sesso

# Wilcoxon rank sum test with continuity correction
#
# data:  sesso$pfos by sesso$sesso
# W = 2406, p-value = 4.017e-06
# alternative hypothesis: true location shift is not equal to 0

# ####### Null hypothesis rejected

# ##########################################
# #######          Area          #######
# ##########################################

test_area <- wilcox.test(area$pfos ~ area$area)
test_area
```

Wilcoxon rank sum test with continuity correction

data: area$pfos by area$area

W = 5038, p-value = 0.00104

alternative hypothesis: true location shift is not equal to 0