PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING OF PCDD/FS AND PFASS IN HUMANS.

Francesc Fabrega Bonadona

Dipòsit Legal: T 1660-2014

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DOCTORAL THESIS

Supervised by

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UNIVERSITAT ROVIRA I VIRGILI

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CERTIFY

That the present doctoral thesis, entitled “Physiologically-based pharmacokinetic (PBPK) modeling of PCDD/Fs and PFASs in humans” presented by Francesc Fàbrega for the award of the degree of Doctor, has been carried out under our supervision at the Department of Chemical Engineering at “Rovira i Virgili” University, and that it fulfills all the requirements to be eligible for the European Doctorate Label.

Tarragona, October 2014

Dr. Martí Nadal Dr. Marta Schuhmacher

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This thesis is dedicated to my beloved Babaji, who guided all my steps in this thesis.

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Abbreviations

AhR: Aryl hydrocarbon receptor

ANN: Artificial neural network

ATSDR: Agency for Toxic Substances and Disease Registry

BCF: Bioconcentration factor

BW: Body weight

CA: Concentration addition

COMMPS: Combined monitoring-based and modeling-based priority

EC: European Commission

ED: Endocrine disruptors

EU: European Union

HI: Hazard Index

HW: Hazardous waste

HWI: Hazardous waste incinerator

IA: Independent action

IARC: International Agency for Research on Cancer

IRICAP: Integrated risk index of chemical aquatic pollution

Kow: Octanol-water partition coefficient

LOD: Limit of detection

LOQ: Limit of quantification

MSWI: Municipal solid waste incinerator

NATO: North Atlantic Treaty Organization

OHC: Organo halogen compound

PBT: Persistence, bioaccumulation and toxicity

PCDDs: Polychlorinated dibenzo-*p*-dioxins

PCDFs: Polychlorinated dibenzofurans

PD: Pharmacodynamic

PK: Pharmacokinetic

PBPK: Physiologically-based pharmacokinetic models

PFASs: Perfluoroalkyl substances

PFOA: Perfluorooctanoic acid

PFOS: Perfluorooctane sulfonate

POP: Persistent organic pollutant

QSAR: Quantitative structure-activity relationship

REACH: Registration, evaluation and authorisation of chemicals

RfD: Reference dose

SC: Stockholm Convention

SOM: Self-organizing maps

TEF: Toxic equivalency factor

TEQ: Toxic equivalent

U.S.EPA: U. S. Environmental Protection Agency

WHO: World Health Organization

CONTENTS

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SUMMARY

Due to the large amount of pollutants produced by the chemical industry, the European Union (EU) approved in 2007 the regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). This aims at improving the identification and knowledge of up to 30,000 existing chemical substances. Among them, 1,500 compounds are considered “substances of very high concern” due to their environmental and human health risk. Animal experimentation is the classical approach to test the harmful effects of chemicals. However, due to the huge amount of chemicals, expensive cost and ethical reasons, alternatives testing methods are being implemented nowadays. According to REACH, the experimentation in animals should be used as a “last resort”. In this framework, *in silico* computational methods are promising tools, being physiologically-based pharmacokinetic (PBPK) models specially relevant. PBPK models are mathematical representations of the human body, where human tissues are considered as compartments linked by the blood flow. These tools allow estimating the concentration and behaviour of environmental contaminants in the human body. The use of PBPK models is still incipient in human health risk assessment. Therefore, there is a lack of validated PBPK models. Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and perfluoroalkyl substances (PFASs) are two of the most harmful groups of chemicals for the human health. These environmental pollutants are well known for their high persistence, bioaccumulation and toxicity (PBT), exhibiting also a long-range transport capacity. Furthermore, PCDD/Fs as well as perfluorooctane sulfonic acid (PFOS), the mostly studied PFAS, are included in the list of persistent organic pollutants (POPs) in the Stockholm Convention.

The objective of the present thesis was to develop a PBPK model to study the behaviour and concentration of POPs in human tissues. PCDD/Fs and PFASs, two notably different POPs, were selected. Since late 1970s, PCDD/Fs have been posing special concern for the regulatory agencies. In contrast, PFASs have not been so extensively studied yet. To identify the potential risk of PFASs, a PBT-based ranking was developed to confirm their potential human health risk in front of other environmental contaminants. Because of their easy pharmacokinetics, the PBPK model was firstly developed for PCDD/Fs. Afterwards, the model was adapted to estimate the accumulation of PFASs in human tissue.

In Chapter I, a ranking to prioritize the risk of chemical compounds in river water was developed. Up to 205 compounds, including pharmaceutic compounds, illicit drugs,

endocrine disruptors, UV filters, pesticides as well as PFASs, were selected. The index was based on the PBT properties of the chemicals and the water concentration of 4 Spanish rivers. Self-organizing maps (SOM) were used to cluster the compounds according to their PBT characteristics, and the final result was a hazard index (HI), where pollutants were prioritized according to their harmful effects. With a HI of 5.58 over 10, PFASs were identified among the most harmful group of compounds for the human health. Afterwards, an integrated risk index for the chemical aquatic pollution (IRICAP) was obtained by multiplying the HI of each compound by its concentration found in river water. The IRICAP was applied to 77 sampling points located in the basins of Ebro, Júcar, Guadalquivir and Llobregat.

Chapter II and III developed the PBPK model for PCDD/Fs, highlighting their presence in blood and adipose tissue, respectively. The main characteristics of the PBPK model were: 1) PCDD/Fs are not metabolized in the human body, 2) the ingestion may be considered only through food and water intake, and 3) the elimination occur only through feces. The simulated compartments were blood, muscle, skin, richly perfused, fat, kidney, and liver. Experimental data on PCDD/F levels in blood and adipose tissue samples from the adult population in Tarragona County (NE of Spain), were collected. The model was validated comparing the simulated and experimental results, which were highly coincident. In plasma, the modelled mean level of PCDD/Fs was 7.95 pg I-TEQ/g lipid, while the experimental concentration was 6.18 pg I-TEQ/g lipid. In adipose tissue, the simulated and experimental levels of PCDD/Fs were also of the same order of magnitude (4.77 vs 11.15 pg I-TEQ/g lipid). Due to the reasonable coincidence between the experimental and simulated results, the PBPK model developed was considered as validated. The PBPK model was simulated in four temporal scenarios, for which experimental data were available: 1998, 2002, 2007, and 2012.

Chapter IV adapted the previously developed PBPK model to perfluorooctanoic acid (PFOA) and PFOS. In this case, the elimination mechanism was considered to occur only through urine by a mechanism of resorption, according to chemicals in urine are resorbed back to plasma through a saturable process. Furthermore, the binding to plasma albumin was also considered in the model equations. The PBPK model simulated the concentration of PFOS and PFOA in humans for a non-occupationally exposed population in Tarragona County. Nine body compartments were considered: plasma, liver, brain, lungs, kidney, gut, filtrate, rest of the body, and fat. Data of PFOS and PFOA in 5 autopsy tissues (liver, brain, lung, kidney and bone marrow) were used to validate the PBPK model. In liver, the target tissue of PFOS and PFOA, the simulated

concentration of PFOA was 3.33 ng/g, while the experimental concentration was 13.6±35.2 ng/g. For PFOS, the concentrations found were 36.4 ng/g and 102.3±122.9 ng/g according to simulated and experimental results, respectively. Because simulated concentrations were within the range of the measured levels of PFOS and PFOA, the model was considered as validated.

Chapter V extended the previous model to other 9 PFASs. The model followed the same structure, and only parameterization data were modified according on each specific compound. Values of partition coefficient (Pks), elimination constants and binding to albumin protein were assessed by using experimental data of biological tissues from residents in Tarragona County. Parameterization data were assessed by fitting the PBPK simulation with the experimental data. For validation purposes, the PBPK model was applied in a case-study. Andøya Island (Norway) was used as a scenario because data on human intake and plasma concentration of PFASs were available for a non-occupationally exposed population. Validation consisted on comparing the range of the simulations and the experimental results. The model was considered validated for PFHxS, PFOS, PFHpA, PFOA and PFNA, due to the important coincidence between the ranges of the simulated and measured concentrations. For instance, for PFNA the simulated concentration ranged from 0.02 to 1.14 ng/g, whereas the mean experimental concentration was 0.95 ng/g, ranging from 0.26 to 2.9 ng/g.

Given the importance of uncertainty, Chapter VI was focused on quantifying the influence of uncertainty in the PBPK model for PFOS and PFOA. The uncertainty analysis was focused on the parametric uncertainty, which is the study of the error associated to the experimental parameters used in the PBPK model. The objective of this chapter was to study the validation process of PBPK models and to mathematically assess their validity. Therefore, the minimum and maximum concentrations for the PBPK simulations were assessed by using the range of data of the most uncertain parameters (elimination parameters, protein binding, and daily intake) to obtain a range of simulated concentrations. The model was considered as visually validated if the experimental results were in the same range of the simulated range. Moreover, the validity of the PBPK model was mathematically studied by using the Student's *t*-test. Finally, the visual and the statistical validation were compared to analyze the influence of the uncertainty in the process of model validation. For few tissues, the model was not statistically validated, indicating that the inclusion of the statistical analysis should be incorporated in the development of PBPK models to ensure the validity of the final outcomes.

The here developed PBPK model has been demonstrated to be a reliable tool to simulate the levels of PCDD/Fs and PFASs in human tissues. The simulations were highly coincident with the experimental results for most of the PCDD/F congeners, whereas for PFASs, the simulated and experimental concentrations were also of the same order of magnitude. Moreover, the statistical study of the uncertainty in the PBPK models was incorporated into the process of model development to ensure the validity of the PBPK models. As a general conclusion, PBPK models are not only a promising tool, but also a reality to simulate the concentrations of environmental pollutants in human tissues, for their subsequent use in human health risk assessment.

RESUM

Degut a la gran quantitat de productes contaminants produïts per la indústria química, la Unió Europea va aprovar a l'any 2007 la regulació sobre registració, avaluació, autorització i restricció de productes químics (REACH), amb l'objectiu de millorar la identificació i coneixement de més de 30,000 compostos químics. Entre aquests, 1,500 son considerats "compostos de molt alt perill" degut al seu risc per la salut humana i pel medi ambient. L'aproximació clàssica per mesurar els efectes perjudicials sobre la salut dels compostos químics és l'experimentació en animals. No obstant, degut a la gran quantitat de compostos químics que es necessiten estudiar i degut al seu cost i raons ètiques, s'estan implementant mètodes alternatius. Segons REACH, l'experimentació en animals hauria de ser l'"última alternativa". En aquest context, les eines computacionals o *in silico* son una bona alternativa. Entre elles, es troben els models farmacocinètics o PBPK (·physiologically-based pharmacokinetic models"). Els models PBPK són representacions matemàtiques del cos humà on els teixits es consideren compartiments units pel flux de sang. L'ús de models PBPK és encara incipient en l'avaluació de risc per a la salut, i per tant hi ha una falta de models per calcular la concentració i el comportament de contaminants ambientals en el cos humà. Les dibenzo-*p*-dioxines policlorades i dibenzofurans policlorats (PCDD/Fs) i compostos perfluorats (PFASs), són dos dels grups de contaminants més perillosos per a la salut humana. Aquest compostos són ben coneguts per la seva alta persistència, bioacumulació i toxicitat (PBT), a més d'una alta capacitat pel transport a llarga distància. A més a més, les PCDD/Fs i l'àcid perfluorooctà sulfònic (PFOS), que és el PFASs més estudiat, estan inclosos a la llista de compostos orgànics persistents (COPs) en la Convenció d'Estocolm.

L'objectiu de la present tesis va ser el desenvolupar d'un model PBPK per estudiar el comportament i concentració de compostos orgànics persistents (COPs) en teixits humans. Es van seleccionar dos grups de compostos notablement diferents, com son els PCDD/Fs i PFASs. Des de finals dels 70, les PCDD/Fs han tingut una preocupació especial per les agencies reguladores. Però, per altra banda, els PFASs encara no han estat gaire estudiats. Per conèixer amb exactitud la perillositat dels PFASs es va desenvolupar un índex de risc humà enfront a contaminants ambientals. Després, el model PBPK va ser primerament desenvolupat per PCDD/Fs degut a la seva relativament simple farmacocinètica en el cos humà. A continuació, el model es va adaptar per estimar l'acumulació de PFASs en teixits humans.

En el Capítol I es va desenvolupar un índex de risc per prioritzar compostos químics en aigua fluvial. Es van estudiar més de 205 compostos incloent farmacèutics, drogues, disruptors endocrins, filtres ultraviolats, pesticides i PFASs. L'índex es va basar en les propietats PBT dels químics i en la concentració en aigua en 4 conques fluvials Espanyoles. Els mapes autoorganitzats (SOM) van ser utilitzats per agrupar els compostos segons les seves característiques PBT. El resultat final va ser un índex de perillositat (HI), on els compostos es van prioritzar en funció dels seus efectes nocius. Els PFASs es van identificar entre els compostos més perillosos per a la salut humana amb un HI de 5.58 sobre 10. A continuació, es va calcular l'índex de risc integrat per la contaminació aquàtica (IRICAP) multiplicant el HI de cada compost per la seva concentració en aigua. L'IRICAP es va aplicar a 77 punts localitzats a l'Ebre, Xúcar, Guadalquivir i Llobregat.

Els Capítols II i III van desenvolupar un model PBPK per PCDD/Fs en sang i en teixit adipós, respectivament. Les principals característiques del model PBPK van ser: 1) els compostos PCDD/Fs no es metabolitzen en el cos humà, 2) la ingestió es va considerar exclusivament per ingestió d'aliments i aigua, 3) l'eliminació és exclusivament per femta. Els compartiments estudiats van ser la sang, múscul, pell, grassa, ronyó, fetge i resta del cos. En aquest estudi, les dades sobre la població adulta es van extreure a l'àrea de Tarragona (NE d'Espanya). El model es va validar comparant dades simulades i experimentals. Es va trobar una gran similitud entre les simulacions i les dades experimentals. En plasma, les dades del model per PCDD/Fs van ser 7.95 pg I-TEQ/g greix, mentre les concentracions experimentals van ser de 6.18 pg I-TEQ/g greix. En teixit adipós els valors simulats i experimentals van ser igualment del mateix ordre de magnitud (4.77 vs 11.15 pg I-TEQ/g greix). Degut a la raonable coincidència entre els resultats experimentals i simulats, el model PBPK es va considerar com a validat per simular les concentracions de PCDD/Fs en sang i en greix per població no ocupacional. Les simulacions amb el model PBPK es van calcular en 4 escenaris on hi havia dades experimentals disponibles: 1998, 2002, 2007 i 2012.

El Capítol IV va adaptar el model PBPK desenvolupat prèviament, a àcid perfluorooctanoic (PFOA) i a PFOS. En aquest cas el mecanisme d'eliminació es va considerar només a través de l'orina, mitjançant un procés de reabsorció, on des del plasma els compostos es reabsorveixen a l'orina, seguint un procés saturable. A més, es va considerar que hi havia una forta unió a l'albúmina del plasma. El model PBPK va simular la concentració de PFOA i PFOS per població no ocupacional de l'àrea de Tarragona. Els teixits que es van estudiar van ser plasma, fetge, cervell, pulmons, ronyó,

estomac, veixiga, resta del cos i teixit adipós. Per validar el model es van utilitzar dades de teixits humans en autòpsia. En el fetge, que és l'òrgan diana per PFOA i PFOS, la concentració simulada de PFOA va ser de 3.33 ng/g, mentre que la concentració experimental va ser de 13.6±35.2 ng/g. Per PFOS les concentracions van ser de 36.4 ng/g i de 102.3±122.9 ng/g, per valors simulats i experimentals, respectivament. Degut a la coincidència entre els valors simulats i els rangs dels valors experimentals, el model es va considerar com a validat.

En el Capítol V es va estendre el model previ a 9 compostos PFASs més. El model va seguir la mateixa estructura i només les dades paramètriques es van modificar en funció del compost. Els coeficients de partició (Pks), les constants d'eliminació i la unió a l'albúmina es va calcular utilitzant dades experimentals de residents de l'àrea de Tarragona. Les dades paramètriques es van calcular ajustant les simulacions del model PBPK amb les dades experimentals. A continuació, per tal de validar el model, el model PBPK es va aplicar en un cas d'estudi. L'illa d'Andøya (Noruega) es va utilitzar com escenari degut a la disponibilitat de dades de concentracions en plasma i d'ingesta en població no ocupacional. El model es va validar per simular les concentracions de PFASs en teixits humans per població sota exposició no ocupacional. Per considerar el model com a vàlid, es va considerar que el rang entre els valors simulats i experimentals havien de ser coincidents. Per exemple per PFNA, la concentració simulada va ser d'entre 0.02 i 1.14 ng/g (mínim i màxim), i el valor experimental va ser de 0.95 ng/g (rang entre 0.26 i 2.9 ng/g). El model es va considerar com a validat per PFHxS, PFOS, PFHpA, PFOA i PFNA degut a la coincidència entre el rang dels valors simulats i els valors experimentals

Donada la gran importància de la incertesa, en el Capítol VI es va estudiar la incertesa en els models PBPK per PFOS i PFOA. L'anàlisi de incertesa es va enfocar en la incertesa paramètrica, que és l'estudi de l'error associat als paràmetres experimentals utilitzats en el model PBPK. Per tant, l'objectiu d'aquest capítol és l'estudi del procés de validació dels models PBPK i calcular matemàticament la seva validesa. Conseqüentment, els valors màxim i mínim dels valors simulats pel model PBPK es van calcular utilitzant el rang de concentració dels paràmetres amb més incertesa (eliminació, ingesta i unió amb proteïnes), per obtenir un rang de concentracions simulades. El model es va considerar com a vàlid quan els resultats experimentals estaven dins del rang dels resultats simulats. A més a més, la validesa del model PBPK es va estudiar utilitzant el test estadístic de la t de Student. Finalment les validacions visual i estadística es van comparar per analitzar la influència de la incertesa en el procés

de validació. Per alguns pocs teixits el model no es va poder considerar com a validat. És per això que la inclusió d'anàlisis estadístic hauria de ser incorporat en el desenvolupament dels models PBPK com una pràctica habitual per assegurar la validesa dels resultats finals.

El model PBPK ha demostrat ser una eina fiable per simular la concentració de PCDD/Fs i PFASs en teixits humans per població no ocupacional. Les simulacions de PCDD/Fs van ser altament coincidents amb els resultats experimentals per la major part del congéners, mentre que per PFASs les concentracions simulades i experimentals van ser raonablement coincidents. A més a més l'estudi estadístic de la incertesa en el model PBPK va ser incorporat en el procés de validació del model. Com a conclusió, els models PBPK son no només una eina prometedora, sinó una realitat per simular les concentracions de contaminants ambientals en teixits humans, pel seu posterior ús en l'avaluació de risc per a la salut humana.

INTRODUCTION

1. New computational methods: *In silico* tools

Classic approaches to estimate the toxicity of chemical compounds for human health have involved the usage of animal testing. However this is a relatively slow technique, subject to a big variability and expensive costs, besides obvious ethical reasons (Robson and Toscano, 2007). Furthermore there is a huge amount of chemicals which required to be tested, making terribly difficult their assessment by means of animal experimentation studies. The European Commission (EC) has estimated that over 30,000 chemicals in 11 years will be regulated under REACH (Registration, Evaluation and Authorisation of Chemicals). Given the impressive number of chemicals, new testing methods have emerged on powerful, reliable and cheaper alternatives. One of these are *in silico* methods, whose main objective is to reduce the usage of animal experimentation (Roncaglioni et al., 2013). *In silico* techniques may be either Expert Systems (ES) or Data Driven Systems (DDS) (Bakhtyari et al., 2013). ES attempts to emulate the decision making ability of the human experts, while DDS are methods to extract and predict models directly from experimental data. DDS vary in sophistication from simple methods (Read-across methods) to more complex methods (QSAR: Quantitative structure-activity relationship).

In silico techniques can extract information easier and quicker than with *in vivo* experiments. Mathematical models are relatively simple to build and with the additional advantage that validated models can be applied to a number of different hypothetical scenarios including new routes, dose, species and the involvement of new chemicals. *In silico* tools also allow to replacing *in vivo* animal studies by extrapolating results between species with a relatively high degree of reliability. Furthermore, modeling seems to be a better technique to extrapolate kinetic or toxicological results from animals to humans, thus increasing confidence in health risk assessment (Bouvier et al., 2007). Despite, modeling is a useful tool, it must be highlighted that they are an oversimplification of the real processes of the body, as was pointed out by Suresh Moolgavkar, and eminent professor of toxicology and risk assessment: “No model can be said to be ‘correct’. The role of any model is to provide a framework for viewing known facts and to suggest experiments”.

2. Physiologically-based pharmacokinetic (PBPK) models

2.1. Introduction and needs

Human health risk assessment is a fundamental step to estimate the impact of chemicals emitted to the environment, and to estimate the exposure. Dose and time of exposure have been considered traditionally as the most important parameters to perform evaluation of the human health risk. However, when a chemical enters the human body, there are complex processes which must be taken into account: absorption, distribution, metabolism and elimination (ADME). Physiologically-based pharmacokinetic (PBPK) models provide a tool to predict these ADME processes inside the body along time. Physiologically-based pharmacokinetic (PBPK) models are mathematical representations of the human body where the tissues are represented as compartments linked by the blood flow. The final result is a set of ordinary differential equations that can be solved using computational software. These models can be used for pharmacological research, health risk assessment and other fields in medicine.

2.2. History of PBPK models

The first PBPK model including a multi-compartmental structure and PK/PD data was introduced by Teodorell (1937). Unfortunately, the lack of mathematical tools to solve differential equations made PBPK models a difficult problem to solve in that time. Moreover, there was a lack of methods to predict the parameters needed for the PBPK model parameterization (Jones and Rowland-Yeo, 2013). The proliferation of PBPK models started in the 1980s due to the improvement of the new *in vitro* technologies, and computational tools (Edginton et al., 2008). Bischoff et al. (1971), published an early report that established the basis for the current PBPK models incorporating metabolism mechanisms in a PBPK model for methotrexate. Another seminal report of the early development of PBPK modeling was developed by Ramsey and Andersen (1984) that applied a PBPK model to assess styrene in rats for several routes of administration. Since the eighties, the PBPK model started to proliferate increasing its complexity and being used in pharmaceutical research and in environmental risk assessment. Over the last two decades, the number of scientific publications about PBPK modeling has gradually increased, demonstrating its utility in drug development and risk analysis (Huang et al., 2013) (Figure 1).

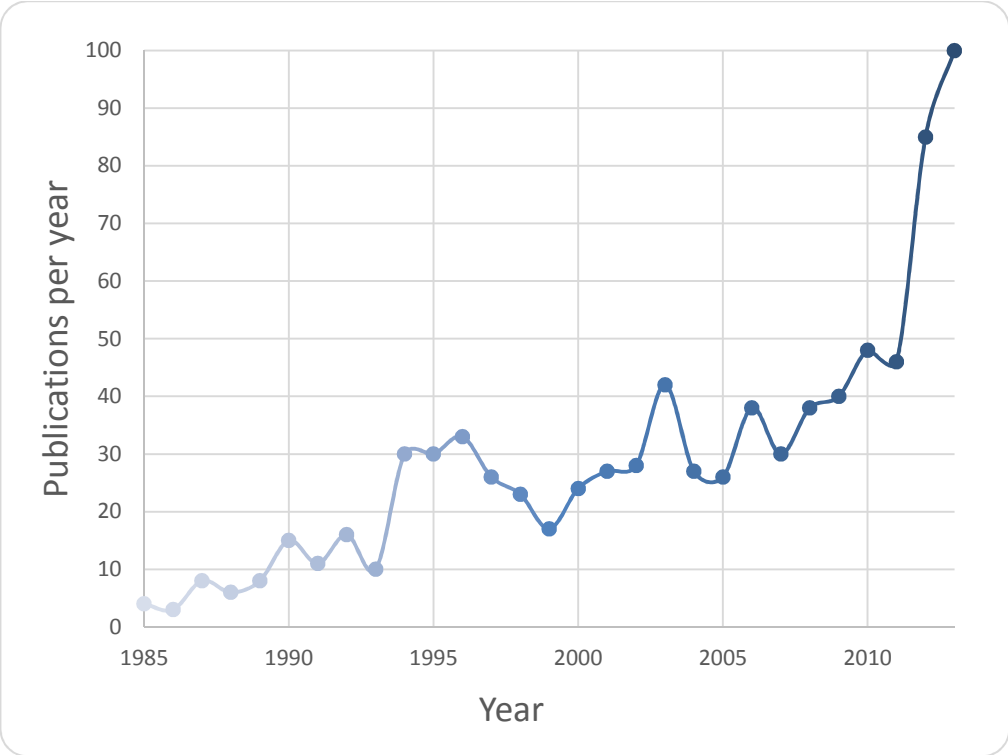


Fig. 1. Annual rate of publications whose title contains the phrase "Physiologically-based pharmacokinetic"; Source: ISI Web of Knowledge.

2.3. Development of PBPK models

The development of PBPK models involves three main steps: 1) definition of the model, 2) data collection and 3) mathematical simulation (Figure 2). Subsequently, the model is compared with experimental data available for calibration and validation purposes, and the model is finally modified according to new suppositions and presumptions.

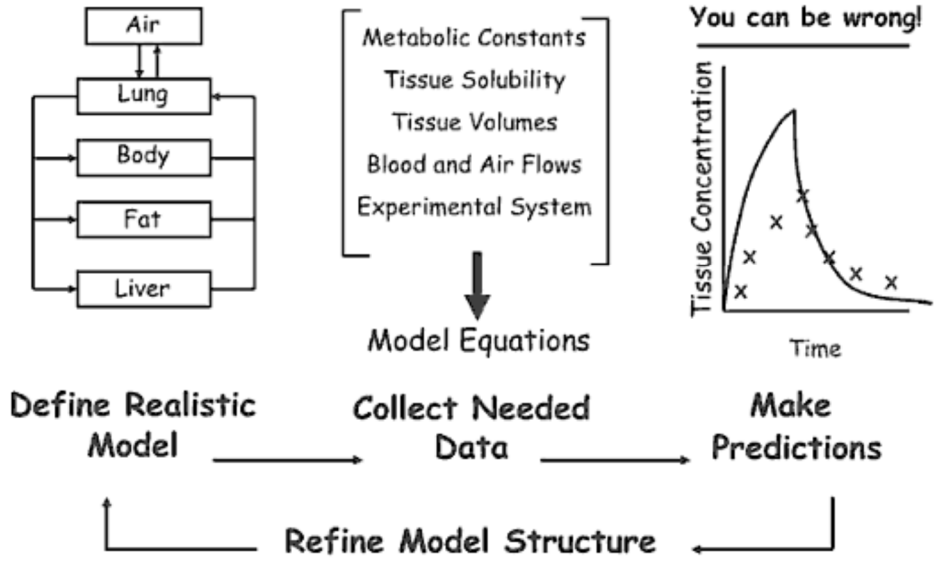


Fig. 2. Steps on the PBPK model development (Reddy et al., 2005).

The first step on the PBPK model building is to identify the organs or tissues involved in the ADME processes. Models generally simplify the biological complexity by subdividing the body in discrete compartments (Bouvier et al., 2007). The construction of the model can be done by considering previously available information about the distribution of the compound inside the body, and the biochemical information on chemicals metabolism. For instance, if a compound is strongly lipophobic, it is senseless to include fat tissues. Realism and simplification must be balanced.

Each organ or tissue is considered a compartment, characterized by its own initial concentration, tissue volumes, solubility, metabolic constants, etc. The different tissues are connected by the arterial and venous blood flow. To maintain the mass balance, the sum of arterial blood flows must to be equal to the venous flow. It is also important to distinguish between interstitial and intracellular space because the compartments are separated from membranes that act as a barrier with a limiting distribution (permeation limiting kinetics), and this distribution can be a limiting step (von and Huisinga, 2007). Usually, metabolism is considered only in liver, although this may also occur in lungs, kidney or gut (Beaudouin et al., 2010). The administration of the compound can be by gastric intake, dermal exposure or inhalation, and therefore all the possible human exposures can be simulated (Di Muria et al., 2010). A model can be as complex as the modeler wants, including all the organs of the body, but the simplicity of the model can avoid unnecessary waste time. In this first step, an overestimation or oversimplification is possible, becoming one of the most problematic steps. If the model does not run well,

a new adjustment will be necessary in the future. In this way, models are useful not only to simulate the behaviour of a chemical, but also to determine the metabolic and biochemical processes inside the human body (Edginton et al., 2008).

Once the conceptual model is created, the next step is to make a mathematical representation of each compartment or tissue. Generally, mass-balance ordinary differential equations are used to explain the change of a chemical in a tissue over the time, describing the transfer between compartments, as well as metabolism and excretion processes. The PBPK compartments are usually defined as a well-stirred tank or two-subcompartment tissues, being defined by flow-limited and permeability-limited equations (Thompson and Beard, 2011; Thompson et al., 2012). These equations establish the rate at which molecules enter and leave a tissue, and the speed of metabolism in the same tissue. Once a set of equations is obtained, the resolution is done by specific computational software (e.g., Berkley Madonna, AcslX) or by programming the equations with generic mathematical programs (e.g., Matlab, Fortran 95) (Schmitt and Willmann, 2004). The building of the model also involves the collection of parametric data. Pharmacokinetic data are available in medical literature, where have been compiled (Brown et al., 1997; Price et al., 2003). In turn, information can be calculated through different algorithms (Peyret et al., 2010; Poulin and Theil, 2009). Sometimes there exist gaps in human body data, and then extrapolation from experimentation on animal data is necessary. There are three kinds of parameters that are necessary to build PBPK models: physiological (e.g., ventilation rate, body weight, blood rate), thermodynamic (e.g., tissue partition coefficients), and biochemical (e.g., Km and Vmax) (Mumtaz, 2010).

Once the model is created, it can be compared with experimental data for calibration and validation purposes. If the model is not in agreement with the experimental observations, some possibilities arise: either a wrong estimation was done in some step of the process or some information had been misunderstood. Then, it is necessary to check which the mistake is and solve the problem or refine much more the model. Sometimes PBPK models are created as a hypothesis-testing in toxicology to conduct *in silico* experiments to know much more about some toxicological processes. Once a model is correctly validated, then it is ready to be used in other scenarios.

2.4. PBPK in human health risk assessment

Firstly, it is important to distinguish between tissue dose or target dose in front of the exposure dose. Exposure dose is the administrated dose via food intake, dermal exposure or air inhalation, while target dose is the dose in a tissue or organ after different ADME processes in the body (Leahy, 2006). Beneficial or adverse effects are not related only to the exposure but also with the different processes inside the body. Pharmacokinetic models allow to evaluating the chemical distribution and to making much more precise approximations than exposure-based models.

PBPK models vary in complexity according to their purpose, while there are not always necessary for human risk assessment. Traditionally, cancer and noncancer risk assessment rarely considers chemical-specific pharmacokinetic information, but PBPK models may improve risk assessment. There are four important uses of PBPK models in risk assessment (Clark et al., 2004; Clewell et al., 2002):

1) Cross-species extrapolation. Classical risk assessment assumes the same mode of action for both animals and humans. PBPK models may be used to extrapolate from animals to humans when the mode of action is different between species.

2) Cross-route extrapolation. It consists on extrapolating data between different exposure pathways. For instance, PBPK modeling can be used to assess the tissue-dose associated to a target tissue in animals through one route, and then assessing the equivalent exposure in human by using another exposure pathway.

3) Dose extrapolation. This incorporates the dose-response of a chemical. As for instance, the toxicity of the vinyl chloride is not lineal due to the saturation of its metabolism. By using PBPK models, the response in the metabolism may be estimated and the accuracy of the model improved.

4) Time extrapolation. PBPK modeling provides more accuracy to extrapolate exposure frames than other methods such as average daily dose. For instance, exposure to one chemical to a single dose is not the same than a repeated exposure to multiple low doses. Moreover, PBPK models may simulate the dosimetry under pregnancy, lactation and developmental age.

3. POPs

Persistent organic pollutants (POPs) are organic compounds intentionally or unintentionally released to the environment. They are characterized by a high persistence in the environment, high bioaccumulation in the human body, high toxicity for both humans and wildlife, as well as long-range transport capacity (Boethling et al., 2009). The Stockholm convention (SC) on POPs is a global treaty for the regulation of such compounds that was adopted in May 2001 and came into force in 2004. The SC aims to reduce or eliminate the use and production of these compounds. The initial SC list included 12 compounds, but this list has increased in recent years. Currently, up to 22 compounds are included.

4. PCDD/Fs

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are a group of halogenated organic compounds, constituted by two rings of benzene linked by two or one oxygen atoms, respectively. This family of compounds is generally known as “dioxins or furans”. It family includes 210 congeners, which depend on the position of the chlorine atom: 75 are dioxins (PCDD), and 135 furans (PCDFs). The general structure of dioxins and furans is depicted in Figure 3.

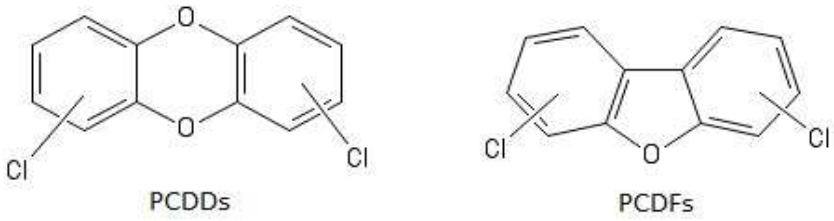


Fig. 3. Chemical structure of PCDDs and PCDFs.

4.1. Sources of exposure

PCDD/Fs are unintentional by-products of industrial and burning processes, being human activities the main emission source. In turn, this can be divided into regulated and non regulated or diffuse source (Fiedler, 1996; Kulkarni et al., 2008). The former group includes municipal solid waste incinerators, chemical waste incinerators, clinical waste incinerators, cement kilns, sinter plants and sewage sludge incinerators

(Alcock et al., 2001). Non regulated emissions come from traffic, domestic combustion and accidental fires. In the last decades, important legislation efforts to reduce the environmental emissions of PCDD/Fs have give place to a substantial reduction of the emissions and the atmospheric concentrations (Hassanin et al., 2006). Consequently, a reduction in human exposure and in body burdens of PCDD/Fs has also been observed (Nadal et al., 2013).

Scientific studies, have indicated that the main source of human exposure to PCDD/Fs is food ingestion, over the >95% of the total intake (Linares et al., 2010; Martí-Cid et al., 2008; Nadal et al., 2011). In general, food from animal origin contains more dioxins that the food coming from vegetal origin (Chan and Wong, 2013), while relatively high levels of PCDD/Fs have also been found in fish and seafood.

4.2. Toxicity

The toxicity of the different PCDD/F congeners may vary in a 1000-folder magnitude depending of the congener. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) is considered the most toxic of the dioxins congeners and a toxic factor of 1 has been assigned to this congener. All the other congeners of PCDD/Fs have a relative toxicity that ranges from 0 to 1 (Table 1). This scale of toxicity is called the Toxicological Equivalent Factor (TEF). It is agreed that the most toxic congeners for the human Health risk are those that contains Cl in the positions 2, 3, 7 and 8 in the ring (Linden et al., 2010). Up to 17 toxic congeners have been selected: 7 dioxins and 10 furans. For the remaining 193 PCDD/F congeners, the toxicity for the human health may be considered as negligible (TEF=0). The most and more extensively used TEF values for human toxicity are I-TEF and WHO-TEF. I-TEF was set up by NATO in 1989 and after that was extended and validated (Kutz et al., 1990). WHO-TEF where set up by the World Health Organization (WHO) in 1998, and reevaluated in 2005 (Van den Berg et al., 2005). I-TEF and WHO-TEF values are summarized in table 1.

Table 1. I-TEF and WHO-TEF valued for the 17 PCDD/Fs congeners

Compound	I-TEF	WHO-TEF
PCDDs		
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDD	0.5	1
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDD	0.001	0.0003
PCDFs		
2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDF	0.05	0.03
2,3,4,7,8-PeCDF	0.5	0.3
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01
OCDF	0.001	0.0003

In order to assess the toxicity of a mixture of dioxins the toxic equivalents (TEQ) is used. TEQ is calculated as a summation of the multiplying the toxicity (WHO-TEQ or I-TEQ) for the concentration of each congener, according the equation 1.

$$TEQ=\sum (TEF_i \cdot C_i) \quad (1)$$

4.3. Health effects

The International Agency for Research on Cancer (IARC) classified the 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) as a carcinogen for the human health (Group 1), and recently, the 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF) was

also included (IARC, 2012). In turn, other PCDD/F congeners, such as 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD have been included in the Group 3 by the IARC, therefore not being classifiable as to its carcinogenicity to humans (IARC, 1997). In turn, USEPA considers there is sufficient evidence in animals but insufficient in humans (carcinogenic B2) to classify PCDD/Fs as carcinogenic, when considered alone (U.S.EPA, 2000). However, in complex mixtures, dioxins and related compounds were classified as likely to be carcinogenic for humans (Group B1) (U.S.EPA, 2000). In spite of these classifications, of some authors point out there is important lack of conclusive data to consider PCDD/Fs as a carcinogenic (Boffetta et al., 2011; Cole et al., 2003).

The hallmark of non-carcinogenic toxicity of PCDD/Fs in humans is chloracne that may be manifested after occupational exposure or high acute exposure (Linden et al., 2010). Children are more sensitive to chloracne than the adult population, according to the experience of the accident of Seveso (Italy) in 1976 (Mocarelli et al., 1991). Moreover, harmful effects on the reproductive system, hepatoxicity, development, immunology, neurotoxicity and endocrine systems have been observed (Linden et al., 2010; Sweeney and Mocarelli, 2000). The aryl hydrocarbon receptor (AhR) seems to be implied in the mode of action (MoA) of PCDD/Fs. Thus, the MoA is similar to the hormonal biochemistry, and PCDD/Fs are considered endocrine disruptors (ED) (Vasseur and Cossu-Leguille, 2006; Wang et al., 2006).

In 1990, the World Health Organization (WHO) established a tolerable daily intake (TDI) of 1-4 pg-TEQ/kg-day for PCDD/Fs (Van et al., 2000). However, it was indicated that human intake should be less than 1 pg-TEQ/kg-day. In 2001, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that, due to the long half-lives in blood, the human doses of PCDD should be evaluated in a monthly period, establishing a Provisional Tolerable Monthly Intake (PTMI) of 70 pg WHO-TEQ/kg BW per month (WHO, 2002). In 2012, a RfD of 0.7 pg/kg-day for TCDD was suggested by U.S.EPA (2012). Such dose was derived based in two previous studies: a study that related the sperm concentration and mobility on men exposed to TCDD, and a second study that related the concentration of TCDD with thyroid hormone levels on newborns (Baccarelli et al., 2008; Mocarelli et al., 2008).

4.4. Pharmacokynetics and Pharmocodynamics (PK/PD)

The absorption of PCDD/Fs is mainly through oral intake, being the inhalation another important route of exposure. In contrast, dermal intake seems to be a minor

pathway according to previous reports (McLachlan, 1993; Schlummer et al., 1998). PCDD/Fs may be absorbed in a range of 80%-90% by oral route. The limiting factor of the absorption is the passage across the intestinal wall, decreasing in relation to molecular size (ATSDR, 1998).

The distribution and disposition of PCDD/Fs is dependent of the congener, dose, route of administration and age. The main storage compartments of dioxins for mammals are the liver and the adipose tissue (mesentery fat), due to the high lipophilicity of these compounds. Furthermore, the blood compartment also works as a storage compartment and correlation between the levels found in blood and other tissues has been established (Iida et al., 2007). The distribution of PCDD/Fs in the lipid fraction of the tissues, and specially for the highly chlorinated compounds, is not equal depending of the depot (ATSDR, 1998). Quantifiable concentration of dioxins have been found in the liver of neonates, suggesting a transplacental transfer in gestational mothers (Schecter et al., 1990). Moreover, detectable concentrations of several PCDD/Fs congeners have been found in breast milk samples (Schuhmacher et al., 2009, 2013).

The half-life of 2,3,7,8-TCDD have been estimated in 7.1 years, according to data from a group of Vietnam veterans highly exposed to TCDD in the Ranch Hand operation of herbicide warfare (Pirkle et al., 1989). After that, in a more recent study, the same value of half-life elimination of 7.1 years was reported in a cohort of women exposed to 2,3,7,8-TCDD in a highly residential exposure in Seveso (Italy) (Warner et al., 2013). Other studies of Vietnam veteran subjects suggested higher values of half-lives, ranging from 8.7 and 11.3 years (Michalek et al., 1996; Wolfe et al., 1994). A correlation between the percentage of adipose content in the human body and the human half-lives has been established in several studies (Flesch-Janys et al., 1996; Wolfe et al., 1994).

PCDD/Fs are poorly metabolized in humans (Hu and Bunce, 1999; Wendling et al., 1990), and the slow metabolism of PCDD/Fs is hypothesized to be the responsible of the long half-lives elimination in humans (Reddy et al., 2005). The major elimination routes of PCDD/Fs are feces, and in a smaller quantity, the urine. In feces, the mechanism of TCDD was proposed to be a passive diffusion into the intestinal lumen from blood to feces and subsequently excreted (Olson, 1986). In urine, detectable amounts of metabolites of TCDD were found in a study conducted in rats after a bolus injection of 2,3,7,8-TCDD (Weber et al., 1993). Furthermore, breast milk can be also an important source of PCDD/Fs elimination. High-chlorinated dioxins are more present in milk, decreasing the concentration with the chlorination of the aromatic rings (ATSDR, 2008). OCDD is usually the main PCDD/F congener found in milk samples, with

percentages over 50% of the total (ATSDR, 1998). The highest concentration in milk is in the first weeks of lactation, with a sharp decline after that (Takekuma et al., 2011). Some studies have demonstrated that dioxins in breast milk may be quickly absorbed by nursing babies (McLachlan, 1993).

4.5. PBPK models for PCDD/Fs

PBPK models have been demonstrated to be useful to predict the distribution of TCDD body burdens. The first PBPK model for TCDD was developed in 1983 by King et al. (1983) using rats, mice and monkeys. Some years later, Leung et al. (1988) improved the model including the hepatic CYP1A2 binding in the PBPK model of TCDD. Currently, more than 25 PBPK models have been elaborated to predict the tissue distribution of TCDD in rats, humans or other animals (Reddy et al., 2005).The degree of complexity in the model for TCDD in rodents has been increasing over the time. Four levels of complexity of PBPK models have been described for TCDD in rodents depending of the protein binding, binding to AhR, the CYP1A1/2 induction, extrahepatic biochemical responses, etc (Figure 4) (Reddy et al., 2005). In summary, Level 1 models feature two binding proteins in liver that are cytosolic AhR and microsomal CYP1A2 (Lawrence and Gobas, 1997; Leung et al., 1988). Level 2 models include the interaction between AhR-TCDD complex and DREs and the induction of CYP1A1/2 (Andersen et al., 1993; Santostefano et al., 1998). Level 3 models features the nonuniform induction of enzymes in liver (Andersen et al., 1997). Level 4 model include extrahepatic responses, like induced effects on thyroid hormones (Kohn et al., 1996). Most of these models were only applicable and validated for acute and subchronic exposure, but not for chronic exposure.

Chronic exposure to PCDD/Fs in humans is obviously in much lower levels than exposure in experimentation animals. At chronic concentrations, TCDD do not induce protein binding, and most of the models do not induce CYP1A1 binding, with some exceptions (Carrier et al., 1995a, b). Thus, the level of complexity is lower than in rodent studies. Most of the human PBPK models have been developed only for TCDD, and only few works developed PBPK model for the 17 PCDD/F congeners (Carrier et al., 1995a, b; Maruyama et al., 2002, 2003). Furthermore, few PBPK models were have been developed to consider the distribution of TCDD from mother to fetus, and to newborns via pregnancy and breast milk feeding (Gentry et al., 2003; Maruyama et al., 2003).

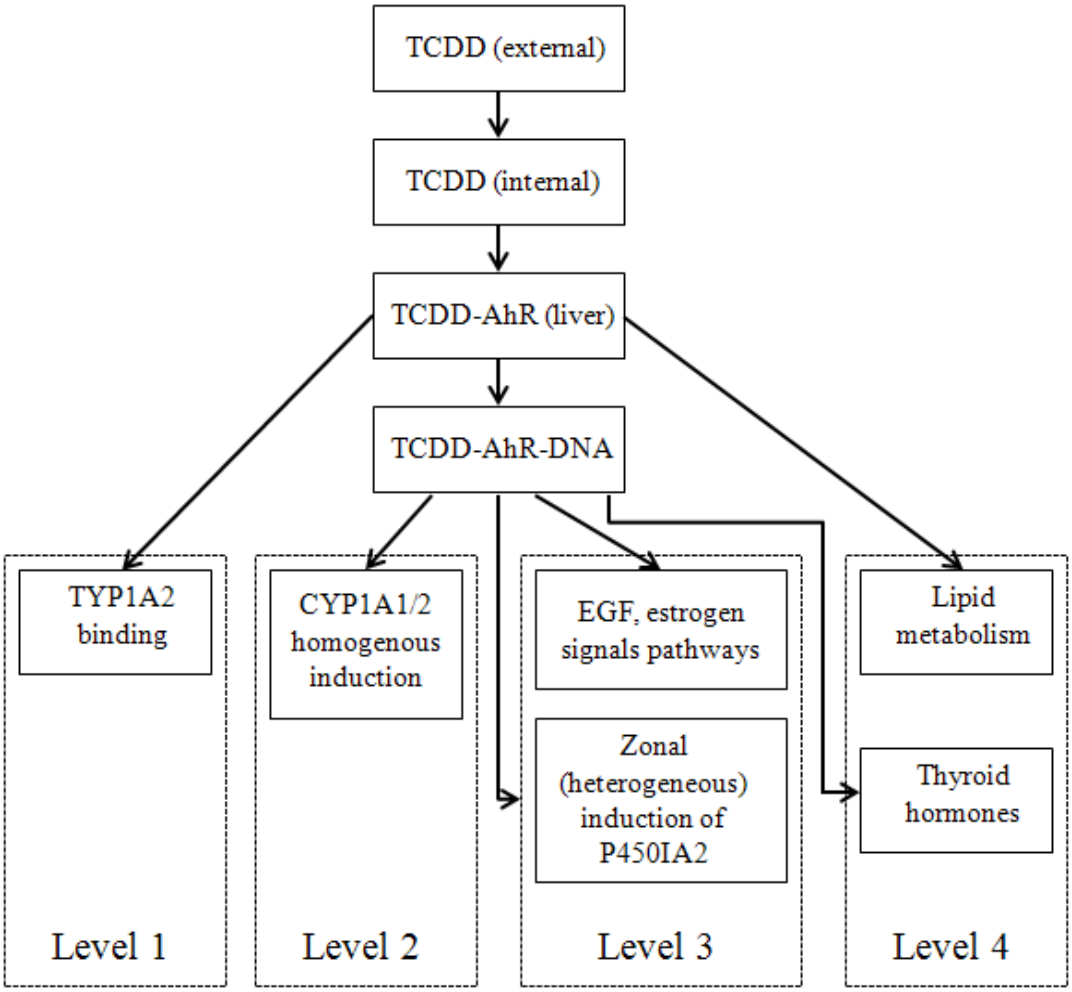


Fig. 4. Diagram of the four levels of PBPK models of TCDD in rats. Adapted from Reddy et al. (2005).

5. Perfluoroalkyl substances (PFASs)

Perfluoroalkyl substances (PFASs) are a group of fluorinated compounds, constituted by a carbon backbone where all the hydrogen atoms are substituted by fluorine, with the exception of those hydrogen atoms that are part of a functional group. The most common PFASs includes carboxylic acid (-COOH) or sulfonic acid ($-SO_3H$) as functional groups (Buck et al., 2011), being perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) the foremost studied PFASs. The structure of PFOS and PFOA is depicted in Figure 5.

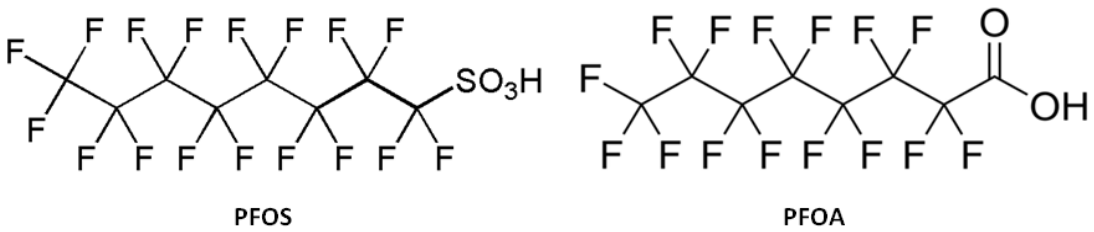


Fig. 5. Chemical structure of PFOS and PFOA.

5.1. Sources of exposure

PFASs are a group of manmade organic environmental pollutants. The sources of emissions are defined as either direct (manufacture, use and consumption) or indirect (impurities, degradation products and precursors) (Prevedouros et al., 2006). More than 80% of the PFASs emitted to the environment come from fluoropolymer manufacture and the use of products such as stain repellent treated carpets, waterproof apparel and aqueous firefighter foams (Paul et al., 2009; Prevedouros et al., 2006). Indirect sources are not well understood yet due to the lack of information about their degradation (Paul et al., 2009). PFASs have been detected in water and biota worldwide (Falk et al., 2012; Rotander et al., 2012). Specifically, PFOS is the most predominant PFAS found worldwide, being detected in animal wildlife like fish, birds and mammals (Giesy and Kannan, 2001). A general decrease of PFASs in the environment has been observed (Paul et al., 2009), since the phase-out of the PFOA production by the 3M company in 2002 (Wang et al., 2009).

The main source of human exposure to PFASs is the oral intake through food and water ingestion (Domingo, 2012; Ericson et al., 2008; Vestergren and Cousins, 2009). PFASs are also present in food packaging materials for oil resistance, and migration of PFASs from packaging materials to food may also occur (Llorca et al., 2010; Zafeiraki et al., 2014). Dust intake of PFASs is negligible in the adult population, whereas in toddlers it is also low (Ericson et al., 2012). Furthermore, inhalation exposure is negligible even in the indoor areas, due to the low volatility of PFASs (Schlummer et al., 2013). In newborn infants, exposure from breast milk may be notable source of exposure (Karrman et al., 2010; Karrman et al., 2007; Llorca et al., 2010). Recently, a decrease of PFASs concentration in food items and drinking water has been observed (Calafat et al., 2007; Ericson et al., 2008; Johansson et al., 2014), in parallel to the environmental reduction of their environmental burdens. As a result of the declining of human intake,

decrease of PFASs concentration in human blood has also been observed in individuals of Western countries (D'eon and Mabury, 2011; Olsen et al., 2008).

5.2. Toxicity and Health effects

The potential toxicity of some PFASs has been characterized in animal studies. On general, the toxicity of PFASs is relatively similar among congeners, being the liver the target tissue (Yeung et al., 2013). PFASs cause hepatotoxicity, reproductive toxicity, toxic effects on body weight (BW), development, cholesterol, thyroid hormone and immunotoxicity (Lau et al., 2007; Stahl et al., 2011). Acute toxicities of PFASs have been studied in animals, but studies have been mainly focused on PFOS and PFOA. The lethal dose [LD_{50}] of PFOS is estimated in 251 mg/kg BW, and that of PFOA in a range between 430 and 680 mg/kg BW in PFOA (EFSA, 2008). The lethal concentration [LC50] in rats for 1h of inhalation of contaminated airbone dust was assessed estimated in 5.2mg/L for PFOS, and 0.98 mg/L for PFOA. In studies of LD_{50} in fresh water organisms such as flea, planaria, snail or shrimp, PFOS has showed to be more toxic than PFOA (Ji et al., 2008; Li, 2009).

Acute exposure to PFOS in mice causes developmental effects like reduction of fetal weight, edema, cardiac abnormalities, delayed ossification and behavioral effects (Fuentes et al., 2006; Fuentes et al., 2007a; Fuentes et al., 2007b; Lau et al., 2004). Exposure to PFOA has been suggested to cause kidney and testicular cancer in human population (Barry et al., 2013). Subchronic and subacute toxicities in rats induce hepatotoxicity, reduction of body weight, reduction of the levels of triglycerides, reduction of cholesterol in serum, liver hypertrophy, thyroid hormones reduction and damage on the neuroendocrine system for PFOS (Austin et al., 2003).

5.3. Pharmacokynetics and Pharmocodynamics (PK/PD)

The pharmacokinetic and pharmacodynamic characteristics of PFASs have been studied in animals (Lau et al., 2007). In general, PFASs 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 plasma concentration. In rats, the concentration of PFOA and PFOS in liver is between 3 and 5 higher than in plasma in animals, and between 1.3 and 2 times higher in humans (Hundley et al., 2006; Seacat et al., 2003). The distribution of PFOS is mainly extracellular (Noker and Gorman, 2003). PFOS and PFOA have a high affinity to bind to

β-lipoproteins, plasma albumin and fatty acid liver proteins (Han et al., 2003; Luebker et al., 2002). The fraction of binding of PFASs to plasma albumin is estimated to be over the 90% in rats and humans (Han et al., 2003). Detectable concentrations of PFASs have been found in umbilical cord suggesting that PFASs can cross the placenta exposing the fetus (Apelberg et al., 2007). Furthermore, PFASs have been detected in breast milk, exposing the neonate to PFASs (Karrman et al., 2007).

PFASs half-lives in plasma have been studied in detail. The plasma/serum elimination in PFOA show notably differences among species, ranging 2-6 h, 17-19 days and 30-21 days in rats, mice and monkey, respectively (Lau et al., 2007). Regarding PFOS, half-lives have been set in 1-2 months, 4 months and 4.8 years in rodents, monkey and humans, respectively (Chang et al., 2012). Moreover, in animals PFOS and PFOA exhibits different elimination rates depending on the gender and the age of the individuals (Lau et al., 2007). For PFOA, the elimination half-lives in adult female rat was assessed in 2-4h, whereas in adult male rat was assessed in 4-6 days (Kemper, 2003). In monkey, the elimination rate of PFOA was assessed in 30 days for adult female and 21 days for adult male (Butenhoff et al., 2004). On the other hand, in mouse and humans, sex differences on the elimination rate have not been observed yet. The explanations of the differences in the elimination rates in rats were not conclusive, but some studies suggested that the transporters in the proximal tube of the kidney may be the responsible (Andersen et al., 2006; Tan et al., 2008; Yang et al., 2009). In humans, blood half-lives of 5.4, 3.8 and 8.5 years have been estimated for PFOS, PFOA and perfluorohexane sulfonic acid (PFHxS), respectively (Lau, 2012). In contrast, half-lives of 32, 30 and 3 days have been observed for perfluorohexanoic acid (PFHxA), perfluorobutane sulfonic acid (PFBS), and perfluorobutanoic acid (PFBA), respectively (Lau, 2012; Olsen et al., 2009). The organic anion transporter proteins are compound, gender and chain length dependent (Han et al., 2011). PBPK models have been used in the past to successfully simulate the elimination of PFOS and PFOA via a resorption mechanism, that once the chemical is in the urine is resorbed back to plasma following a saturable mechanism (Andersen et al., 2006; Loccisano et al., 2011; Tan et al., 2008).

5.4. PBPK models for PFASs

Recently, a number of PBPK models have been developed for PFASs. However, most of them are limited to the study of PFOS and PFOA. One of the first PBPK model for PFOS and PFOA was developed by Andersen et al. (2006), who described the elimination of PFOS and PFOA in monkey. The model included three compartments

(central compartment, tissue and filtrate) and included two key features of the ADME characteristics of PFOS and PFOA. First, the elimination was mainly urinary, following a resorption mechanism, that when the chemicals are in the urine are resorbed back to the plasma following a saturable process. This kinetic is analogous to the Michaelis-Menten kinetics. The second feature of that PBPK model was the binding of PFOS and PFOA to plasma albumin. As a consequence, 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 (Loccisano et al., 2012a; Lou et al., 2009; Tan et al., 2008). 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).

Other PBPK models have considered other pharmacokinetic mechanisms, different from that firstly suggested by Andersen et al. (2006). A PBPK model was developed for PFOS by Harris and Barton (2008). In rats the structure of the model differentiated blood, liver, rest of the body, and two compartments for the gastrointestinal tract (GI), being tissues described as diffusion-limited compartments (Thompson and Beard, 2011). For validation purposes, experimental data of concentration of PFOS in rats were used, and the model was based on the elimination via urinary and biliary excretion. Sonne et al. (2009) developed a PBPK model to estimate the burdens of PFOS in Greenland polar bears (*Ursus Maritimus*). This PBPK model was based on a previous generic model applied to organohalogen contaminants (OHC) (Cahill et al., 2003). Another approach was done for cows, for which PBPK model was developed to assess the transfer of PFOS from contaminated feed to milk (van Asselt et al., 2013). Two tissues were considered in the model (carcass and serum), while the elimination of PFOS occurred mainly in milk, instead of urine and feces. The estimated half-life of PFOS in milk was 56 days.

It must be highlighted that most PBPK models have been exclusively developed for PFOS and PFOA. Unfortunately, pharmacokinetic information about other PFASs is particularly scarce. Instead of some PBPK models were elaborated for PFOS and PFOA

in animals and in humans, none of them were elaborated for other PFASs. Thus, there is a lack of PBPK models to describe the pharmacokinetics of PFAS in the human body. Due to the lack of knowledge of the pharmacokinetics of compounds different of PFOS and PFOA this is a challenging area of study.

HYPOTHESIS

The human health risk assessment of exposure to environmental pollutants may be improved if the time course concentrations of these chemicals in the human body are well known. *In silico* tools are cheap, quick and reliable techniques to estimate the body burdens of chemicals, being a serious complement to *in vivo* or *in vitro* investigations.

PBPK models may simulate and predict the distribution and accumulation of POPs in the human body. Therefore, they may be a good alternative to biological monitoring of PCDD/Fs and PFASs.

OBJECTIVES

General objective

To develop and validate a PBPK model to simulate and predict the time course concentration of PCDD/Fs and PFASs in human tissues.

Specific objectives

- To develop a Ranking Index to prioritize those environmental pollutants posing the highest health risk for humans.
- To develop a PBPK model to predict the tissue concentration of 17 PCDD/F congeners in human plasma and adipose tissue.
- To determine the main biochemical parameters needed to develop a PBPK model for the estimation of PFASs in the human body.
- To develop a PBPK model to predict the tissue concentration of 11 PFASs in human tissues, including plasma, liver, kidney, brain and lungs.
- To study the parametric uncertainty of a PBPK model for PFOS and PFOA.

CHAPTER 1

INTEGRATED RISK INDEX OF CHEMICAL AQUATIC POLLUTION (IRICAP): CASE STUDIES IN IBERIAN RIVERS

Abstract

The hazard of chemical compounds can be prioritized according to their PBT (persistence, bioaccumulation, toxicity) properties by using Self-Organizing Maps (SOM). The objective of the present study was to develop an Integrated Risk Index of Chemical Aquatic Pollution (IRICAP), useful to evaluate the risk associated to the exposure of chemical mixtures contained in river waters. Four Spanish rivers basins were considered as case-studies: Llobregat, Ebro, Jucar and Guadalquivir. A SOM-based hazard index (HI) was estimated for 205 organic compounds. IRICAP was calculated as the product of the HI by the concentration of each pollutant, and the results of all substances were aggregated. Finally, Pareto distribution was applied to the ranked lists of compounds in each site to prioritize those chemicals with the most significant incidence on the IRICAP. According to the HI outcomes, perfluoroalkyl substances, as well as specific illicit drugs and UV filters, were among the most hazardous compounds. Xylazine was identified as one of the chemicals with the highest contribution to the total IRICAP value in the different river basins, together with other pharmaceutical products such as loratadine and azaperol. These organic compounds should be proposed as target chemicals in the implementation of monitoring programs by regulatory organizations.

1. Introduction

Due to the massive use of chemicals in industrial and agricultural activities, as well as their content in home products, water pollution in rivers has been growing in recent decades. Detectable and quantifiable amounts of chemicals can be found in rivers, some-times at harmful concentrations for the environment and especially for the human health (Carafa et al., 2011; Fernández-Turiel et al., 2003). Furthermore, Mediterranean rivers are characterized by a strong rainfall and temperature seasonality, with periods of severe drought and floods (López-Doval et al., 2012; Petrovic et al., 2011). Because of these special characteristics, climate change models conclude that Mediterranean regions will be among the most impacted regions in a near future (Giorgi

et al., 2008). Therefore, climate change and the anthropogenic manipulation of water resources in Mediterranean rivers may lead to enhance human health risks of river water exposure.

As humans are exposed to chemical mixtures rather than individual substances, new realistic approaches have been developed to assess the risks associated to combined exposure to sets of pollutants (McCarty et al., 2006; Sumpter et al., 2006). Classically, two main approaches have been used to evaluate the toxicity of chemical mixtures: concentration addition (CA) and independent action (IA), which assume a similar or different mode of action (MoA), respectively. Although these approaches have been successfully applied in the past (Backhaus et al., 2012), mixtures of compounds can interact, modifying therefore the final toxicity (Boobis et al., 2011). In 2004, the Agency of Toxic Substances and Disease Registry (ATSDR) elaborated a guide manual for the evaluation of join action of chemical mixtures (ATSDR, 2004), which contained flows charts to help toxicologists (Wilbur et al., 2004). Nevertheless, proper schemes of the MoA considering possible interactions among pollutants, are clearly necessary (Teuschlet et al., 2007).

Ranking systems allow the prioritization of chemicals according to their chemical, physical or toxicological properties. Ranking methods have become useful tools for stakeholders involved in water management. The European Union (EU) developed a combined monitoring-based and modeling-based priority settings (COMMPS) methodology (EC, 1999). COMMPS procedure is based on the exposure of pollutants in freshwater, as well as the effects of the pollutants on aquatic organisms and humans. Among ranking methods, an extended method to prioritize organic chemicals is the use of 3 basic properties: persistence, bioaccumulation and toxicity commonly known as PBT (Arnot et al., 2008; Pennington et al., 2001). The main outcome of this exercise was the list of 33 priority substances identified under the Water Framework Directive (Directive 2000/60/EC) and its “daughter” specifically devoted to this issue (Directive 2008/105/EC). However, PBT models cannot be used to study interactions among compounds. Alternatively, Self-Organizing Maps (SOM) may be a good option. SOM are a kind of artificial neural network (ANN) extensively used in data analysis, which are able of friendly visualizing large amounts of information (Alvarez Guerra et al., 2008; Arias et al., 2008; Mari et al., 2008). Data can be analyzed and the extracted results studied in a two-dimensional grid. In addition to information visualization, SOM has been also used for environmental modeling (Kalteh et al., 2008). Due to the ability of the SOM algorithm to group data according to similar characteristics, it has been previously used to create

PBT-based rankings pollutants (Nadal et al., 2006; Nadal et al., 2008). Recently, SOM was also applied to elaborate an ecological hazard index of a series of pollutants found in Ebro River waters (Spain) (Ocampo-Duque et al., 2012).

In recent years, some statistical and mathematical tools have been used to detect the chemicals with a higher contribution. Some examples are h-index (Hirsch index) and Zipf's law, which were successfully applied in a recent study performed to prioritize pharmaceuticals in a wastewater treatment plant (Ginebreda et al., 2011). H-index is capable to identify the most relevant compounds in a list of ranked chemicals by means of a Pareto distribution, according to some specific parameters. In turn, Zipf's law, based on the inverse proportion between the frequency of one event and its ranking position, has been also widely applied in different domains (Hisano et al., 2011).

The objective of the present study was to rank the hazard of 205 organic compounds analyzed in water samples from 4 river basins in Spain (Llobregat, Ebro, Júcar and Guadalquivir), according to the PBT properties of the pollutants. Firstly, a hazard index (HI) was developed and applied to each individual compound by using SOM. Secondly, an Integrated Risk Index of Chemical Aquatic Pollution (IRICAP) was elaborated to rank the human health risks depending on the HI and the concentration of each individual chemical. Finally, the compounds with the highest contribution were characterized using the h index and the power law exponent of the Zipf's law.

2. Materials and Methods

2.1. Data and study area

As part of a large monitoring program (Navarro-Ortega et al., 2012), a total amount of 205 organic pollutants were analyzed in four Spanish rivers with different pressures and impacts: Llobregat, Ebro, Júcar and Guadalquivir. The former three are located in the Mediterranean catchment basin, while Guadalquivir waters discharge into the Atlantic Ocean (Fig. 1). A network of representative 77 sampling points was previously established to assess the main stressors of the river basins. The geographical distribution of the sampling sites is presented in Table S1 (Annex 1).

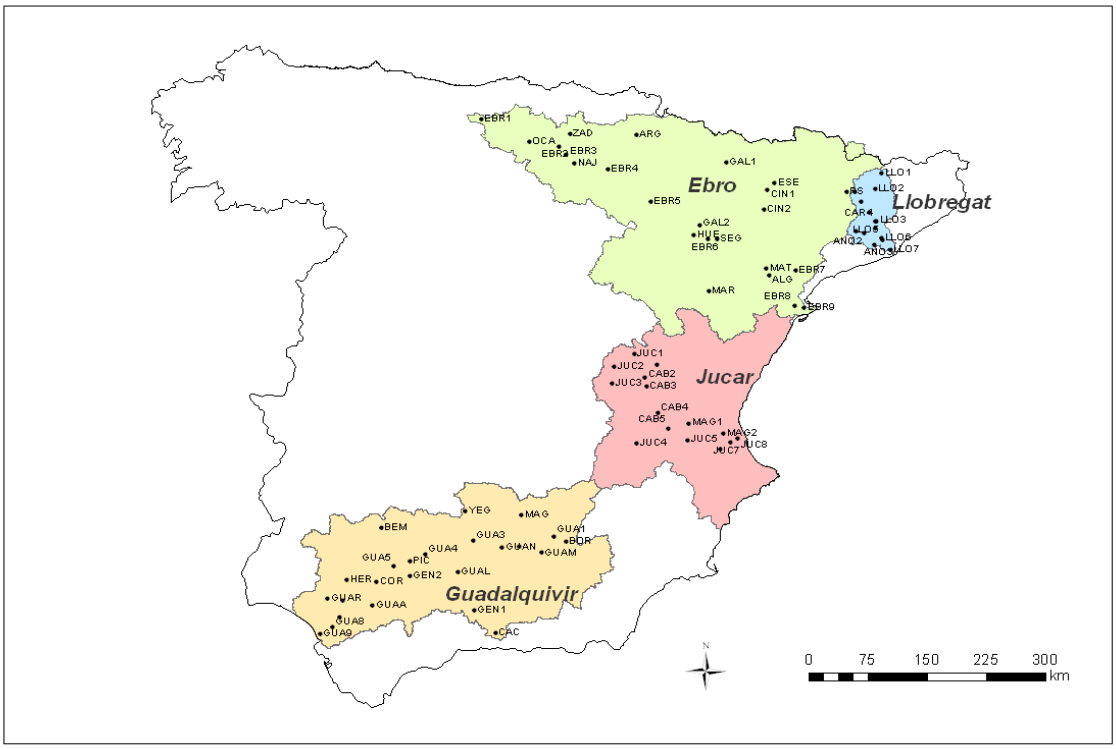


Fig. 1. Area of study.

The list of analyzed compounds included pharmaceutical products, illicit drugs, endocrine disruptors (ED), pesticides, perfluoroalkyl substances (PFAS), and UV filters. Eighty-one pharmaceutics, including analgesics such as ibuprofen and acetaminophen, antibiotics such as ofloxacin and amoxicilin, and antihypertensives such as enalapril and losartam, were determined. Pharmacy products, like psychiatric drugs, diuretic or veterinary pharmaceuticals, were also analyzed. Nineteen illicit drugs including cannabinoids, and recreational drugs such as cocaine, LSD or amphetamine, were also studied. Thirty-one endocrine disruptors were added to the set of target pollutants, including diethylstilbestrol and nonylphenol. Forty-two different pesticides (insecticides and herbicides) were analyzed, including ethion and propanil, among others. Finally, 21 perfluoroalkyl substances (PFASs) such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), and 11 UV filters, including benzophenone and octocrylene, were also determined. Full names and abbreviations are summarized in Table S2 (Annex 1). Water samples were collected in Autumn of 2010 and 2011.

The Llobregat river basin is located in the north-east of Spain. It has a drainage basin of 1948 km^2 and a total length of 170 km, being the main drinking water resource of Barcelona and surrounding cities. Because of its proximity to Barcelona, the lowest course of the river receives strong anthropogenic pressures. Urban and industrial waste

water are discharged in the Llobregat river, in addition to the surface run off coming from agricultural and salt mining areas (Cabeza et al., 2012). Furthermore, the Mediterranean climate of this area usually changes the water flow, and consequently the natural capacity of the river water dilution. The Ebro river basin is the largest in Spain in terms of water flow, with a drainage basin of 85,550 km^2 and a length of 928 km. Situated in the north of Spain, it is regulated by numerous dams and channels in all the river flow. At the end of the 20th century, approximately 30% of the river flow had decreased due to surface water extractions for irrigation, land use change (reforestation) and rainfall decrease as well as due to the impact of industrial activities, which are concentrated in the main cities along the river. An important “hot spot” of chemical pollution has been also identified in the Flix (Catalonia) reservoir, where thousands of tons of toxic substances (e.g., radio nuclides, heavy metals, organo chlorine compounds, etc.) have been accumulated (Ferré-Huguet et al., 2009a; Ferré-Huguet et al., 2009b; Mola et al., 2011). These pressures have also altered the sediment regimes and the water quality. The Jucar river basin, located in the east of Spain, covers a drainage area of 21,632 km^2, and the mainstream shows a length of 500 km. Agriculture pressures are located in the medium and lower parts of the river basin, where there is also a notable industrial activity. Since it flows in a semiarid zone, the most important problems of this basin are the hydrological modification of the river course, the aquifers overexploitation, and the river contamination. The Guadalquivir river basin is located in the south of Spain, with a drainage basin of 57,071 km^2 and a length on the main stream of 657 km. This area covers a population of approximately 7 million inhabitants, and the river consequently receives many anthropogenic pressures. The total agriculture area of the river basin is 700,000 ha (mainly rice, olives and fruit trees), with the consequent environmental effects. The regime of the river is highly modified by dams and reservoirs, especially in the lower course. Doñana National Park, a natural area severely affected by metal inputs after a mining accident (Gómez et al., 2004), is located at the mouth of the river.

2.2. Hazard Index

Originally developed by Kohonen (Kohonen, 1982), Self-Organizing Maps (SOM) use an unsupervised learning algorithm that reduces large amounts of input data (Kohonen, 2013). The results are generally visualized in two-dimension maps, allowing clustering the input information by grouping similar data. The final result is, on one hand, a Kohonen’s map showing the distribution of the input values on a two dimensional grid, and on the other hand, a set of component planes (c-planes) showing the clusters

created by the algorithm in the Kohonen's grid. In previous studies, we successfully used SOM in order to create prioritization rankings of pollutants (Nadal et al., 2006; Nadal et al., 2008).

A hazard index (HI) was elaborated for the whole set of 205 compounds, using the PBT properties of each individual chemical. Data of persistence, bioaccumulation and toxicity were gathered using Estimation Program Interface (EPI) Suite™ for Microsoft Windows®, v 4.1 (EPI Suit™). EPI Suit™ is a set of tools developed by the US Environmental Protection Agency (US EPA) to estimate physical and chemical properties, environmental fate and aquatic toxicology of chemicals. It uses a database of more than 41,000 chemical compounds, coming from the PHYSPROP© database. The predictive methods and the equation used for each calculation parameter have been described elsewhere (Boethling et al., 2004). Half-lives were assessed by using Biowin™ tool. Based on a previously developed model (Boethling et al., 1994), Biowin™ tool predicts the primary aerobic degradation of the organic compounds. The result is a semi-quantitative rate of times with the following units: 5-h, 4-days, 3-weeks, 2-months and 1-years. Bioaccumulation was estimated by using BCFBAF™ tool. The method was initially developed by Meyland et al., (1997; 1999), and subsequently improved by Arnot and Gobas et al., (2006), who added experimental values of bioconcentration factor (BCF). Finally, toxicity was estimated by applying the ECOSAR™ tool, which let assess the aquatic (fish) toxicology based on Kow levels (Sanderson et al., 2003). As low levels of persistence and toxicity derive in a higher hazard, inverse values obtained from the Biowin™ and ECOSAR™ tools, respectively, were considered in the HI building. Final data constituted a matrix of 205 compounds and 3 parameters (Table S2, Annex 1), which was run with the SOM toolbox for Matlab® (Alhoniemi et al., 1999). Values were normalized using the same toolbox to obtain a variance equal to one for each parameter. A linear initialization was applied. The learning phase consisted on 10,000 steps, while the tuning phase added other 10,000 steps. HI was considered as the sum of the PBT values for each compound, after the SOM training. Default range (from 0 to 3) was re-scaled to 0–10.

2.3. Integrated Risk Index of Chemical Aquatic Pollution (IRICAP)

The Integrated Risk Index of Chemical Aquatic Pollution (IRICAP) was calculated by applying the following formula:

$$IRICAP = \frac{\sum Hazard\ Index \times Chemical concentration}{Number of\ chemicals} \quad (1)$$

The HI of each individual compound was multiplied by the normalized water concentration found in each sampling point (unpublished data), being the final sum divided by the number of pollutants (205). Concentrations were normalized to avoid any overestimation. They were re-scaled from 0 to 10, being the same as the HI. The concentrations were normalized for each chemical by using the following formula:

$$C\ norm\ = \frac{C\ i - C\ \min}{C\ \max - C\ \min} \quad (2)$$

2.4. H-index and Zipf's law

The list of 205 compounds was initially ordered according to their IRICAP. The percentage of the product of the normalized concentration by the HI was obtained for all the individual compounds studied in each sampling point. Afterwards, data were distributed following a Pareto distribution, using h-index and Zipf's law were used to study some of the characteristics of the distribution. The h-index was suggested by Hirsch (Hirsch et al., 2005) in order to evaluate the quality of the scientific and academic publications. In the academic area, the number h is the number of papers published by an author that have the same number (h) of citations. However, this can be translated to other research areas. In the present study, all compounds were ranked according to the HI and the subsequent h-index was obtained. Furthermore, the h-content or percentage of IRICAP covered by h-compounds, was assessed. The h-compounds are defined as those located in the first h positions of the list of compounds, ordered according to the HI. Finally, the number of compounds representing the 90% of HI (referred to as P 90) was assessed by means of Pareto distributions.

The Zipf's law was formulated for the first time by the linguist Zipf, and it has been extensively used in experimental and social sciences (Newman et al., 2005). It assumes that there is a relationship on the frequency of one event that varies depending of some attribute of the event. This mathematical relationship is called a power law. The power law usually follows this formula:

$$y(x) = c \times x^{-\alpha} \quad (3)$$

where y is the frequency of the event of rank x, c is a constant factor, and α is the power parameter. The α exponent is numerically determined by linear regression of the log–log

transformed experimental data ($\log y = -\alpha \cdot \log x + \log c$). It may give a measure of the complexity of the distribution in the following sense: When $\alpha = 0$, there is a situation of "minimum complexity", as $x^0 = 1$. In contrast, when $\alpha = \infty$ there is a situation of "maximum complexity", because $x^\infty = \infty$. Because α gives important information about the curve shape, this power parameter was assessed in each sampling point.

3. Results and Discussion

3.1. Hazard Index

The final result for the HI was a two dimensional grid of 96 hexagons (12 × 8), where the compounds are spread around the cells according to PBT similarities. Moreover, three c-planes maps were obtained, one for each of the PBT parameters (Fig. 2). C-planes show the normalized values of half-lives, bioaccumulation and toxicity, obtained after the SOM training of the initial PBT data. The numerical values of the c-planes were extracted for the HI calculation developing specific commands using the SOM Toolbox for Matlab® (Table S3, Annex 1). HI outcomes are summarized in Table 1. Half-lives ranged from 0.04 to 2.48. A cluster of 16 PFASs was identified as the group of compounds with highest values of half-lives. The environmental persistence of some PFASs has been reported in previous studies (Thomas et al., 1990). Surprisingly, neither PFOS nor PFOA, two of the most known and well-studied PFASs, were among the perfluoroalkyl substances presenting a highest persistence. Half-life scores for other compounds were comparatively lower, being distributed in a list with no appreciable trends.

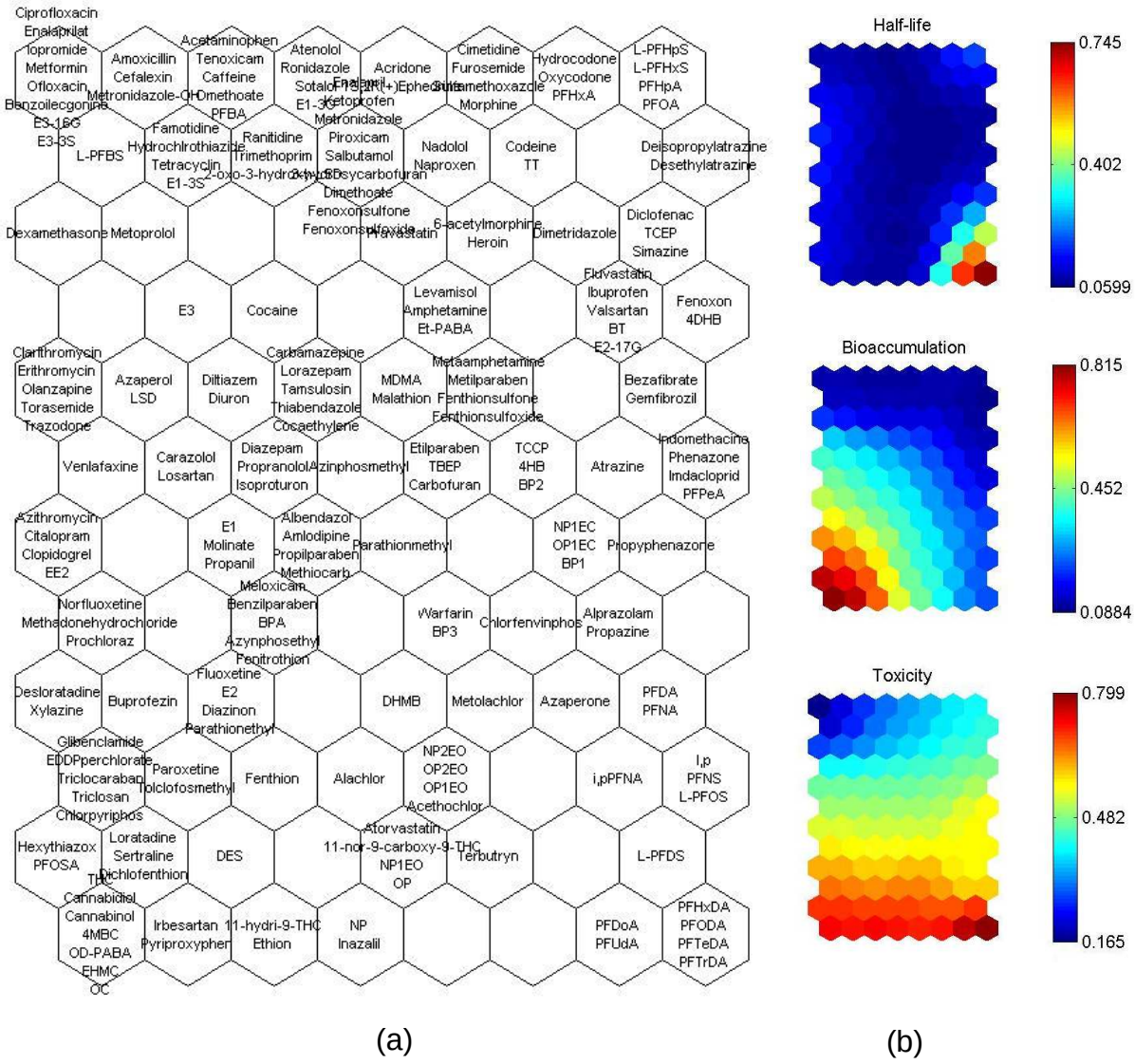


Fig. 2. Self-Organizing Map of the Hazard Index: a) Kohonen's map, and b) c-planes of PBT.

Table 1. SOM-based Hazard Index (HI) developed for 205 organic compounds.

Compound	HI	Compound	HI	Compound	HI	Compound	HI	Compound	HI	Compound	HI
*PFHxDA	5.58	¶NP	4.34	*PFDA	3.52	#Cocaethylene	3.02	*PFOA	2.30	¥3-hydroxycarbofuran	1.76
*PFODA	5.58	¥Inazalil	4.34	*PFNA	3.52	¥Diuron	3.02	¥Fenoxon	2.29	¥Dimethoate	1.76
*PFTeDA	5.58	¥Fenthion	4.26	¥Metolachlor	3.46	¥Azinphos methyl	2.96	Δ4DHB	2.29	¥Fenoxon sulfone	1.76
*PFTrDA	5.58	*I,p-PFNS	4.20	¶E1	3.45	¶NP1EC	2.92	⊥Fluvastatin	2.21	¥Fenoxon sulfoxide	1.76
*PFDoA	5.58	*L-PFOS	4.20	¥Molinate	3.45	¶OP1EC	2.92	⊥Ibuprofen	2.21	⊥Acridone	1.75
*PFUdA	5.58	⊥Norfluoxetine	4.13	¥Propanil	3.45	ΔBP1	2.92	⊥Valsartan	2.21	#1S,2R(+)Ephedrine	1.75
#THC	5.45	#Methadonehydrochloride	4.13	⊥Azaperone	3.45	⊥Propyphenazone	2.90	¶BT	2.21	⊥Ranitidine	1.69
#Cannabidiol	5.45	¥Prochloraz	4.13	⊥Clarithromycin	3.40	¶Etilparaben	2.80	¶E2-17G	2.21	Trimethoprim	1.69
#Cannabinol	5.45	⊥Fluoxetine	4.06	⊥Erithromycin	3.40	¶TBEP	2.80	¥Deisopropylatrazine	2.21	#2-oxo-3-hydroxy-	1.69
Δ4MBC	5.45	¶E2	4.06	⊥Olanzapine	3.40	¥Carbofuran	2.80	¥Desethylatrazine	2.21	⊥Metoprolol	1.64
ΔOD-PABA	5.45	¥Diazinon	4.06	⊥Torasemide	3.40	#Metaamphetamine	2.72	⊥Hydrocodone	2.15	⊥Atenolol	1.64
ΔEHMC	5.45	¥Paratión ethyl	4.06	⊥Trazodone	3.40	¶Metilparaben	2.72	⊥Oxycodone	2.15	⊥Ronidazole	1.64
ΔOC	5.45	*I,p-PFNA	3.99	⊥Carazolol	3.32	¶TCCP	2.72	*PFHxA	2.15	⊥Sotalol	1.64
¥Hexythiazox	5.32	⊥Atorvastatin	3.98	⊥Losartan	3.32	¥Fenthion sulfone	2.72	⊥Dimetridazole	2.14	¶E1-3G	1.64
*PFOSA	5.32	#11-nor-9-carboxy-9-THC	3.98	⊥Warfarin	3.28	¥Fenthion sulfoxide	2.72	⊥Diclofenac	2.12	⊥Famotidine	1.57
⊥Irbesartan	5.26	¶NP1EO	3.98	ΔBP3	3.28	Δ4HB	2.72	¶TCEP	2.12	⊥Hydrochlrothiazide	1.57
¥Pyriproxyphen	5.26	¶OP	3.98	⊥Albendazol	3.27	ΔBP2	2.72	¥Simazine	2.12	⊥Tetracyclin	1.57
⊥Loratadine	5.13	¥Terbutryn	3.94	⊥Amlodipine	3.27	¶E3	2.71	#6-acetylmorphine	2.10	¶E1-3S	1.57
⊥Sertraline	5.13	⊥Azithromycin	3.90	¶Propilparaben	3.27	#MDMA	2.68	#Heroin	2.10	⊥Acetaminophen	1.53
¥Dichlofenthion	5.13	⊥Citalopram	3.90	¥Methiocarb	3.27	¥Malathion	2.68	⊥Codeine	2.06	⊥Tenoxicam	1.53
*L-PFDS	5.06	⊥Clopidogrel	3.90	Azaperol	3.23	Indomethacine	2.66	¶TT	2.06	¶Caffeine	1.53
⊥Glibenclamide	4.91	¶EE2	3.90	#LSD	3.23	Phenazone	2.66	⊥Dexamethasone	2.02	¥Omethoate	1.53
#EDDPperchlorate	4.91	¥Alachlor	3.90	⊥Diazepam	3.17	¥Imdacloprid	2.66	⊥Pravastatin	1.99	*PFBA	1.53
¶Triclocaraban	4.91	¶NP2EO	3.74	⊥Propranolol	3.17	*PFPeA	2.66	⊥Cimetidine	1.91	*L-PFBS	1.46
¶Triclosan	4.91	¶OP2EO	3.74	¥Isoproturon	3.17	¥Atrazine	2.60	⊥Furosemide	1.91	⊥Amoxicillin	1.41
¥Chlorpyriphos	4.91	¶OP1EO	3.74	¥Chlorfenvinphos	3.17	#Cocaine	2.55	⊥Sulfamethoxazole	1.91	⊥Cefalexin	1.41
¶DES	4.86	¥Acethochlor	3.74	⊥Alprazolam	3.16	⊥Bezafibrate	2.42	#Morphine	1.91	⊥Metronidazole-OH	1.41
#11-hydri-9-THC	4.85	⊥Meloxicam	3.62	¥Propazine	3.16	⊥Gemfibrozil	2.42	⊥Nadolol	1.86	⊥Ciprofloxacin	1.24
¥Ethion	4.85	¶Benzilparaben	3.62	¥Paratión methyl	3.08	⊥Levamisol	2.35	⊥Naproxen	1.86	⊥Enalaprilat	1.24
⊥Paroxetine	4.64	¶BPA	3.62	⊥Carbamazepine	3.02	#Amphetamine	2.35	⊥Enalapril	1.76	⊥Iopromide	1.24
¥Tolclofos methyl	4.64	¥Azynphos ethyl	3.62	⊥Diltiazem	3.02	ΔEt-PABA	2.35	⊥Ketoprofen	1.76	⊥Metformin	1.24
⊥Desloratadine	4.57	¥Fenitrothion	3.62	⊥Lorazepam	3.02	*L-PFHpS	2.30	⊥Metronidazole	1.76	⊥Ofloxacin	1.24
⊥Xylazine	4.57	ΔDHMB	3.61	⊥Tamsulosin	3.02	*L-PFHxS	2.30	⊥Piroxicam	1.76	#Benzoilecgonine	1.24
¥Buprofezin	4.37	⊥Venlafaxine	3.58	⊥Thiabendazole	3.02	*PFHpA	2.30	⊥Salbutamol	1.76	¶E3-16G	1.24
										¶E3-3S	1.24

*PFCs, #Illicit drugs, ⊥Pharmaceutics, ¥Pesticides, ¶Endocrine disruptors, ΔUV filters

Bioaccumulation scores ranged from 0.29 to 2.70. In contrast to persistence, PFASs were not especially found to be bioaccumulative compounds, excepting PFOSA. In turn, 3 illicit drugs (THC, cannabidiol and canabinol) and 4 UV filters (4MBC, OD-PABA, EHMC, OC) presented the highest bioaccumulation factor (2.70).With regard to this, cannabinoids could present a high bioaccumulation potential due to their important lipophilicity (Thomas et al., 1990). Toxicity ranges were within 0.57 and 2.53. Two PFAS, PFDoA and PFUdA, seemed to be the most toxic compounds, with a toxicity score of 2.53. Moreover, some cannabinoids (e.g., THC, cannabidiol, and cannabinol) and UV filters (e.g., 4MBC, OD-PABA, EHMC and OC) were also among the most toxic chemicals among the 205 com-pounds analyzed (2.40). In general terms, most pesticides, such as pyriproxiphen, ethion and anazalil, also presented relatively high levels of toxicity. By contrast, and with the exception of irbesartan, pharmaceutical products mostly were in the lowest part of the list.

HI was obtained by summing the individual score of persistence, bioaccumulation, and toxicity, and re-scaling to 0–10. The final HI values ranged from 1.24 to 5.58. Some trends and clusters can be visually established by means of the SOM. Six PFASs (PFHxDA, PFODA, PFTeDA, PFTrDA, PFDoA, and PFUdA) were identified as the most hazardous pollutants, in terms of PBT parameters, reaching a HI value of 5.58. These perfluoroalkyl substances were characterized by a high environmental persistence and aquatic toxicity, but a relatively low bioaccumulation potential. However, PFOS and PFOA were not included in this set of hazardous pollutants, as their HI was lower (4.20 and 2.30, respectively). A group of cannabinoids (THC, cannabinol and cannabidiol) and UV filters (4 MBC, OD-PABA, EHMC, OC) showed also a high HI (5.45), given their high bioaccumulation and toxicity. Since pharmaceutical compounds generally have low half-lives and toxicity, most of them also showed a comparatively low HI. Bioaccumulation of each particular pharmaceutic determined its final position in the HI ranking. Thus, irbesartan, loratadine, and sertraline were identified as hazardous compounds (HI > 5), which agree with previous results (Roos et al., 2012). In turn, ciprofloxacin, enalaprilat, iopromide, metformin andofloxacin, were selected as pharmaceutical compounds with a low hazard (HI = 1.24). Illicit drugs did not show any special trend, being well distributed in the whole range of HI values. As abovementioned, cannabinoids were among the most hazardous com-pounds. In contrast, benzoilecgonine hazard was poor (HI = 1.24). In the group of pesticides, hexythiazox was the most hazardous (HI = 5.53), while Omethoate presented the lowest HI (1.53).

3.2. IRICAP: Case-studies

An IRICAP value was estimated in each one of the 77 sampling points of the four Spanish rivers above described. IRICAP was estimated by the HI and the water concentration of each one of the 205 compounds (unpublished data). Final IRICAP values are summarized in Table 2, and also depicted for all the four river basins in Fig. 3. A high value of IRICAP is an indicator of more hazard, while a small value of IRICAP indicates water hazardless in terms of risks.

In the Llobregat area, sampling was performed in 14 points across the river basin, including the main course and two tributaries: Anoia and Cardener. In general, the water hazard in Cardener was lower than that in the 2 remaining rivers, as expected owing to its location in the upper basin. The IRICAP score for Cardener ranged between 5.26 (CAR2) and 5.59 (CAR3). CAR1 is considered a reference station with no (or minimum) anthropogenic pressures. Water presented a low risk, with an IRICAP score of 5.58. CAR4, located after a wastewater treatment plant in Manresa, showed an IRICAP value of 5.88. In contrast, highest levels of IRICAP were found in the lowest part of the river basin. Specifically, ANO2, which receives agricultural pressures and the discharges of an industrial pole located in the town of Igualada, showed a high IRICAP value (10.29). ANO3, located at the mouth of the tributary river, is also impacted by the waste waters from a population living close to the river (IRICAP = 7.09). Finally, the Llobregat river had an IRICAP ranging from 6.03 (LLO2) to 11.62 (LLO7). The IRICAP showed a logical tendency in Llobregat, as values increased downriver. The up river area (LLO1 and LLO2) is a mountainous zone with no agricultural and industrial activities. In the mid part of the river course (from LLO3 to LLO6), there are urban and industrial areas, while the final point on the mainstream (LLO7) is located near the mouth of the river close to the Barcelona town area, with high urban, industrial and agricultural activities in the surroundings. The IRICAP value in this last site was 11.62. It is the highest value of all the river basins, indicating a high degree of risk. The Ebro is the largest river of Spain and water was sampled in 24 points across the whole river basin. IRICAP ranged from 4.08 (GAL1) to 11.36 (ZAD). The Ebro river basin is constituted by several tributaries such as Arga, Cinca, Gallego and Matarranya, among others. GAL1 could be considered as a reference point (IRICAP = 4.08), whereas GAL2 is much more affected by agricultural activities (IRICAP = 5.93). The river mainstream, from EBR2 to EBR9, is severely affected by the anthropogenic activities of some cities located nearby: Miranda de Ebro (EBR2), Logroño (EBR4), Tudela (EBR5), and Tortosa (EBR8), all of them with important populations. Furthermore, some sites also receive the impact of agricultural and industrial activities, such as EBR3 (Haro) and EBR7 (Flix), respectively. Despite Flix

reservoir was previously identified as a "hot spot" of pollution due to accumulation of toxic sediments formerly discharged by an existing chloro-alkali industry, the sampling point presenting the highest risk was ZAD. It strongly receives the influence of the city of Vitoria, located a few kilometers upstream. It is important to highlight that the current IRICAP was exclusively developed taking into account the levels in the water compartment, while other environmental relevant compartment such as sediments or biota were disregarded. Pollutant levels of organic chemicals near Flix were not found to be of especial concern (Ferré-Huguet et al., 2009a; Ferré-Huguet et al., 2009b).

Table 2. Values for the IRICAP in the 77 sampling points studied.

Llobregat		**Ebro**		**Júcar**		**Guadalquivir**	
Site	**IRICAP**	**Site**	**IRICAP**	**Site**	**IRICAP**	**Site**	**IRICAP**
LLO7	11.62	ZAD	11.36	JUC8	7.80	GUAA	6.32
ANO2	10.29	HUE	8.47	JUC7	7.63	GUA3	5.93
LLO5	8.35	ARG	8.35	JUC4	7.07	GEN1	5.93
LLO6	7.75	EBR6	7.51	MAG1	6.97	GUA6	5.91
LLO1	7.60	EBR1	7.26	JUC6	6.79	GUA4	5.84
ANO3	7.09	EBR3	7.24	JUC1	6.52	HER	5.65
LLO4	6.92	EBR4	7.17	JUC2	6.47	GUA2	5.64
LLO3	6.88	EBR2	7.13	JUC5	6.43	GEN2	5.48
ANO1	6.76	OCA	7.10	CAB1	6.09	COR	5.41
LLO2	6.03	SEG	7.03	MAG2	6.04	GUAR	5.32
CAR4	5.88	EBR5	6.92	CAB3	6.01	GUAL	5.32
CAR3	5.59	EBR9	6.85	JUC3	5.79	CAC	5.26
CAR1	5.58	EBR8	6.59	CAB4	5.74	GUAN	5.16
CAR2	5.26	MAT	6.54	CAB5	5.64	GUA5	5.14
		MAR	6.40	CAB2	4.41	PIC	5.13
		EBR7	6.30			GUA8	5.11
		NAJ	6.25			GUA7	5.06
		CIN2	6.21			BOR	5.05
		ESE	6.01			GUA1	5.03
		GAL2	5.93			BEM	4.84
		RS	5.78			MAG	4.82
		ALG	5.53			GUAM	4.79
		CIN1	5.41			GUA9	4.71
		GAL1	4.08			YEG	4.69

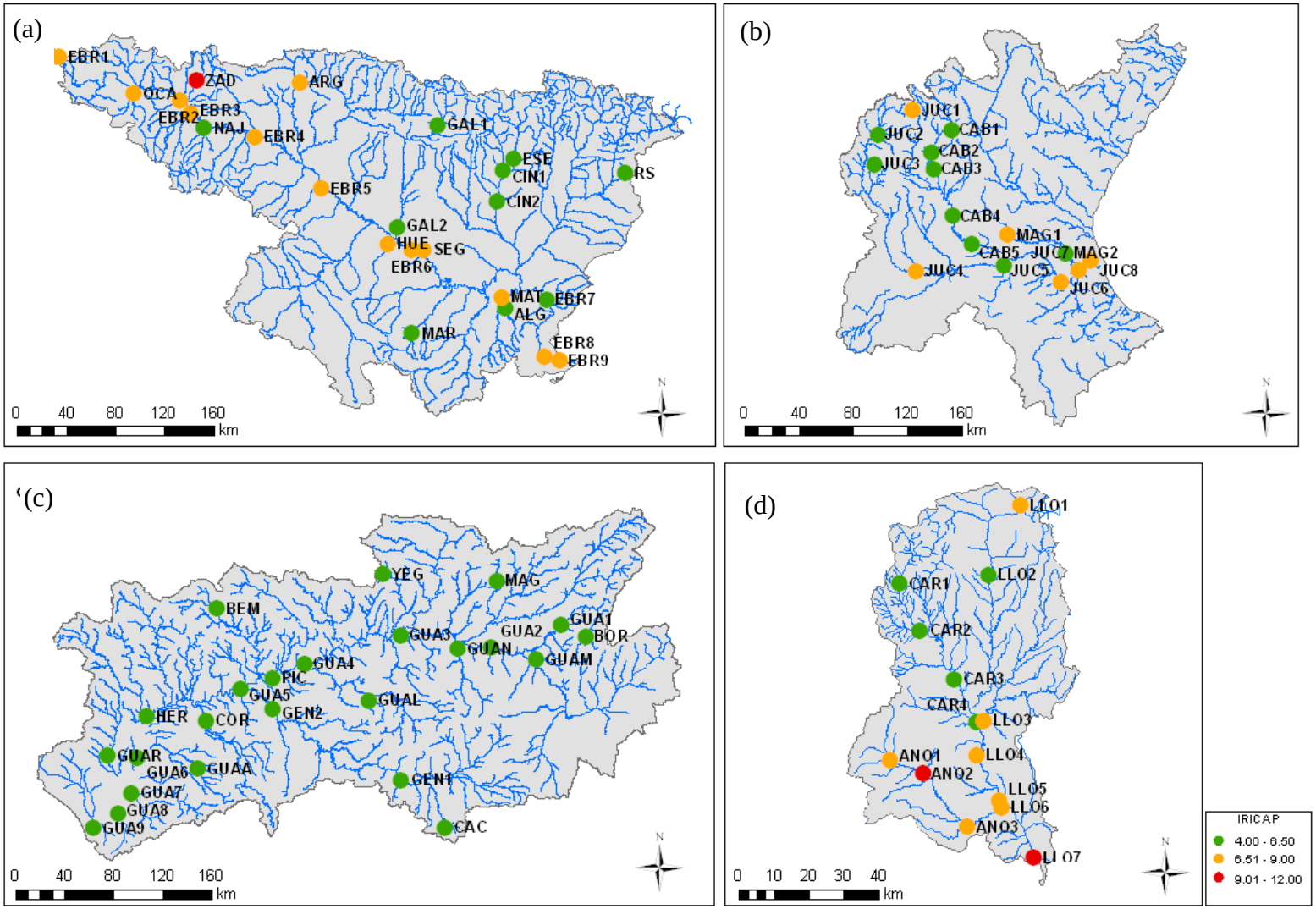


Fig. 3. IRICAP score of 4 Spanish river basins: (a) Ebro, (b) Jucar, (c) Guadalquivir, and (d) Llobregat.

Fifteen points were sampled in the Jucar river basin: 8 in the mainstream, 5 in Cabriel tributary, and the remaining 2 in Magro tributary. IRICAP ranged from 4.41 (CAB2) to 7.80 (JUC8). Cabriel showed lower IRICAP values (range: 4.41–6.09), indicating the quality of its waters. Cabriel River is located in a semiarid area with a low industrial/agricultural pressure. In Magro River, MAG1 showed the highest IRICAP score (6.97). It corresponds to a point just located after a village with no wastewater treatment plant. Therefore, sewage is directly discharged to the river waters, influencing the environmental conditions of the river at that point. The Jucar has several scenarios in the mainstream with some agricultural and industrial areas. JUC1 should be considered as a reference point (IRICAP = 6.52), although the risk score was somehow higher than that obtained in reference sites of other river basins. This value does not really differ from the IRICAP in JUC2 (6.47), which his affected by the Cuenca city, whose population exceeds 50,000 inhabitants. In JUC 3, the contamination from Cuenca city is diluted, resulting in a final IRICAP outcome of 5.79. From JUC4 to JUC 6, the IRICAP again increased (range: 7.07–7.63) as a consequence of the immediate affection of some small village and agricultural practices. Finally, JUC7 and JUC8 showed the highest IRICAP values (7.63 and 7.80, respectively) in the basin, which presents a high industrial activity in its mouth. In general terms, Guadalquivir was the river basin with the lowest IRICAP values, and consequently, with lower human health risks. The Guadalquivir river basin is constituted by other sub-river basins such as Yeguas, Bembézar, Guadaira or Genil, among others. Twenty-four sampling points were included. The IRICAP values ranged from 4.69 (YEG) to 6.32 (GUAA). Although notable high levels of metals were previously reported in Sanlcar de Barrameda (GUA9) (Kraus et al., 2006), a high degree of pollution is not reflected in our index, as only organic contaminants were considered in the development of the IRICAP. However, quantifiable amounts of some pharmaceutically active compounds have been recently reported in the waters of Doñana National Park (Camacho-Muñoz et al., 2010; Dowse et al., 2013).The most polluted area of the basin was GUAA (IRICAP = 6.32), a sampling point downstream a military area. On the other hand, the less polluted area corresponded to YEG (IRICAP = 4.69).

The results obtained after application of h-index and Zipf's law are summarized in Table 3. The h-index ranged between 2 and 3 in all the sampling points, except for ZAD, whose h-index was 1. This means that 2–3 compounds are the most important contributors to the IRICAP score, being the determination of their levels in river water sufficient as indicators of the total risk in terms of both hazard and pollutant concentration. More specifically, the contribution percentage of the 2–3 compounds with respect to the total IRICAP, defined as the percentage of h-values content, would range

from 1.74% to 13.68% (ZAD and CAB2, respectively). Between 35 and 96 compounds (CAB2 and ZAD, respectively) summed up to 90% of the IRICAP (Table 3, P90). The current results denote that more than one-half of the 205 compounds here assessed play a minor role, since their aggregated contribution to the risk is less than 10%. Finally, the specific h-compounds for each sampling point are given in Table 4. In 3 of the river basins, xylazine was identified as one of the h-compounds. Xylazine is a veterinary drug used for sedation, anesthesia, muscle relaxation, and analgesia in animals. Loratadine, pyriproxyphen and azaperol were also cataloged as h-compounds (in Ebro, Jucar and Guadalquivir, respectively). In contrast, the h-compounds in Llobregat river basin were loratadine and azaparone. According to this, we strongly suggest that xylazine, firstly, as well as other major pharmaceutical products (loratadine, azaperol, loratadine, and azaperone) and pesticides (pyriproxyphen) should be definitively included in the set of pollutants that are routinely measured in river waters. As top contributors to health risks, their levels should be controlled when implementing water monitoring programs in rivers with typical Mediterranean regimes. Power-law equations generally fitted well to the ranked list of compounds in each sampling site (regression coefficient R^2 between 0.47 and0.93). However, it must be also highlighted that ranges of the exponent (from 0.99 to 2.44) were relatively small (Table 3). Values of α exponent obtained in all the sampling points denote relatively flat curves, thus indicating that there is a lack of compounds with a prominent dominating weight. Therefore, the IRICAP-based risk load is not clustered with a few compounds.

Table 3. Results of *h*-index and Zipf's law applied on the IRICAP.

Sampling	*h*-index	*h*-content	P 90	α	Sampling	*h*-index	*h*-content	P 90	α
ANO1	3	9.98	62	1.74	CAB1	3	10.86	50	2.37
ANO2	2	4.66	90	1.25	CAB2	3	13.68	35	1.97
ANO3	2	6.41	84	1.45	CAB3	3	11.80	52	2.19
CAR1	2	6.48	56	1.87	CAB4	3	10.78	54	2.23
CAR2	2	6.87	53	1.95	CAB5	3	11.26	52	2.28
CAR3	2	6.46	57	1.88	JUC1	3	10.63	52	2.19
CAR4	2	6.14	68	1.54	JUC2	3	10.32	52	2.19
LLO1	3	10.36	60	2.00	JUC3	2	7.02	54	1.99
LLO2	3	10.43	52	1.87	JUC4	3	9.62	58	1.97
LLO3	2	6.72	63	1.67	JUC5	3	9.91	53	2.24
LLO4	2	6.32	63	1.65	JUC6	3	9.79	55	2.09
LLO5	2	6.52	76	1.38	JUC7	2	6.42	58	1.94
LLO6	2	5.38	79	1.36	JUC8	2	6.36	63	2.07
LLO7	2	4.36	95	1.11	MAG1	2	6.10	63	1.82
ALG	3	9.61	52	2.00	MAG2	2	6.73	58	2.08
ARG	2	4.13	83	1.34	BEM	3	11.10	50	2.20
CIN1	3	10.95	50	2.05	BOR	3	9.61	53	2.04
CIN2	2	6.69	56	1.88	CAC	2	8.18	48	2.40
EBR1	3	9.63	59	1.88	COR	2	7.07	52	2.11
EBR2	2	6.49	62	1.68	GEN1	2	6.70	61	1.84
EBR3	2	5.75	68	1.42	GEN2	2	7.20	59	1.93
EBR4	2	5.80	67	1.47	GUA1	3	10.68	50	2.22
EBR5	2	6.01	65	1.53	GUA2	2	7.39	59	1.88
EBR6	2	4.92	74	1.53	GUA3	2	7.09	60	2.02
EBR7	2	6.15	56	1.69	GUA4	2	7.46	58	1.79
EBR8	2	6.31	60	1.74	GUA5	3	10.42	55	1.99
EBR9	3	8.65	61	1.79	GUA6	2	7.12	63	1.85
ESE	3	9.87	51	1.96	GUA7	3	10.58	51	2.07
GAL1	3	13.44	36	1.95	GUA8	3	10.52	53	2.06
GAL2	2	6.19	59	1.86	GUA9	3	10.07	50	2.14
HUE	3	6.99	78	1.45	GUAA	2	6.48	64	1.81
NAJ	2	6.66	54	1.88	GUAL	2	9.18	53	2.13
MAR	2	6.50	52	1.92	GUAM	3	11.22	48	2.23
MAT	2	6.59	55	1.92	GUAN	3	10.41	59	1.89
OCA	2	5.86	63	1.75	GUAR	2	7.89	51	2.16
RS	3	10.26	49	2.14	HER	2	5.61	58	1.97
SEG	2	5.91	68	1.50	MAG	3	11.39	56	2.33
ZAD	1	1.74	96	0.99	PIC	3	10.49	52	2.03
					YEG	3	11.40	49	2.15

Table 4. List of *h*-compounds according to the IRICAP in 77 sampling sites in Spanish river basins.

Site	*h*-compounds			Site	*h*-compounds		
ANO1	Triclocaraban	Xylazine		CAB1	Pyriproxyphen	Xylazine	NP
ANO2	Triclosan	EDDPperchlorate		CAB2	Pyriproxyphen	NP	Dichlofenthion
ANO3	Loratadine	L-PFOS		CAB3	PFUdA	Xylazine	Pyriproxyphen
CAR1	Loratadine	Azaperone		CAB4	Xylazine	Pyriproxyphen	Hexythiazox
CAR2	Loratadine	Azaperone		CAB5	Xylazine	Pyriproxyphen	Buprofezin
CAR3	Loratadine	Azaperone		JUC1	Pyriproxyphen	Xylazine	Buprofezin
CAR4	Loratadine	Azaperone		JUC2	Pyriproxyphen	Xylazine	Desloratadine
LLO1	OC	EHMC	Hexythiazox	JUC3	NP	Hexythiazox	
LLO2	Xylazine	Paroxetine	Atorvastatin	JUC4	Dichlofenthion	Pyriproxyphen	Xylazine
LLO3	Triclocaraban	Xylazine		JUC5	Xylazine	Pyriproxyphen	Buprofezin
LLO4	Xylazine	Desloratadine		JUC6	Pyriproxyphen	Xylazine	Desloratadine
LLO5	PFTrDA	PFTeDA		JUC7	Dichlofenthion	Chlorpyriphos	
LLO6	Xylazine	Atorvastatin		JUC8	Hexythiazox	Ethion	
LLO7	Irbesartan	Sertraline		MAG1	Xylazine	Pyriproxyphen	
ALG	Triclocaraban	Xylazine	Paroxetine	MAG2	Xylazine	Pyriproxyphen	
ARG	Meloxicam	Azaperone		BEM	Xylazine	Azaperol	Desloratadine
CIN1	Xylazine	Loratadine	Meloxicam	BOR	Azaperone	Warfarin	Azaperol
CIN2	Xylazine	Loratadine		CAC	Xylazine	4MBC	
EBR1	Triclocaraban	Diethylstilbestrol	Xylazine	COR	Xylazine	Warfarin	
EBR2	Triclocaraban	Xylazine		GEN1	Xylazine	4MBC	
EBR3	Xylazine	Loratadine		GEN2	Xylazine	PFNA	
EBR4	Xylazine	Loratadine		GUA1	Xylazine	Azaperol	Desloratadine
EBR5	Xylazine	Loratadine		GUA2	Xylazine	11-nor-9-carboxy-9-	
EBR6	Loratadine	Meloxicam		GUA3	Xylazine	Estradiol (E2)	
EBR7	i,p-PFNA	Loratadine		GUA4	Xylazine	Desloratadine	
EBR8	Xylazine	Loratadine		GUA5	Xylazine	Azaperol	Diazepam
EBR9	Xylazine	Loratadine	Meloxicam	GUA6	Xylazine	Diazinon	
ESE	Xylazine	Loratadine	Meloxicam	GUA7	Xylazine	Azaperol	Diazepam
GAL1	Xylazine	Azaperone	Azaperol	GUA8	Xylazine	Azaperol	Desloratadine
GAL2	Loratadine	NP2EO		GUA9	terbutryn	Desloratadine	Alprazolam
HUE	Xylazine	Loratadine	Meloxicam	GUAA	Xylazine	NP	
NAJ	Xylazine	Loratadine	Meloxicam	GUAL	4MBC	Xylazine	
MAR	Xylazine	Loratadine		GUAM	Xylazine	Azaperol	Desloratadine
MAT	Xylazine	Fenthion		GUAN	Xylazine	Azaperol	Desloratadine
OCA	Xylazine	Loratadine		GUAR	Xylazine	NP	
RS	Xylazine	Loratadine	Meloxicam	HER	Propilparaben	Azaperol	
SEG	Xylazine	Loratadine		MAG	Xylazine	Estrone (E1)	Azaperol
ZAD	Fluoxetine			PIC	Xylazine	Azaperol	Desloratadine
				YEG	Xylazine	Desloratadine	Alprazolam

4. Conclusions

IRICAP means an important effort to elaborate methods for assessing human health risks associated to exposure to chemical mixtures, or the aggregated exposure to chemicals. Although interactions have not been considered in the current study, this tool was able to easily integrate a large amount of compounds to establish similar patterns, with the ultimate goal of prioritizing contaminants in terms of health risks. IRICAP showed logical and reliable results in most sampling points, taking into account the chemical characteristics of each site. Furthermore, IRICAP considers the joint effect of the chemical mixture, but not single groups of pollutants. Consequently, although some places (e.g., agricultural areas) are known to have high concentrations of pesticides, this effect is not reflected in IRICAP because of their low weight vs. other contaminants with a higher HI or concentration. An important limitation of the IRICAP is the use of theoretical values as HI parameters. Data on persistence, bioaccumulation and toxicity for each one of the 205 compounds were derived by applying the US EPA EPI Suit™ software, which is a very powerful tool to get estimative values when experimental information is not available. However, the process of modeling PBT data may be inherently associated to a high uncertainty. This is especially remarkable in the variable "toxicity". In this case, only fish toxicity values were used to build the HI by means of the ECOSAR™ tool, which is in turn based on Kow levels. This approach may lead to a significant bias, as the impact on aquatic species of other steps in the food web, or even on the human health, is not taken into account. In this framework, further improvements of the IRICAP, in general, and the hazard index, in particular, should be focused on incorporating as many species as possible. Species Sensitivity Distributions (SSDs) are distributions of species' responses to a given toxicant (Dowse et al., 2013). Using SSDs, instead of point toxicity values, could reduce the exclusion of key pollutants, for which fish toxicity is not significant, but the effects on other species may be notable. In order to solve other limitations of this index, as well as to improve the robustness of the model, further studies should include other groups of pollutants, such as heavy metals and POPs and include, if possible, other environmental compartments (sediments and biota). In addition, validation of the index should be considered in future studies by comparing IRICAP values with scores obtained by applying biological indices. Pareto distribution-based indices have been proven to be a useful complement for risk assessment. In the current study, the final results allowed estimating the distribution of the compounds in the curve. Furthermore, the h-compounds, this is, those with a highest contribution on the hazard/concentration-based risk, were identified. Xylazine, as well as loratadine, azaperol, loratadine, azaperone, and pyriproxyphen, should be selected as key

pollutants when measuring the chemical pollution of fresh waters, at least in rivers of similar characteristics to those here evaluated. In conclusion, these chemicals must be priority pollutants in quality control monitoring networks of river basins. IRICAP may be a useful tool for stakeholders involved in water management, for its capabilities to evaluate and compare human risks in water river samples.

References

Alhoniemi, E., Hollmén, J., Simula, O., Vesanto, J., 1999. Process monitoring and modeling using the Self-Organizing Map. Integrated Computer Aided Engineering 6, 3–14.

Alvarez-Guerra, M., González-Piñuela, C., Andrés, A., Galán, B., Viguri, J.R., 2008. Assessment of Self-Organizing Map artificial neural networks for the classification of sediment quality. Environment International 34, 782–790.

Arias, R., Barona, A., Ibarra-Berastegi, G., Aranguiz, I., Elías, A., 2008. Assessment of metal contamination in dregded sediments using fractionation and Self-Organizing Maps. Journal of Hazardous Materials. 151, 78–85.

Arnot, J.A., Gobas, F., 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environmental Reviews 14, 257–297.

Arnot, J.A., Mackay, D., 2008. Policies for chemical hazard and risk priority setting: Can persistence, bioaccumulation, toxicity, and quantity information be combined? Environmental Science & Technology 42, 4648–4654.

ATSDR, The Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures, Agency for Toxic Substances and Disease Registry, US,2004, http://www.atsdr.cdc.gov/interactionprofiles/IP-ga/ipga.pdf (accessed 25.03.2013).

Backhaus, T., Faust, M., 2012. Predictive environmental risk assessment of chemical mixtures: A conceptual framework. Environmental Science & Technology 46, 2564–2573.

Boethling, R.S., Howard, P.H., Meylan, W.M., 2004. Finding and estimating chemical property data for environmental assessment. Environmental Toxicology & Chemistry 23, 2290–2308.

Boethling, R.S., Howard, P.H., Meylan, W., Stiteler, W., Beauman, J., Tirado, N., 1994. Group contribution method for predicting probability and rate of aerobic biodegradation. Environmental Science & Technology 28, 459–465.

Boobis, A., Budinsky, R., Collie, S., Crofton, K., Embry, M., Felter, S., Hertzberg, R., Kopp, D., Mihlan, G., Mumtaz, M., Price, P., Solomon, K., Teuschler, L., Yang, R., Zaleski, R., 2011. Critical analysis of literature on low-dose synergy for use in screening chemical mixtures for risk assessment. Critical Reviews in Toxicology 41, 369–383.

Cabeza, Y., Candela, L., Ronen, D., Teijon, G., 2012. Monitoring the occurrence of emerging contaminants in treated wastewater and groundwater between 2008 and 2010. The Baix Llobregat (Barcelona, Spain). Journal of Hazardous Materials 239–240, 32–39.

Camacho-Muñoz, M.D., Santos, J.L., Aparicio, I., Alonso, E., 2010. Presence of pharmaceu-tically active compounds in Doñana Park (Spain) main watersheds. Journal of Hazardous Materials 177, 1159–1162.

Carafa, R., Faggiano, L., Real, M., Munné, A., Ginebreda, A., Guasch, H., Flo, M., Tirapu, L., von der Ohe, P.C., 2011. Water toxicity assessment and spatial pollution

patterns identification in a Mediterranean river basin district. Tools for water management and risk analysis. Science of the Total Environment 409, 4269–4279.

Dowse, R., Tang, D., Palmer, C.G., Kefford, B.J., 2013. Risk assessment using the species sensitivity distribution method: Data quality versus data quantity. Environmental Toxicoogy & Chemistry 32, 1360–1369.

EC, Revised proposal for a list of priority substances in the context of the WaterFramework Directive (COMMPS Procedure) 98/788/3040/DEB/E1, Fraunhofer-Institut Umweltchemie und Ökotoxicologie, 1999.

Fernández-Turiel, J.L., Gimeno, D., Rodriguez, J.J., Carnicero, M., Valero, F., 2003. Spatial and seasonal variations of water quality in a Mediterranean catchment: The Llobregat river (NE Spain). Environmental Geochemistry & Health 25, 453–474.

Ferré-Huguet, N., Bosch, C., Lourencetti, C., Nadal, M., Schuhmacher, M., Grimalt, J.O., Domingo, J.L. 2009a. Human health risk assessment of environmental exposure to organochlorine compounds in the Catalan stretch of the Ebro River, Spain. Bulletin of Environmental Contamination & Toxicology 83, 662–667.

Ferré-Huguet, N., Nadal, M., Schuhmacher, M., Domingo, J.L., 2009b. Human health risk assessment for environmental exposure to metals in the Catalan stretch of the Ebro River, Spain. Human & Ecological Risk Assessment 15, 604–623.

Ginebreda, A., Jelic, A., Petrovic, M., de Alda, M.L., Barceló, D., 2011. New indexes for compound prioritization and complexity quantification on environmental monitoring inventories. Environmental Science & Pollution Research 19, 958–970.

Giorgi, F., Lionello, P., 2008. Climate change projections for the Mediterranean region. Global & Planetary Change 63, 90–104.

Gómez, G., Baos, R., Gómara, B., Jiménez, B., Benito, V., Montoro, R., Hiraldo, F., González, M.J., 2004. Influence of a mine tailing accident near Doñana National Park (Spain) on heavy metals and arsenic accumulation in 14 species of waterfowl (1998–2000). Archives of Environmental Contamination & Toxicology 47, 521–529.

Hirsch, J.E., 2005. An index to quantify an individual's scientific research output. Proceedings of the National Academy of Sciences U.S.A. 102, 16569–16572.

Hisano, R., Sornette, D., Mizuno, T., 2011. Predicted and verified deviations from Zipf's law in ecology of competing products. Physical Review E, Statistical Nonlinear & Soft Matter Physics 84 (2011) 026117.

Kalteh, A.M., Hjorth, P., Berndtsson, R., 2008. Review of the Self-Organizing Map (SOM) approach in water resources: analysis, modelling and application. Environmental Modelling & Software 23, 835–845.

Kohonen, T., 1982. Self-organized formation of topologically correct feature maps. Biological Cybernetics 43, 59–69.

Kohonen, T., 2013. Essentials of the Self-Organizing Map. Neural Networks 37, 52–65.

Kraus, U., Wiegand, J., 2006. Long-term effects of the Aznalcóllar mine spill–heavy metal content and mobility in soils and sediments of the Guadiamar river valley (SW Spain). Science of the Total Environment 367, 855–871.

López-Doval, J.C., De Castro-Català, N., Andrés-Doménech, I., Blasco, J., Ginebreda, A., Muñnoz, I., 2012. Analysis of monitoring programmes and their suitability for ecotoxicological risk assessment in four Spanish basins. Science of the Total Environment 440, 194–203.

Nadal, M., Kumar, V., Schuhmacher, M., Domingo, J.L., 2006. Definition, GIS-based characterization of an integral risk index applied to a chemical/petrochemical area. Chemosphere 64, 1526–1535.

Nadal, M., Kumar, V., Schuhmacher, M., Domingo, J.L., 2008. Applicability of a neuro-probabilistic integral risk index for the environmental management of polluted areas: A case study. Risk Analysis 28, 271–286.

Navarro-Ortega, A., Acuña, V., Batalla, R.J., Blasco, J., Conde, C., Elorza, F.J., Elosegi, A., Francés, F., La-Roca, F., Muñoz, I., Petrovic, M., Picó, Y., Sabater, S., Sanchez-Vila, X., Schuhmacher, M., Barceló D., 2012. Assessing and forecasting the impacts of global change on Mediterranean rivers. The SCARCE Consolider project on Iberian basins. Environmental Science & Pollution Research 19, 918–933.

Newman, M.E.J., 2005. Power laws, Pareto distributions and Zipf's law. Contemporary Physics 46, 323–351.

Mari, M., Nadal, M., Schuhmacher, M., Domingo, J.L., 2008. Application of Self-Organizing Maps for PCDD/F pattern recognition of environmental and biological samples to evaluate the impact of a hazardous waste incinerator. Environmental Science & Technology 44, 3162–3168.

McCarty, L.S., Borgert, C.J., 2006. Review of the toxicity of chemical mixtures: theory, policy, and regulatory practice. Regulatory Toxicology & Pharmacology 45, 119–143.

Meylan, W.M., Howard, P.H., Aronson, D., Printup, H., Gouchie, S., 1997. Improved method for estimating bioconcentration factor (BCF) from octanol-water partition coefficient. U.S. Environmental Protection Agency, SRC TR-97-006.

Meylan, W.M., Howard, P.H., Boethling, R.S., Aronson, D., Printup, H., Gouchie, S., 1999. Improved method for estimating bioconcentration/bioaccumulation factor from octanol/water partition coeficient. Environmental Toxicology & Chemistry 18, 664–672.

Mola, M., Palomo, M., Peñalver, A., Aguilar, C., Borrull, F., 2011. Distribution of naturally occurring radioactive materials in sediments from the Ebro river reservoir in Flix (Southern Catalonia, Spain). Journal of Hazardous Materials 198, 57–64.

Myers, A.L., Crozier, P.W., Helm, P.A., Brimacombe, C., Furdui, V.I., Reiner, E.J., Burniston, D., Marvin, C.H., 2012. Fate, distribution, and contrasting temporal trends of perfluoroalkyl substances (PFASs) in Lake Ontario, Canada. Environment International 44, 92–99.

Ocampo-Duque, W., Juraske, R., Kumar, V., Nadal, M., Domingo, J.L., Schuhmacher, M., 2012. A concurrent neuro-fuzzy inference system for screening the ecological risk in rivers. Environmental Science & Pollution Research 19, 983–999.

Pennington, D.W., Bare, J.C., 2001. Comparison of chemical screening and ranking approaches: the waste minimization prioritization tool versus toxic equivalency potentials. Risk Analysis 21, 897–912.

Petrovic, M., Ginebreda, A., Acuña, V., Batalla, R.J., Elosegi, A., Guasch, H., de Alda, M.L., Marcé, R., Muñoz, I., Navarro-Ortega, A., Navarro, E., Vericat, D., Sabater, S., Barceló, D., 2011. Combined scenarios of chemical and ecological quality under water scarcity in Mediterranean rivers. Trends in Analytical Chemistry. 30, 1269–1278.

Roos, V., Gunnarsson, L., Fick, J., Larsson, D.G.J., Rudén, C., 2012. Prioritising pharmaceuticals for environmental risk assessment: Towards adequate and feasible first-tier selection. Science of the Total Environment 421-422,102–110.

Sanderson, H., Johnson, D.J., Wilson, C.J., Brain, R.A., Solomon, K.R., 2003. Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids and algae by ECOSAR screening. Toxicology Letters 144, 383–395.

Sumpter, J.P., Johnson, A.C., Williams, R.J., Kortenkamp, A., Scholze, M., 2006. Modeling effects of mixtures of endocrine disrupting chemicals at the river catchment scale. Environmental Science & Technology 40, 5478–5489.

Teuschler, L.K., 2007. Deciding which chemical mixtures risk assessment methods work best for what mixtures. Toxicology & Applied Pharmacology 223, 139–147.

Thomas, B.F., Compton, D.R., Martin, B.R., 1990. Characterization of the lipophilicity of natural and synthetic analogs of delta-9-tetrahydrocannabinol and its relationship to pharmacological potency. Journal of Pharmacology & Experimental Therapeutics 255, 624–630.

Wilbur, S.B., Hansen, H., Pohl, H., Colman, J., McClure, P., 2004. Using the ATSDR guidance manual for the assessment of joint toxic action of chemical mixtures. Environmental Toxicology and Pharmacology 18, 223–230.

Discussion of Chapter I

Up to 205 environmental contaminants were ranked according their Hazard Index (HI), which, in turn was basedon the PBT properties of the chemicals. The whole list included 81 pharmaceutical compounds, 19 illicit drugs, 31 endocrine disruptors (ED), 42 pesticides, 11 UV filters and 21 perfluoroalkyl substances (PFASs). PBT data were derived by applying EpiSuit^{TM}, due to the impossibility to found experimental data in the scientific literature. PFASs were characterized by a high environmental persistence and aquatic toxicity, but relatively low bioaccumulation potential. A cluster of 16 PFASs was identified as the group of compounds with the highest half-lives. PFASs were ranked as the most harmful familiy of compounds, with a HI of 5.58 in a scale from 0 to 10. PFASs were subsequently chose to develop the PBPK model, due to the harmful effects of the whole family of compounds, and the relatively similar behaviour with PCDD/Fs. The groups of cannabinoids (THC, cannabinol and cannabidiol) and UV filters (4 MBC, OD-PABA, EHMC and OC) also showed a high HI (5.45).

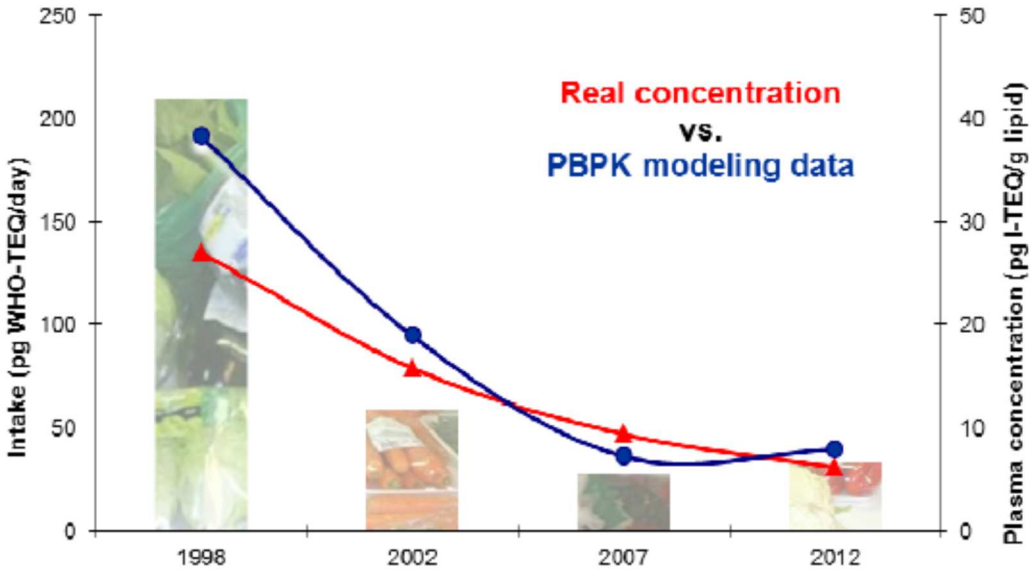
A specified risk ranking (IRICAP) was designed to identify the environmental pollutants posing the highest risk for the human health in freshwater. IRICAP was the product of the HI and the respective concentration of each chemical in water. This tool allows assessing the aggregated effect of chemical mixtures. IRICAP showed logical and reliable results in most sampling points, taking into account the chemical characteristics of each site. Although the interactions of chemical pollutants were not taken into account in the IRICAP, this index improved previous PBT-based rankings.

CHAPTER 2

PCDD/Fs IN PLASMA OF INDIVIDUALS LIVING NEAR A HAZARDOUS WASTE INCINERATOR. A COMPARISON OF MEASURED LEVELS AND ESTIMATED CONCENTRATIONS BY PBPK MODELING

Abstract

The construction of the first and, until now, only hazardous waste incinerator (HWI) in Spain finished in 1998. To assess its potential impact on the population living in the vicinity, a surveillance program was established. It includes the periodical biomonitoring of PCDD/Fs body burden. On the basis of this program, in 2012 we determined the levels of PCDD/Fs in plasma of nonoccupationally exposed individuals living near the HWI. The results were compared with those of the baseline study, and with those of two previous surveys (2002 and 2007). A multicompartment, physiologically-based pharmacokinetic (PBPK) model was also applied to estimate the levels of PCDD/Fs in plasma. The model was validated by comparing the results with our experimental data (baseline, 2002, 2007, and 2012). The current mean concentration was 6.18 pg I-TEQ/g lipid, with a range between 2.03 and 18.8 pg I-TEQ/g lipid. In 1998 (baseline), the mean concentration of PCDD/Fs in plasma was 27.0 pg I-TEQ/g lipid (reduction of 77%, $p< 0.001$). Significant reductions were also noted in our previous 2002 and 2007 surveys, with mean concentrations of 15.7 and 9.36 pg I-TEQ/g lipid, respectively. However, the comparison between simulated (using the PBPK model) and experimental results was very successful, as PCDD/F values in plasma were very similar (7.95 vs 6.18 pg I-TEQ/g lipid). The levels of PCDD/Fs in plasma of nonoccupationally exposed individuals living near the HWI here assessed are comparatively lower than most recently reported values.



1. Introduction

If not safely managed and disposed, hazardous waste (HW) may mean risk to human health and the environment. According to Eurostat (European Commission, 2012) in 2010, a total of 94.5 million tons of HW were generated in the EU-27, that amount being higher than that generated in 2004 (89 million tons), but lower than the amounts generated in 2006 (101 million tons) and 2008 (98 million tons). For the treatment of HW, Eurostat indicates that about 9.8 million of tons (or 13.2% of all HW) were incinerated or used for energy recovery, while 29.1 million of tons (or 39.2% of all HW) were recovered (European Commission, 2012). Although waste combustion, including HW, offers a number of advantages (i.e., volume reduction and energy recovery) with respect to other waste management strategies (Domingo et al., 2002), the construction and/or operation of incinerators has been, and remains, an issue of considerable public concern. It is well-known that waste incinerators emit pollutants such as heavy metals and organic chemicals, whose potential toxicity at certain environmental concentrations is well-established. With respect specifically to HW incineration, although the number of HW incinerators (HWIs) is notably lower than that of municipal solid waste incinerators (MSWIs), and the volume of waste to be treated is remarkably different, special attention has been paid to those facilities (García-Pérez et al., 2013), as a consequence of the notable pressure that plants managing hazardous waste receive from the administration and the people living nearby.

In 1999, the first HWI in Spain initiated its regular operations in Constantí (Tarragona County, Catalonia). This facility started its activities before the Waste

Incineration Directive (WID) entered into force in December 2000 (European Commission, 2000). However, since the construction of the facility generated an important concern among the local population, on behalf of the Catalan Agency of Waste, we initiated a wide environmental surveillance program during the construction of the HWI (baseline study, 1996−1998). This program was focused on evaluating the environmental impact of the stack emissions, particularly of metals and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) on the environment, as well as assessing the health risks for the population living in the area under potential influence of the HWI. Although metals and PCDD/Fs are produced in many processes, incineration has received prolonged special attention by scientists and politicians (Domingo et al., 2002; Mari et al., 2010; Tian et al., 2012). Our surveillance program is in addition to the requirements of the WID, which sets emission limit values and monitoring requirements for air pollutants such as dust, nitrogen oxides (NOx), sulfur dioxide (SO_2), hydrogen chloride (HCl), hydrogen fluoride (HF), heavy metals, and PCDD/Fs. Since then, the program has been continuously maintained, measurements of the pollutants in different environmental and/ or biological matrixes being carried out annually (Bocio et al., 2005; Ferré-Huguet et al., 2005; Ferré-Huguet et al., 2009; Nadal et al., 2005; Nadal et al., 2009; Schuhmacher et al., 2002a; Schuhmacher et al., 2002b; Schuhmacher et al., 2004a; Schuhmacher et al., 2004b; Vilavert et al., 2010).

The aim of the present study was to determine, after approximately 13 years of regular operations in the HWI, the concentrations of PCDD/Fs in plasma samples of individuals living in the neighborhood of the facility. In order to assess the temporal trend of these pollutants in the general, nonoccupationally exposed population of the area, the results of this survey have been compared with those of the baseline survey (Schuhmacher et al., 1999), and also with those of two previous campaigns carried out after the plant initiated its regular activities (2002 and 2007) (Agramunt et al., 2005; Nadal et al., 2008). We have also applied a multicompartment physiologically-based pharmacokinetic (PBPK) model in order to estimate the levels of PCDD/Fs in plasma. The model was validated by comparing the results with our experimental data, not only from the current, but also from our previous surveys (1998, 2002, and 2007) (Agramunt et al., 2005; Nadal et al., 2008; Schuhmacher et al., 1999).

2. Materials and Methods

2.1. Area of Study

The HWI herein studied is located in the industrial park of Constantí, relatively close to other significant industrial sources of environmental pollutants, such as a big oil refinery, an important complex of petrochemical industries, and a municipal solid waste incinerator among others. The plant is situated 5 km from the closest populated nuclei (Constantí and Reus), and 10 km from Tarragona city. Despite the presence of some chemical industries, the closest land to the plant is mainly used for agricultural purposes, in which hazel and olives are the predominant vegetal species. A few chicken farms are also sporadically situated in the surrounding zone. The number of farm workers is relatively reduced, as the main activities in the zone are oriented to the secondary and tertiary sectors of the economy.

2.2. Sampling

Blood collection was done in collaboration with the Joan XXIII and Santa Tecla Hospitals (Tarragona, Spain), where people living in the zone under the potential influence of the HWI emissions are mostly treated. Some samples were also obtained through blood donors of the area, with the collaboration of the Blood and Tissue Bank of the Vall d'Hebron Hospital (Barcelona, Spain). Plasma samples (80 mL per sample) were obtained from 40 individuals. All participants in the study had lived at least during the last 10 years in zones near the HWI. All individuals with potential occupational exposure to PCDD/Fs, including welders and smelters, stations workers, PVC workers, and workers in waste incinerators, were excluded. No further consideration was done according to the eating habits of the subjects. People followed regular Mediterranean diets, characterized by a higher contribution of fruits, vegetables, and cereals. In turn, eggs and pulses showed the lowest values in the weekly intake.

Blood samples were collected from individuals of both genders (20 men and 20 women) and different ages (22−67 years). As in our previous surveys, 18−20 volunteers were also classified according to the specific place of residence: urban (living in Tarragona City) or industrial (living near incinerators and chemical/petrochemical industries) areas. Once collected, samples were kept at −20 °C until analysis.

2.3. Analytical Procedure

PCDD/F concentrations in plasma samples were determined following a methodology derived from the U.S. EPA methods 1613 and 8290A. Forty mL of a standard solution containing a mixture of $^{13}C_{12}$−PCDD/Fs was added to 75 g of plasma. Lipid extraction was carried out using a mixture of isopropanol/hexane through a solid phase (marine plankton diatomite). The concentrated lipids were calculated by gravimetry. For cleaning, extracts were passed through a column of acid/basic silica gel. Then, 40 mL of tetradecane was added as a control. After being concentrated to 1 mL, solutions were passed through a second column with activated Al_2O_3 and Na_2SO_4. Fractionation was performed with hexane. The third fraction, which contained PCDD/Fs, was immediately passed through a column of charcoal. A mixture (18%/82%) of 0.5 g of Carbopack C (Supelco Inc., Bellefonte, PA, U.S.) and Celite 545 (Fluka AG, Buchs, Switzerland) was used. Finally, the extract was evaporated to dryness with a stream of nitrogen, being redissolved with a solution containing $^{13}C_6$-1,2,3,4- TCDD until a final volume of 20 mL with ultrasonic agitation.

Cleaned extracts were analyzed by high resolution gas chromatography/high resolution mass spectrometry (HRGC/ HRMS), with selected ion recording at resolution >10000 and working with SIR mode (Selected Ion Recording). The calculation of the toxic native compounds was made by using the corresponding $^{13}C_{12}$−PCDD/F internal standards, automatically correcting for any potential losses occurring during the processes of extraction, cleanup, and analysis. To check the reliability of the method and the apparatus, blanks and standards were set during the analytical procedure. The average recovery percentages ranged between 53% and 95%.

2.4. Data treatment

Toxic equivalents (TEQs) of the analyzed PCDD/Fs were calculated using both international NATO/ CCMS toxic equivalency factors (I-TEFs) and WHO-$TEFs_{2005}$ (Van den Berg et al., 2006; Van Zorge et al., 1989). Data were statistically evaluated using the SPSS 19.0 software package. The Levene test was applied to study the equality of variances. Subsequently, statistical significance between groups was determined by using analysis of variance (ANOVA) or a Kruskal−Wallis test, respectively, depending on whether data followed a normal distribution or not. A probability lower than 0.05 ($p < 0.05$) was considered as statistically significant. For calculations, the concentrations of those PCDD/F congeners with a value below the limit of detection (LOD) were considered to be one-half of the respective LOD (ND = 1/2 LOD).

3. Results and Discussion

3.1. Concentration of PCDD/Fs in human plasma

Table S1 (Annex 2) summarizes the individual concentrations of PCDD/Fs in plasma of 40 subjects living in the vicinity of the HWI here assessed. The mean concentration was 6.18 pg I-TEQ/g lipid (6.26 pg WHO-TEQ/g lipid), with a range between 2.03 and 18.8 pg I-TEQ/g lipid (2.16−25.4 pg WHO-TEQ/g lipid). Table 1 shows a general comparison between the results obtained in the baseline (1998) and the current surveys. In 1998, the mean concentration of PCDD/Fs in plasma was 27.0 pg I-TEQ/g lipid ($n = 20$), which significantly decreased to 6.18 pg I-TEQ/g lipid (reduction of 77%, $p < 0.001$) in the present study. Considerable and significant reductions in the PCDD/F levels in plasma were already noted in our previous 2002 and 2007 surveys, with mean concentrations of 15.7 and 9.36 pg I-TEQ/g lipid, respectively (Agramunt et al., 2005; Nadal et al., 2008). There was a significant ($p < 0.001$) reduction in the PCDD/F concentrations for both genders between the baseline survey (1998) and the current study. Pearson correlation was applied to analyze the influence of age on body levels of PCDD/Fs. A significant decrease in the levels of these pollutants was noted for all age groups. In contrast to other studies (Huang et al., 2007), no correlation between the concentrations of PCDD/Fs in plasma and age was found. However, in the present study, PCDD/F levels in plasma of the residents in urban zones were slightly higher than the mean level of subjects living in industrial areas (7.15 and 5.21 pg I-TEQ/g lipid, respectively). However, the difference did not reach a level of statistical significance ($p > 0.05$). A very notable and significant decrease in the mean concentrations of PCDD/Fs in plasma between the baseline (1998) and the current survey was found for the individuals living in both areas. The temporal evolution of the PCDD/F levels in plasma of the population living near the HWI is depicted in Figure S1 (Annex 2).

Table 1. Total concentration of PCDD/Fs (in pg I-TEQ/g lipid) in plasma of residents nearby the HWI, according to gender, age and specific area of residence (industrial or urban).

	No. Samples		Mean		Standard deviation		Range		Variation %
	1998	2012	1998	2012	1998	2012	1998	2012	1998-2012
Total	20	40	27.0	6.18	8.2	4.06	14.8-49.0	2.03-18.8	-77**
Gender									
Male	13	20	26.7	6.48	2.5	4.52	14.8-49.0	2.11-18.8	-76**
Female	7	20	27.8	5.88	2.7	3.63	14.9-34.7	2.03-17.5	-79**
Age									
18-30	4	4	17.2	6.26	3.8	3.98	14.8-22.9	3.82-12.1	-64*
31-45	4	10	25.2	4.93	5.0	2.85	17.9-29.2	2.04-9.21	-80**
46-55	8	19	32.0	5.18	8.4	3.18	21.2-48.9	2.03-13.3	-84**
>56	4	7	28.8	10.4	5.1	5.45	23.9-33.8	5.72-18.8	-64**
Area									
Industrial	15	20	26.4	5.21	8.8	3.18	14.8-49.0	2.03-12.1	-80**
Urban	5	20	28.7	7.15	7.1	4.66	16.5-34.7	2.04-18.8	-75**

Significant differences at: *p < 0.01; **p < 0.001.

Nowadays, it is well-known that for nonoccupationally exposed populations, diet is the main route of exposure to PCDD/Fs, being the percentages of the daily intake of PCDD/Fs through food consumption even higher than 95% (Domingo et al., 2007; Linares et al., 2010; Perelló et al., 2012). On the basis of this, we have also periodically measured the levels of PCDD/Fs in the most consumed food items in the zone under evaluation, and determined the dietary intake of these pollutants by the local population (Bocio et al., 2005; Domingo et al., 1999; Llobet et al., 2008). In the baseline survey (1998), the dietary intake of PCDD/Fs was 210.1 pg I-TEQ/ day, while the current dietary intake was estimated in 33.1 pg WHO-TEQ/day (Domingo et al., 2012). This intake was similar to that obtained in our previous survey (27.8 pg WHO-TEQ/day) (Llobet et al., 2008), and lower than that found in the 2002 study (59.6 pg I-TEQ/day). It is evident that there is a parallel decrease and a direct relationship between the exposure to PCDD/Fs through the diet, and the concentrations of PCDD/Fs in the plasma of the population living in the area under the current evaluation. It has been suggested that there might be

some delay between the dietary intake of PCDD/Fs and the detection of these contaminants in biological tissues, such as plasma or breast milk (Nadal et al., 2008). The reduction in the levels of PCDD/Fs in foodstuffs and the corresponding decrease in the concentration of PCDD/Fs in human tissues and biological matrixes agree well with the results of other recent international studies (Consonni et al., 2012; Lakind et al., 2009; Lopez-Espinosa et al., 2008). Moreover, it is important to note that in an additional monitoring program, which we annually perform to determine the levels of PCDD/ Fs in blood of the workers at the same HWI, we have also observed a significant and continued reduction in these levels, which decreased from an initial (baseline values) mean concentration of 26.7 pg I-TEQ/g lipid to 4.6 pg I-TEQ/g lipid in 2011 (Domingo et al., 2001; Mari et al., 2013). The profiles of the PCDD/F congeners in the 4 campaigns since the baseline (1998) study are depicted in Fig. 1. In the current (2012) survey, OCDD was the predominant congener, followed by 1,2,3,6,7,8 HxCDD and 1,2,3,4,6,7,8-HpCDD, while the lowest concentrations corresponded to 2,3,7,8-TCDD and 1,2,3,7,8,9-HxCDF.

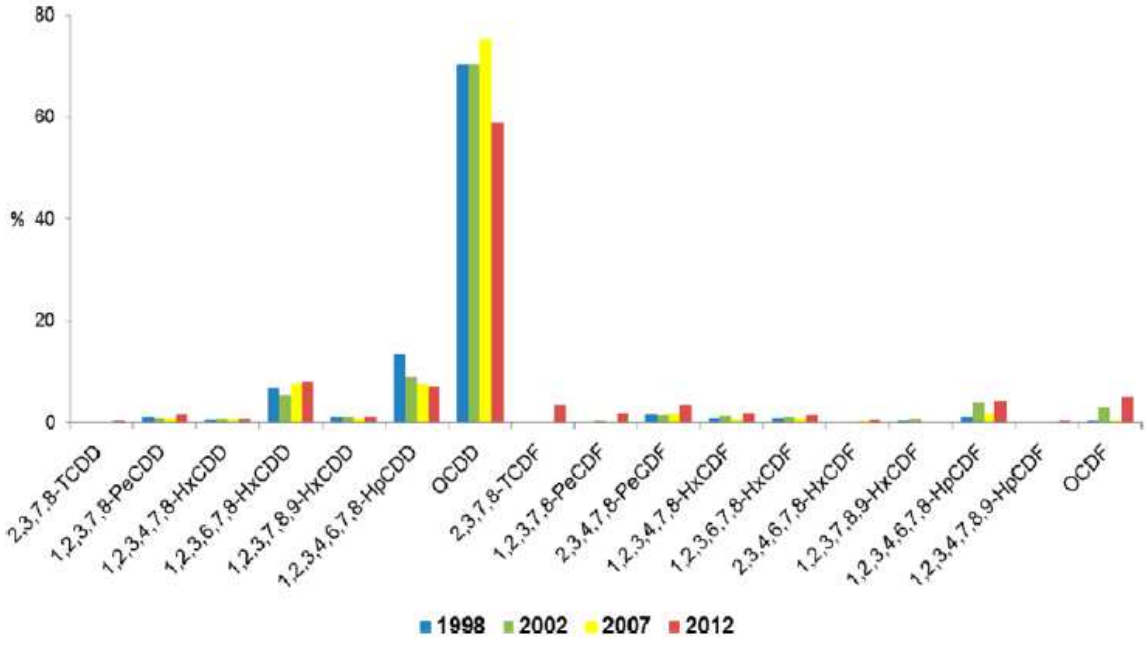


Fig. 1. PCDD/F congener profiles in plasma of non-occupationally exposed subjects living in the vicinity of a HWI. Temporal trends.

Data on PCDD/F concentrations in blood of nonoccupationally exposed populations are rather scarce. While in the scientific literature, a number of reports concerning the levels of PCDD/Fs in human blood of populations living in the neighborhood of MSWIs has been published, information regarding PCDD/F levels in subjects living near HWIs and industrial incinerators is tremendously limited. In Missouri

(U.S.), Evans et al., (2000) assessed whether living in the vicinity of a HWI in Times Beach, which was burning 2,3,7,8-TCDDcontaminanted material, increased TCDD and toxicity equivalencies (TEQ) in individuals living near the facility. Subjects were randomly chosen from an area close to the HWI and compared to individuals outside the exposure area. TCDD and related compounds were measured in blood serum before incineration, four months after incineration started, and at the end of incineration. The results showed that incineration of TCDD did not result in any measurable exposure to the population surrounding the incinerator, as indicated by the biomarkers TCDD and TEQ serum levels. In Korea, Leem et al., (2003) analyzed blood samples of 45 subjects living near two MWSIs at Seoul, and an industrial waste incinerator at Pyongtaek, where high emissions of PCDD/Fs were suspected. The industrial waste incinerator began its operations in 1988 and ceased in 2001. The average TEQ concentration of PCDD/Fs in residents near the industrial waste incinerator was 53.4 pg I-TEQ/g lipid, whereas that in residents near MSWIs was 12.2 pg I-TEQ/g lipid. In a subsequent study performed in the vicinity of the same Korean incinerator, Park et al., (2004) assessed the spatial changes in the influence of that facility. A total of 47 soil samples (in continuous manner with distance) and 60 human blood samples (40 within 5 km, and 20 at 7 and 12 km) were collected and analyzed for the levels of PCDD/Fs. The mean PCDD/F value in the blood samples from the near-site zone was 12.2 pg I-TEQ/g lipid, while the mean concentration in the blood samples collected at the far-site zone was 11.0 pg I-TEQ/g lipid. Influence of the incinerator was not clearly observed both on soil and blood concentrations of PCDD/Fs, as the levels in the zone within 5 km were not significantly different from those at 7 and 12 km. However, the congener patterns of PCDD/Fs in both soil and blood changed with the distance to the facility. In the area under the potential influence of that same Korean incinerator, Leem et al., (2006) estimated whether blood levels and isomer patterns of PCDD/Fs in residents living near the facility were affected by its presence. Blood levels and homologue patterns of PCDD/Fs were determined in a group of 40 residents living within 5 km of the industrial waste incinerator, and in a group of 20 residents living 20 km away from the facility. The average concentration of PCDD/Fs in the near-site zone was 11.9 pg ITEQ/ g lipid, whereas that of the far-site zone was 11.2 pg ITEQ/g lipid, the difference not being statistically significant. The authors observed that the group living next to the industrial incinerator presented the typical isomer pattern, in which the proportions of OCDDs were lower, while those of PCDFs were higher. These scarce international data, together with those obtained for the population living in the vicinity of the Spanish HWI here assessed, are summarized in Table 2. It can be seen that, among those reported, the current and previous mean PCDD/F levels (6.26 and

9.36 pg I-TEQ/g lipid, respectively) correspond to the lowest concentrations of these chemicals in human plasma of populations living near industrial or hazardous waste incinerators. Moreover, the continued decrease in the mean PCDD/F concentrations is well correlated with the reductions in the dietary intake of PCDD/Fs, which have been also noted in this same surveillance program.

For comparison purposes only, we next summarize the results of some recent studies from different scenarios, in which the levels of PCDD/Fs were also determined in human blood. (Zubero et al., 2011) analyzed the concentrations of PCDD/Fs in 16 “composite” samples of individuals living near a MSWI in Zabalgarbi (Bilbao, Spain), and established the temporal changes between 2006 and 2008. The average concentrations of PCDD/Fs were 13.4 and 13.2 pg WHO-TEQ/g lipid, respectively. No significant differences based on the specific place of residence, near or far from the facility, were found. In Germany, the Integrated Exposure Assessment Survey (INES) is designed to obtain data on exposure of the German population, including the analysis of several persistent organic pollutants (POPs) in biological matrixes. The latest campaign, conducted in 2005, showed an average PCDD/F concentration of 8.9 pg WHO-TEQ/g lipid (Fromme et al., 2009). Also in Germany, and specifically in the region of Baden−Württemberg, recently Link et al., (2012) observed a progressive reduction of the levels of PCDD/Fs in blood of children between 1996 and 2009. In that survey, PCDD/F levels ranged between 4.9 and 7.6 pg WHOTEQ/g lipid, depending on the type of breastfeeding. In turn, Turrio-Baldassarri et al., (2008) estimated in 22 pg WHO-TEQ/g lipid the average concentration of PCDD/Fs (assuming ND = LOD) in human serum of differently exposed population groups living in Brescia (Italy). In Japan, Arisawa et al., (2011) found a median concentration of 10 pg WHO-TEQ/g lipid in 1656 Japanese individuals, while in Australia, Staff et al., (2012) reported concentrations of PCDD/Fs between 9.5 and 10.1 pg WHOTEQ/g lipid in blood of a control group. Interestingly, the calculated concentration of PCDD/Fs in plasma samples of 43 workers who had been assigned to work in the vicinity of the World Trade Center (New York, U.S.) during the week after the collapse of the buildings was, on average, 41.2 pg WHOTEQ/g lipid (Horii et al., 2010). The levels of PCDD/Fs in plasma of the nonoccupationally exposed population, and living near the HWI here assessed, were lower than all of the above recently reported values.

Table 2. Summary of studies reporting PCDD/F levels in plasma of non-occupationally exposed populations living near industrial or hazardous waste incinerators.

Location	Year of collection	Number of participants	PCDD/F level (pg I-TEQ/g lipid)	Exposed vs. control	Reference
Times Beach, MO, USA	n.a.	n=76 close to the HWI; n=74 outside the area of exposure	9.89	No measurable exposure to the population surrounding the HWI was found in biomarkers (TCDD and TEQ serum levels)	Evans et al. (2000)[36]
Pyoungtaek, South Korea	n.a.	10 residents living near an industrial incinerator, and 29 workers and residents near MSWIs	53.42 vs. 12.23	Significant differences in the exposure to PCDD/Fs between both groups.	Leem et al. (2003)[37]
Pyoungtaek, South Korea	2002-2003	n=40 close (< 5 km) to an industrial incinerator; n=74 far (7-12 km) the facility	12.2 (near) 11.0 (far)	No statistically significant difference between groups	Park et al. (2004)[38]
Pyoungtaek, South Korea	n.a.	n=40 close (< 5 km) to an industrial incinerator; n=74 far (20 km) the facility	11.9 (near) 11.2 (far)	No statistically significant difference between groups	Leem et al. (2006)[39]
Tarragona, Spain	1998	20 non-occupationally exposed subjects living in the area under influence of the HWI emissions	27.0	Baseline study	Schuhmacher et al. (1999)[18]
Tarragona, Spain	2002	20 non-occupationally exposed subjects living in the area under influence of the HWI emissions	15.7	First survey of the surveillance program (4 years after starting regular operations in the HWI)	Agramunt et al. (2005)[19]
Tarragona, Spain	2007	20 non-occupationally exposed subjects living in the area under influence of the HWI emissions	9.36	Second survey of the surveillance program (9 years after starting regular operations in the HWI)	Nadal et al. (2008)[20]
Tarragona, Spain	2012	40 non-occupationally exposed subjects living in the area under influence of the HWI emissions	6.26	Third survey of the surveillance program (13 years after starting regular operations in the HWI)	This study

n.a.: not available.

3.2. Multicompartment physiologically-based pharmacokinetic (PBPK) model

PBPK models are mathematical representations of the human body, where organs are considered as compartments (Spear et al., 1994). These models allow prediction of the theoretical concentration of chemicals along the time in different human tissues (Fàbrega et al., 2011), taking into account species-specific physiological, chemical, and biochemical parameters. PBPK models are based on a system of differential equations which estimate the concentration or amount of individual substances of sets of chemicals in each body compartment. These tools are increasingly used in the field of human health risk assessment, particularly in quantifying the relationship between measures of external exposure and internal dose (Thompson et al., 2008). In the present study, a multicompartment PBPK model developed by Maruyama et al., (2003) was applied to estimate the levels of PCDD/Fs in plasma. The model was validated by comparing the results with experimental data, not only from the current survey, but also from our previous surveys (1998, 2002, and 2007) (Agramunt et al., 2005; Nadal et al., 2008; Schuhmacher et al., 1999). As mentioned above, dietary intake is the most important pathway of exposure to PCDD/Fs, with contribution percentages higher than 95%. Therefore, we assumed that: (1) food intake was the only source of PCDD/F exposure, and (2) this route of exposure was constant along time. Data about

dietary intake of PCDD/Fs were obtained from previous studies conducted in the same area (Bocio et al., 2005; Domingo et al., 1999; Domingo et al., 2012; Llobet et al., 2008). The conceptual representation of the PBPK model, which included 7 individual compartments, is depicted in Figure S2 (Annex 2). Fat, liver, kidney, skin, muscle, and richly perfused tissues (lung, spleen, and brain) were considered as independent compartments, while blood was the only matrix interrelated with the others. The predicted concentration of PCDD/Fs in fat, kidney, skin, muscle, and richly perfused tissues was based on the following mass balance equation:

$$Vi \times \frac{dCi}{dt} = Qi \times \left(Ca - \frac{Ci}{Pi} \right) \quad (1)$$

where Vi is the volume of the tissue i (L), Ci is the concentration of PCDD/Fs in the tissue i (pg/L), Qi is the blood flow (L/h), Pi is the tissue i:blood partition coefficient (unitless), and Ca is the arterial concentration (pg/L). In turn, the predicted level of PCDD/Fs in blood was determined by applying the following expression:

$$Vi \times \frac{dCblood}{dt} = \sum_i \left(\frac{Qi \times Ci}{Pi} \right) - \left(Ca - \sum_i Qi \right) + D \times Abs \quad (2)$$

where Vi is the volume of blood (L), Cblood is the PCDD/F concentration in blood (pg/L), Ci is the PCDD/F concentration in the tissue i (pg/L), Qi is the blood flow (L/h), Pi is the tissue i:blood partition coefficient (unitless), Ca is the arterial concentration (pg/L), D is the daily intake of PCDD/Fs (in pg/day), and Abs is the gastrointestinal absorption factor (unitless). In order to estimate the blood concentration of PCDD/Fs (pg/g lipid), the lipid percentage (weight:weight) of each sample was analyzed (mean: 0.79%; range: 0.39%− 1.39%). As liver was considered as an elimination organ, the predicted concentration of PCDD/Fs in this tissue was estimated by the following equation:

$$Vliver \times \frac{dCliver}{dt} = Qliver \times \left(Ca - \frac{Cliver}{Pliver} \right) - Cliver \times Vliver + Ki \quad (3)$$

where Vliver is the liver volume (L), Cliver is the PCDD/F concentration in liver (pg/L), Qliver is the blood flow (L/h), Ca is the arterial concentration (pg/L), Pliver is the liver:blood partition coefficient (unitless), and K1 is the elimination constant. Values of tissue volume, cardiac output, partition coefficient, absorption rates, and elimination constant were obtained from Maruyama et al., (2003). As the model was restricted to a simulation period of 4 years and applied to an adult population, no variations in volumes and cardiac output along the time were considered. The final system of differential equations was coded in Matlab using the Stiff method. Calculations were made for the

17 toxic 2,3,7,8-chlorinated PCDD/Fs, being the total levels estimated according to I-TEF values.

Simulated concentrations of PCDD/Fs in each one of the 7 compartments, taking into account intake data of 2012, are summarized in Table 3. The highest levels were found in fat and blood (17.6 and 7.95 pg I-TEQ/g lipid, respectively). In contrast, kidney and skin showed the minimum levels (0.14 and 0.24 pg I-TEQ/g tissue, respectively). Adipose tissue is considered an accumulation reservoir of PCDD/Fs in the human body (Moon et al., 2011), while plasma is also a good biomarker of PCDD/Fs, given its distribution capacity. When comparing the modeled and experimental concentrations of PCDD/Fs in plasma, very similar values were observed: 7.95 vs 6.18 pg ITEQ/g lipid, respectively. This corroborates the validity and suitability of this particular model to predict the PCDD/F burdens in plasma. Moreover, the simulation was also performed on the basis of the dietary intake of PCDD/Fs in previous monitoring campaigns. The results are depicted in Fig. 2, together with experimental values of PCDD/Fs in plasma and the dietary intake. Because food intake was assumed to be the only single exposure pathway, a significant reduction of plasma concentrations of PCDD/Fs was noted, which is in agreement with the significant decrease in the dietary intake of PCDD/Fs. In our preoperational study (1998), the dietary intake of PCDD/F ingestion through food consumption by the general population was 210.1 pg I-TEQ/day (Domingo et al., 1999), while in subsequent surveys (2002 and 2007) the intake decreased to 27.8 pg WHO-TEQ/day (Bocio et al., 2005; Llobet et al., 2008). In our last assessment period (2007−2012), the dietary intake slightly increased, reaching 33.1 pg WHO-TEQ/day. The increase was attributed to the inclusion of a new food group (industrial bakery), whose incidence had not been previously evaluated. The daily intake of PCDD/Fs, exclusively due to the consumption of industrial bakery, was estimated in 3.49 pg WHO-TEQ (10.5% of the total). Considering only the food groups evaluated in both 2007 and 2012 surveys, it was noted that the dietary intake had remained nearly constant, from 27.8 to 29.4 pg WHO-TEQ/ day (Domingo et al., 2012).

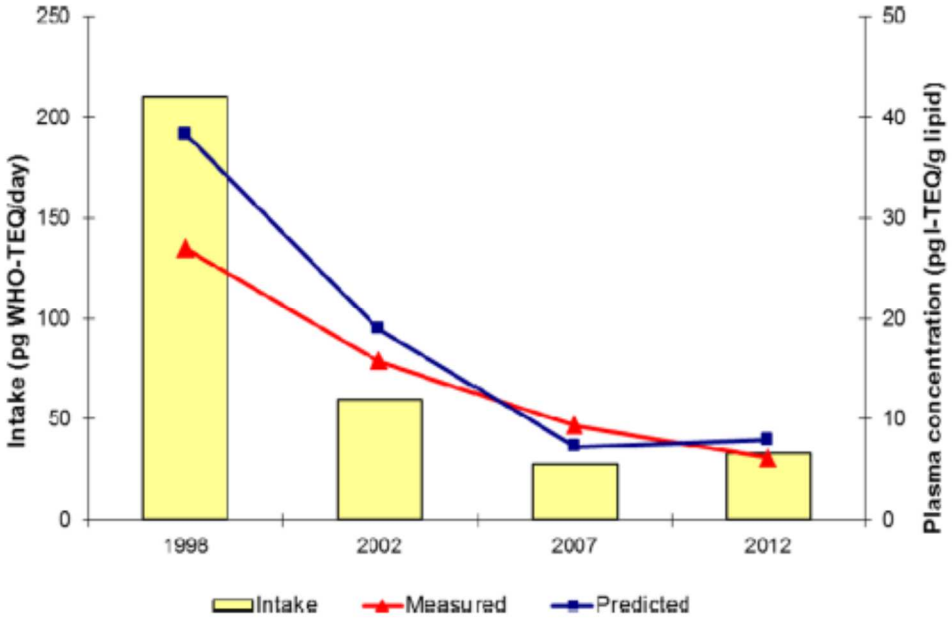


Fig. 2. Dietary intake of PCDD/Fs, and comparison between measured and simulated PCDD/F concentrations in plasma, between 1998 (baseline survey) and 2012 (current data). PCDD/F intake in 1998 is given as pg I-TEQ/day.

Table 3. PBPK modeling results: Simulated concentrations of PCDD/Fs in 7 human compartments according to dietary intake data of 2012.

	Blood	Liver	Fat	Kidney	Muscle	Richly perfused	Skin
2,3,7,8-TCDD	1.06	0.08	2.09	0.03	0.14	0.03	0.02
1,2,3,7,8-PeCDD	3.78	0.51	13.1	0.09	0.57	0.14	0.06
1,2,3,4,7,8-HxCDD	1.10	0.26	1.03	0.01	0.04	0.02	0.02
1,2,3,6,7,8-HxCDD	6.78	2.96	12.0	0.16	0.71	0.15	0.09
1,2,3,7,8,9-HxCDD	0.27	0.61	3.32	0.02	0.29	0.06	0.01
1,2,3,4,6,7,8-HpCDD	18.5	5.01	21.3	0.34	5.78	2.07	2.07
OCDD	16.3	7.22	7.18	0.36	3.38	2.08	1.82
2,3,7,8-TCDF	4.08	0.59	1.79	0.03	0.15	0.08	0.31
1,2,3,7,8-PeCDF	1.49	0.23	1.55	0.02	0.12	0.08	0.11
2,3,4,7,8-PeCDF	4.11	1.47	11.1	0.07	1.25	0.12	0.09
1,2,3,4,7,8-HxCDF	3.45	0.69	2.07	0.04	0.30	0.17	0.06
1,2,3,6,7,8-HxCDF	1.41	0.50	1.47	0.02	0.28	0.02	0.03
1,2,3,7,8,9-HxCDF	3.95	0.06	5.44	0.01	0.09	0.12	0.35
2,3,4,6,7,8-HxCDF	3.80	0.12	1.46	0.03	0.09	0.11	0.19
1,2,3,4,6,7,8-HpCDF	13.2	2.32	14.7	0.09	0.77	0.24	1.16
1,2,3,4,7,8,9-HpCDF	3.58	0.25	3.23	0.01	0.09	0.05	0.31
OCDF	17.2	2.06	19.8	0.23	0.76	0.62	1.92
I-TEQ	7.95	1.75	17.6	0.14	1.33	0.26	0.24

Concentrations in plasma are given in pg I-TEQ/g fat, while those of the remaining tissues are shown in pg I-TEQ/g tissue.

4. Conclusions

In conclusion, the PBPK model has demonstrated to be a very reliable tool to predict the levels of PCDD/Fs in plasma. The comparison between simulated and experimental results was very successful, as PCDD/F values in plasma were very similar (7.95 vs 6.18 pg I-TEQ/g lipid). For validation purposes, modeling results for other compartments may also be compared with the levels of PCDD/Fs found in different human tissues. In this sense, it must be highlighted that, in addition to plasma, periodically we also use other biological monitors, such as breast milk and adipose tissue, as biomonitors of accumulation of PCDD/Fs in subjects living in the zones under potential influence of the HWI. In previous studies, the concentrations of PCDD/Fs in samples of adipose tissue of autopsied subjects living in these zones were 40.1, 9.89, and 14.6 pg I-TEQ/g lipid in 1998, 2002, and 2007, respectively (Nadal et al., 2009). These values are very similar to those obtained after executing the model (31.0, 9.27, and 14.0 pg I-TEQ/g lipid, respectively). As part of the current biological surveillance program of the HWI, the concentration of PCDD/Fs will again be determined in adipose tissue during 2013. The outcomes of that survey will be fundamental in the design and implementation of future monitoring programs of the HWI. If the PBPK model results are sufficiently effective in adipose tissue, as they already seem to be in plasma, then the cost of surveillance studies might be notably reduced by minimizing the number of biological samples, something critical in the context of the current economic crisis.

References

Agramunt, M.C., Schuhmacher, M., Hernandez, J.M, Domingo, J.L., 2005. Levels of dioxins and furans in plasma of nonoccupationally exposed subjects living near a hazardous waste incinerator. Journal of Exposure Analysis and Environmental Epidemiology 15, 29−34.

Arisawa, K., Uemura, H., Hiyoshi, M., Kitayama, A., Takami, H., Sawachika, F., Nishioka, Y., Hasegawa, M., Tanto, M., Satoh, H., Shima, M., Sumiyoshi, Y., Morinaga, K., Kodama, K., Suzuki, T.I., Nagai, M., 2011. Dietary patterns and blood levels of PCDDs, PCDFs, and dioxin-like PCBs in 1656 Japanese individuals. Chemosphere 82, 656−662.

Bocio, A., Domingo, J.L., 2005. Daily intake of polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans (PCDD/PCDFs) in foodstuffs consumed in Tarragona, Spain: A review of recent studies (2001−2003) on human PCDD/PCDF exposure through the diet. Environmental Research 97, 1−9.

Bocio, A., Nadal, M., Domingo, J.L., Human exposure to metals through the diet in Tarragona, Spain: Temporal trend. Biological Trace Element Research 104, 193−201.

Consonni, D., Sindaco, R., Bertazzi, P.A., 2012. Blood levels of dioxins, furans, dioxin-like PCBs, and TEQs in general populations: A review, 1989−2010. Environment International 44, 151−162.

Domingo, J.L., Schuhmacher, M., Granero, S., Llobet, J.M., 1999. PCDDs and PCDFs in food samples from Catalonia, Spain. An assessment of dietary intake. Chemosphere 38, 3517−3528.

Domingo, J.L., Schuhmacher, M., Agramunt, M.C., Müller, L., Neugebauer, F., 2001. Levels of metals and organic substances in blood and urine of workers at a new hazardous waste incinerator. International Archives of Occupational and Environmental Health 74, 263−269.

Domingo, J.L, 2002. Human health risks of dioxins for populations living near modern municipal solid waste incinerators. Reviews on Environmental Health 17, 135−147.

Domingo, J.L., Agramunt, M.C., Nadal, M., Schuhmacher, M., Corbella, J., 2002. Health risk assessment of PCDD/PCDF exposure for the population living in the vicinity of a municipal waste incinerator. Archives of Environmental Contamination & Toxicology 43, 461−465.

Domingo, J.L., Bocio, A., 2007. Levels of PCDD/PCDFs and PCBs in edible marine species and human intake: A literature review. Environment International 33, 397−405.

Domingo, J.L., Perelló, G., Nadal, M., Schuhmacher, M., 2012. Dietary intake of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) by a population living in the vicinity of a hazardous waste incinerator. Assessment of the temporal trend. Environment International 50, 22−30.

European Commission. The Waste Incineration Directive, 2000. Available at: http://ec.europa.eu/environment/air/pollutants/stationary/wid/legislation.htm. Accessed in Jan 31, 2013.

European Commission. Waste Statistics, 2012. Available at: http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Waste_statistics. Accessed in Jan 31, 2013.

Evans, R.G., Shadel, B.N., Roberts, D.W., Clardy, S., Jordan-Izaguirre, D., Patterson, D.G., Needham, L.L., 2000. Dioxin incinerator emissions exposure study Times Beach, Missouri. Chemosphere 40, 1063−1074.

Fàbrega, F., Nadal, M., Schuhmacher, M., Domingo, J.L, 2011. PBPK modeling of PFOS and PFOA using a whole body pharmacokinetic model: A preliminary approach and validation test. Organohalogen Compounds 73, 1169−1172.

Ferré-Huguet, N., Nadal, M., Schuhmacher, M., Domingo, J.L., 2006. Environmental impact and human health risks of polychlorinated dibenzo-p-dioxins and dibenzofurans in the vicinity of a new hazardous waste incinerator: A case study. Environmental Science & Technology 40, 61−66.

Ferré-Huguet, N., Nadal, M., Schuhmacher, M., Domingo, J.L., 2009. Monitoring metals in blood and hair of the population living near a hazardous waste incinerator: Temporal trend. Biological Trace Element Research 128, 191−199.

Fromme, H., Albrecht, M., Boehmer, S., Büchner, K., Mayer, R., Liebl, B., Wittsiepe, J., Bolte, G., 2009. Intake and body burden of dioxin-like compounds in Germany: The INES study. Chemosphere 76, 1457−1463.

García-Pérez, J., Fernández-Navarro, P., Castelló, A., López- Cima, M.F., Ramis, R., Boldo, E., López-Abente, G., 2013. Cancer mortality in towns in the vicinity of incinerators and installations for the recovery or disposal of hazardous waste. Environment International 51, 31−44.

Horii, Y., Jiang, Q., Hanari, N., Lam, P.K S., Yamashita, N., Jansing, R., Aldous, K.M., Mauer, M.P., Eadon, G.A., Kannan, K., 2010 Polychlorinated dibenzo-*p*-dioxins, dibenzofurans, biphenyls, and naphthalenes in plasma of workers deployed at

the World Trade Center after the collapse. Environmental Science & Technology 44, 5188−5194.

Huang, H.Y., Jeng, T.Y., Lin, Y.C., Ma, Y.C., Kuo, C.P., Sung, F.C., 2007. Serum dioxin levels in residents living in the vicinity of municipal waste incinerators in Taiwan. Inhalation Toxicology 19, 399−403.

Lakind, J.S., Hays, S.M., Aylward, L.L., Naiman, D.Q., 2009 Perspective on serum dioxin levels in the United States: An evaluation of the NHANES data. Journal of Exposure Science and Environmental Epidemiology 19, 435-41.

Leem, J.H., Hong, Y.C., Lee, K.H., Kwon, H.J., Chang, Y.S., Jang, J.Y., 2003. Health survey on workers and residents near the municipal waste and industrial waste incinerators in Korea. Industrial Health 41, 181−188.

Leem, J.H., Lee, D.S., Kim, J., 2006. Risk factors affecting blood PCDDs and PCDFs in residents living near an industrial incinerator in Korea. Archives of Environmental Contamination & Toxicology 51, 478−484.

Linares, V., Perelló, G., Nadal, M., Gómez-Catalán, J., Llobet, J.M., Domingo, J.L., 2010. Environmental versus dietary exposure to POPs and metals: A probabilistic assessment of human health risks. Journal of Environmental Monitoring 12, 681−688.

Link, B., Gabrio, T., Zöllner, I., Jaroni, H., Piechotowski, I., Schilling, B., Felder-Kennel, A., Flicker-Klein, A., König, M., Maisner, V., Schick, K.H., Fischer, G., 2012 Decrease of internal exposure to chlororganic compounds and heavy metals in children in Baden− Württemberg between 1996/1997 and 2008/2009. International Journal of Hygiene & Environmental Health 215, 196−201.

Llobet, J.M., Martí-Cid, R., Castell, V., Domingo, J.L., 2008. Significant decreasing trend in human dietary exposure to PCDD/ PCDFs and PCBs in Catalonia, Spain. Toxicology Letters 178, 117−126.

Lopez-Espinosa, M.J., Kiviranta, H., Araque, P., Ruokojärvi, P., Molina-Molina, J.M., Fernandez, M.F., Vartiainen, T., Olea, N., 2008. Dioxins in adipose tissue of women in Southern Spain. Chemosphere 73, 967−971.

Mari, M., Nadal, M., Schuhmacher, M., Domingo, J.L., 2010. Application of self-organizing maps for PCDD/F pattern recognition of environmental and biological samples to evaluate the impact of a hazardous waste incinerator. Environmental Science & Technology 44, 3162−3168.

Mari, M., Nadal, M., Schuhmacher, M., Domingo, J.L., 2013. Body burden monitoring of dioxins and other organic substances in workers at a hazardous waste incinerator. International Journal of Hygiene & Environmental Health 216, 728-734.

Maruyama, W., Yoshida, K., Tanaka, T., Nakanishi, J., 2003. Simulation of dioxin accumulation in human tissues and analysis of reproductive risk. Chemosphere 53, 301−313.

Moon, H.B., Lee, D.H., Lee, Y.S., Kannan, K., 2011. Concentrations and accumulation profiles of PCDDs, PCDFs and dioxin-like PCBs in adipose fat tissues of Korean women. Journal of Environmental Monitoring 13, 1096−1101.

Nadal, M., Perelló, G., Schuhmacher, M., Cid, J., Domingo, J.L., 2008. Concentrations of PCDD/PCDFs in plasma of subjects living in the vicinity of a hazardous waste incinerator: Follow-up and modeling validation. Chemosphere 73, 901−906.

Nadal, M., Domingo, J.L., García, F., Schuhmacher, M., 2009. Levels of PCDD/F in adipose tissue on non-occupationally exposed subjects living near a hazardous waste incinerator in Catalonia, Spain. Chemosphere 74, 1471−1476.

Nadal, M., Bocio, A., Schuhmacher, M., Domingo, J.L., 2005. Trends in the levels of metals in soils and vegetation samples collected near a hazardous waste incinerator. Archives of Environmental Contamination & Toxicology 49, 290−298.

Park, S., Kim, S.J., Kim, K.S., Lee, D.S., Kim, J.G., 2004. Influence of an industrial waste incinerator as assessed by the levels and congener patterns of polychlorinated

dibenzo-*p*-dioxins and polychlorinated dibenzfurans. Environmental Science & Technology 38, 3820−3826.

Perelló, G., Gómez-Catalán, J., Castell, V., Llobet, J.M., Domingo, J.L., 2012. Assessment of the temporal trend of the dietary exposure to PCDD/Fs and PCBs in Catalonia, over Spain: Health risks. Food & Chemical Toxicology 50, 399−408.

Tian, H., Gao, J., Lu, L., Zhao, D., Cheng, K., Qiu, P., 2012. Temporal trends and spatial variation characteristics of hazardous air pollutant emission inventory from municipal solid waste incineration in China. Environmental Science & Technology 46, 10364−10371.

Schuhmacher, M., Domingo, J.L., Llobet, J.M., Lindström, G., Wingfors, H., 1999. Dioxin and dibenzofuran concentrations in blood of a general population from Tarragona, Spain. Chemosphere 38, 1123−1133.

Schuhmacher, M., Rodriguez-Larena, M.C., Agramunt, M.C., Diaz-Ferrero, J., Domingo, J.L., 2002. Environmental impact of a new hazardous waste incinerator in Catalonia, Spain: PCDD/PCDF levels in herbage samples. Chemosphere 48, 187−193.

Schuhmacher, M., Agramunt, M.C., Rodriguez-Larena, M.C., Díaz-Ferrero, J., Domingo, J.L., 2002. Baseline levels of PCDD/FS in soil and herbage samples collected in the vicinity of a new hazardous waste incinerator in Catalonia, Spain. Chemosphere 46, 1343−1350.

Schuhmacher, M., Domingo, J.L., Kiviranta, H., Vartiainen, T., 2004. Monitoring dioxins and furans in a population living near a hazardous waste incinerator: Levels in breast milk. Chemosphere 57, 43−49.

Schuhmacher, M., Domingo, J.L., Hagberg, J., Lindström, G., 2004. PCDD/F and non-ortho PCB concentrations in adipose tissue of individuals living in the vicinity of a hazardous waste incinerator. Chemosphere 57, 357−364.

Spear, R.C., Bois, F.Y., 1994. Parameter variability and the interpretation of physiologically-based pharmacokinetic modeling results. Environmental Health Perspectives 102, 61−66.

Staff, M., Sheppeard, V., Abeywardana, S., Forssman, B., Rutherford, A., Mueller, J.F., Paepke, O., 2012. Blood dioxin biomonitoring to assess local residents' exposure from a large urban remediation project. Chemosphere 88, 316−322.

Thompson, C.M., Sonawane, B., Barton, H.A., DeWoskin, R.S., Lipscomb, J.C., Schlosser, P., Chiu, W. A., Krishnan, K., 2008. Approaches for applications of physiologically-based pharmacokinetic models in risk assessment. Journal of Toxicology & Environmental Health, Part B: Critical Reviews 11, 519−547.

Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicological Sciences 93, 223−241.

Turrio-Baldassarri, L., Abate, V., Battistelli, C.L., Carasi, S., Casella, M., Iacovella, N., Indelicato, A., Rocca, C.L., Scarcella, C., Alivernini, S., 2008. PCDD/F and PCB in human serum of differently exposed population groups of an Italian city. Chemosphere 73, S228−S234.

Van Zorge, J.A., Van Wijnen, J.H., Theelen, R.M.C., Olie, K., Van den Berg, M., 1989. Assessment of the toxicity of mixtures of halogenated dibenzo-p-dioxins and dibenzofurans by use of toxicity equivalency factors (TEF). Chemosphere 19, 1881−1895.

Vilavert, L., Nadal, M., Mari, M., Schuhmacher, M., Domingo, J.L., 2010. Monitoring temporal trends in environmental levels of polychlorinated dibenzo-p-dioxins and dibenzofurans: Results from a 10-year surveillance program of a hazardous

waste incinerator. Archives of Environmental Contamination & Toxicology 59, 521−531.

Zubero, M.B., Aurrekoetxea, J.J., Ibarluzea, J.M., Rivera, J., Parera, J., Abad, E., Rodríguez, C., Sáenz, J.R., 2011. Evolution of PCDD/Fs and dioxin-like PCBs in the general adult population living close to a MSW incinerator. Science of the Total Environment 410−411, 241−247.

CHAPTER 3

A PBPK MODEL TO ESTIMATE PCDD/F LEVELS IN ADIPOSE TISSUE: COMPARISON WITH EXPERIMENTAL VALUES OF RESIDENTS NEAR A HAZARDOUS WASTE INCINERATOR

Abstract

This study was aimed at determining the concentrations of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) in 15 samples of adipose tissue from subjects who had been living in the vicinity of a hazardous waste incinerator (HWI). The results were compared with levels obtained in previous surveys carried out in 1998 (baseline study), 2002 and 2007. The current (2013) concentrations of PCDD/Fs in adipose tissue ranged from 2.8 to 46.3 pg WHO-TEQ/g fat (mean and median concentrations: 11.5 and 7.4 pg WHO-TEQ/g fat, respectively), being significantly lower (64%) than those observed in 1998. In contrast, no significant differences in the mean PCDD/F concentrations were noted in the period 2002-2013. The significant decrease of the PCDD/F content in fat, also noted in other biological monitors such as plasma and breast milk, is in agreement with the reduction in the dietary intake of PCDD/Fs found in the same area of study. Similarly to other investigations across Europe, an increase of PCDD/F levels in adipose tissue in relation to age was observed, while no significant differences were noted according to gender. A multicompartmental physiologically-based pharmacokinetic (PBPK) model was also applied to estimate the levels of PCDD/Fs in adipose tissue. When comparing the modelled and experimental concentrations of PCDD/Fs in that tissue, very similar values were obtained for the four surveys, which indicates this can be a reliable tool to predict the internal dose of PCDD/Fs.

1.Introduction

Due to their toxic potential for humans and wildlife, as well as bioaccumulation and persistence capacity, contamination by polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) is an environmental problem of global concern. PCDD/Fs are released to the environment from a number of sources, including traffic, chemical manufacturing, iron and steelmaking, as well as open burning of materials in forest fires, accidental fires and unintentional landfill fires (Schuhmacher and Domingo, 2006; Estrellan and Iino, 2010; Ooi and Lu, 2011). In the past, incinerators

were catalogued as important sources of toxic emissions, particularly PCDD/Fs and heavy metals (Hu et al., 2004). However, the installation of modern cleaning technologies to comply with maximum emission levels, according to European standards (0.1 ng I-TEQ Nm^3), has substantially reduced the environmental impact of these facilities, not only in terms of PCDD/Fs, but also other air pollutants (Glorennec et al., 2005; Vilavert et al., 2010).

Incineration, also known as waste-to-energy, has become one of the most widely used alternatives for waste management, being considered as a serious option for the disposal of municipal solid, hazardous and medical wastes. In comparison to other waste treatments, incineration has a wide range of advantages, of which volume reduction, energetic recovery and microbial elimination are the most evident. Moreover, the incineration of hazardous waste is often selected as the most desirable disposal method, when these cannot be properly recycled (Ferré-Huguet et al., 2005; Mari et al., 2013). Unfortunately, this process may release a wide range of chemicals to air, therefore contaminating water, soil and biota by pollutant deposition, and ultimately affecting the human health of residents living in the surrounding of hazardous waste incinerators (HWIs). Despite of the heterogeneity of stack emissions, which include heavy metals, semivolatile and volatile organic compounds, especial attention has been paid to PCDD/Fs (Kulkarni et al., 2008; Mari and Domingo, 2010), becoming one of the chemicals of most scientific and social interest. Because of this, the European Union (EU) imposed strict operating conditions and technical requirements on waste incineration plants and co-incineration plants, according to the EU Directive 2000/76/EC. After application of this regulatory measures and implementation of Best Available Techniques (BAT), a gradual decrease in the concentrations of PCDD/Fs in human biological tissues has been noted worldwide (Hagmar et al., 2006; Nadal et al., 2013), being plasma and breast milk two of the most visible monitors. This fact has been associated to the parallel reduction in the dietary intake of these pollutants (Domingo et al., 2012). Notwithstanding, the potential health risks derived from HWI stack emissions still generate a considerable concern among the population (Liu et al., 2012; Bunsan et al., 2013). PCDD/Fs accumulate in human adipose tissues over lifetime. These contaminants are slowly metabolized in the human body and elicit adverse effects including developmental and reproductive toxicity, cancer, and endocrine disruption (Mocarelli et al., 2008; White and Birnbaum, 2009). The levels of contaminant residues in adipose tissue can provide valuable information on steady-state concentrations, as a way to integrate the body burdens of lipophilic chemicals accumulated overtime. For instance, the Stockholm convention (SC) of persistent organic pollutants (POPs) have

the goal to reduce the emissions of PCDD/Fs in a 50% in 10 years. To prove this, SC establishes a global monitoring system to prove the reduction of PCDD/Fs in environmental matrices as well as human tissues, like adipose tissue.

The objective of this study was to determine the concentrations of PCDD/Fs in samples of adipose tissue of individuals living in the neighborhood of a HWI in Catalonia (Spain), 14 years after the facility started its regular operations. The results of this survey were compared with data from previous campaigns carried out before (1998) and after (2002 and 2007) the plant began to operate. Data from the biological monitoring study was also used to validate a multicompartment physiologically-based pharmacokinetic (PBPK) model to estimate the levels of PCDD/Fs in adipose tissue.

2. Material and Methods

2.1. Area of study

The HWI herein studied is located in Constantí (Tarragona County, Catalonia, Spain), relatively close to other potential industrial sources of environmental pollutants, such as a big oil refinery, an important complex of chemical industries, and a municipal solid waste incinerator (MWSI), among others. In addition, an intense traffic is present in the zone, which is crossed by a highway, a motorway and a number of roads. The facility is located 5 km from the closest populated nuclei (Constantí and Reus), and 10 km from Tarragona downtown. Information about the HWI, as well as more characteristics of the surrounding area were described in detail elsewhere (Schuhmacher et al., 1999a,b,c).

2.2. Sampling

During 2012-2013, adipose tissue samples of 15 autopsied subjects living in zones of Tarragona County (Catalonia, Spain) under potential impact of the HWI were collected. At the time of death, all the individuals had lived for at least the last 10 years in the area under evaluation. All samples were obtained from the same body compartment (abdominal adipose tissue). Samples were stored in polyethylene containers and kept at -20 °C until analysis. Samples were classified according to sex (9 men and 6 women) and age (mean age of the subjects was 52 years, ranging from 30 to 74). Individual and medical information of the participants were collected by passing a questionnaire to the relatives. No occupational exposure to PCDD/F was found for any of the subjects. Sampling was conducted in collaboration with forensic physicians of the

Institute of Legal Medicine of Catalonia (Tarragona Division), whose Research Committee evaluated and approved the study.

2.3. Analytical procedure

Analysis of PCDD/Fs was done according to a procedure derived from the US EPA methods 1613 and 8290A. Samples were dissolved in hexane, and a mixture of $^{13}C_{12}$-PCDD/F standards was spiked in order to control potential losses during the extraction and clean-up processes. Lipids were destructed by adding acid silica. In order to remove interfering components, a multi-step clean-up was performed. The first step consisted on a multilayer silica column clean-up. Further, the extract was eluted on a basic alumina column by passing different solvents in order to separate PCDD/Fs from other compounds. The PCDD/F fraction was collected and concentrated to near dryness with a nitrogen flux. Finally, 25 µL of $^{13}C_{12}$-PCDD/F injection standards were added.

The analysis of PCDD/Fs was carried out by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). The extract obtained after extraction and clean-up was injected on an Agilent 6890 gas chromatograph equipped with a ZB5-MS capillary column and coupled to a Waters Autospec Ultima mass spectrometer. The chromatographic process separated the 17 toxic 2,3,7,8-substituted congeners from each other. The mass spectrometer measured (via “selected ion recording” at a resolution of >10 000) two selected ions per congener group for both the native and labelled compounds. The calculation of the concentrations was done by using the corresponding ^{13}C congener level, automatically correcting according to the recovery percentage specific for each congener. In addition, the relative standard deviation (RSD) was calculated as a measure of the uncertainty. In all cases, the RSD of the control sample was lower than 10%.

2.4. Data treatment

The SPSS 19.0 statistical software package was used for data analysis. Total PCDD/F concentrations were calculated according to the 2005 WHO-TEFs (van den Berg et al., 2006). PCDD/F levels obtained in previous campaigns (1998, 2002 and 2007) were also recalculated according to the 2005 WHO-TEFs. The Levene test was applied to study the equality of variances. Furthermore, the ANOVA or Kruskal–Wallis tests were executed. A probability lower than 0.05 ($p < 0.05$) was considered as statistically significant. For calculations, when a PCDD/F congener presented a level below the

respective limit of detection (LOD), the concentration was assumed to be one-half of that limit (ND = 1/2 LOD).

3. Results and Discussion

3.1. Concentrations of PCDD/Fs in adipose tissue

The individual PCDD/F concentrations in adipose tissue samples from 15 subjects from Tarragona County, are summarized in Table 1. Age and sex of the individuals are also given. The mean levels of the 17 substituted-PCDD/F congeners, as well as the total PCDD/F concentration, in adipose tissue samples collected in the baseline (1998), previous (2002 and 2007) and current (2013) surveys, are summarized in Table 2. In the baseline (1998) study, a mean PCDD/F concentration in adipose tissue of 32.1 pg WHO-TEQ/g fat was found (median: 26.7 pg WHO-TEQ/g fat; range: 14.2-70.1 pg WHO-TEQ/g fat) (Schuhmacher et al., 1999b). In the 2002 survey, the mean and median levels of PCDD/Fs were 9.9 and 6.8 pg WHO-TEQ/g fat, with minimum and maximum values of 1.4 and 36.1 pg WHO-TEQ/g fat, respectively (Schuhmacher et al., 2004). In the immediately previous campaign (2007), the mean PCDD/F concentration in adipose tissue was 14.6 pg WHO-TEQ/g fat (median: 7.5 WHO-TEQ/g), with values ranging from 3.3 to 55.4 pg WHO-TEQ/g fat (Nadal et al., 2008).

In the current (2013) survey, mean and median PCDD/F concentrations of 11.5 and 7.4 pg WHO-TEQ/g fat, respectively, were observed, while minimum and maximum concentrations were 2.8 and 46.3 pg WHO-TEQ/g fat, respectively. Comparing the mean PCDD/F concentrations of the baseline (1998) study with those of the current (2013) survey, an important significant reduction was found (64%). However, during the period 2002-2013 no significant differences in the mean PCDD/F concentrations were noted, being median levels very similar. This temporal trend is in agreement with the significant reduction of PCDD/Fs in other biological tissue samples from non-occupationally exposed people of the same area.

Table 1. Individual PCDD/F concentrations (pg WHO-TEQ/g fat) in adipose tissue samples of individuals who had been living for at least the last 10 years in Tarragona County, Spain.

Sample	Gender	Age (years)	pg WHO-TEQ/g fat
1	M	56	10.2
2	M	53	7.4
3	F	74	21.4
4	M	37	2.9
5	F	40	6.7
6	M	50	18.2
7	M	59	11.3
8	M	46	2.8
9	M	56	12.8
10	F	72	46.3
11	F	40	5.1
12	M	64	6.0
13	M	36	2.9
14	F	73	13.9
15	F	30	5.1

M: Male; F: Female

Table 2. Mean levels of PCDD/F congeners (in pg/g fat) in adipose tissue samples of residents near a HWI in Tarragona County (Catalonia, Spain).

Congener	1998	2002	2007	2013
2,3,7,8-TCDD	4.13 ± 3.03[a]	1.39 ± 1.53[b]	1.68 ± 1.86[b]	1.24 ± 1.14[b]
1,2,3,7,8-PeCDD	11.37 ± 4.74[a]	3.73 ± 3.51[b]	5.28 ± 4.80[b]	4.11 ± 3.48[b]
1,2,3,4,7,8-HxCDD	5.61 ± 2.86[a]	2.78 ± 1.73[b]	3.30 ± 3.61[a,b]	2.26 ± 2.26[b]
1,2,3,6,7,8-HxCDD	59.4 ± 30.2[a]	19.2 ± 18.9[b]	28.1 ± 29.3[b]	25.3 ± 26.6[b]
1,2,3,7,8,9-HxCDD	8.12 ± 6.45[a]	2.08 ± 2.03[b]	3.55 ± 4.47[b]	2.63 ± 4.37[b]
1,2,3,4,6,7,8-HpCDD	84.9 ± 60.9[a]	10.2 ± 8.0[b]	20.0 ± 28.9[b]	20.3 ± 52.9[b]
OCDD	477.5 ± 320.2[a]	53.6 ± 51.0[b]	151.9 ± 187.8[b]	113.3 ± 195.6[b]
2,3,7,8-TCDF	0.94 ± 0.58[a]	0.34 ± 0.4[b]	0.40 ± 0.40[b]	0.35 ± 0.27[b]
1,2,3,7,8-PeCDF	0.92 ± 0.47[a,b]	0.5 ± 0.45[b]	1.4 ± 1.33[a]	0.31 ± 0.26[b]
2,3,4,7,8-PeCDF	21.1 ± 11.5[a]	5.71 ± 5.95[b]	9.94 ± 9.43[b]	7.43 ± 6.93[b]
1,2,3,4,7,8-HxCDF	7.02 ± 3.33[a]	2.32 ± 1.75[b]	3.29 ± 3.25[b]	2.83 ± 2.97[b]
1,2,3,6,7,8-HxCDF	8.22 ± 3.99[a]	2.03 ± 1.86[b]	3.31 ± 3.47[b]	2.69 ± 2.86[b]
1,2,3,7,8,9-HxCDF	0.62 ± 0.35[a]	0.39 ± 0.41[b]	0.06 ± 0.05[b]	0.07 ± 0.05[b]
2,3,4,6,7,8-HxCDF	2.2 ± 1.28[a]	0.38 ± 0.44[a]	0.88 ± 0.73[a]	0.69 ± 1.15[a]
1,2,3,4,6,7,8-HpCDF	4.81 ± 2.17[a]	2.06 ± 0.65[a]	2.99 ± 2.54[a,b]	2.34 ± 3.13[b]
1,2,3,4,7,8,9-HpCDF	0.39 ± 0.10[a]	0.31 ± 0.52[a,b]	0.10 ± 0.06[b]	0.11 ± 0.05[b]
OCDF	0.72 ± 0.27[a]	2.59 ± 1.27[b]	0.49 ± 0.30[a]	0.31 ± 0.15[a]
Total WHO-TEQ	32.1 ± 15.3[a]	9.9 ± 9.3[b]	14.6 ± 14.2[b]	11.5 ± 11.1[b]

[a,b]Different superscripts indicate statistically significant differences at $p < 0.05$. In parenthesis, median values.

Between 1998 and 2012, a significant decrease of mean PCDD/F concentrations in plasma from residents in Tarragona County was found. Levels were reduced from 27.0 to 6.18 pg I-TEQ/g lipid (77%; $p<0.001$) (Nadal et al., 2013). Similarly, the concentrations of PCDD/Fs in breast milk of women living in the same area were significantly lower in 2012 than those obtained in the 1998 survey (12.2 and 4.8 pg WHO-TEQ/g fat, respectively; $p<0.001$). Although the overall assessment of the data indicate a similar decreasing trend of PCDD/F burdens in the 3 biomonitors (plasma, breast milk, and adipose tissue), the profile of this latter is slightly different from the remaining two. The temporal decline of PCDD/Fs in plasma and breast milk has been progressive, being the values of the intermediate sampling campaigns lower than that of the baseline survey (1998), but higher than that of the most recent study (2012-2013). Thus, mean plasma concentrations of 15.7 and 9.36 pg I-TEQ/g lipid were found in 2002 and 2007, respectively, while PCDD/F levels in breast milk in 2002 and 2007 were 10.6 and 7.6 pg WHO-TEQ/g fat, respectively. On the other hand, the most important reduction of PCDD/Fs in fat tissue was only detected in the period 1998-2002, while no significant changes have been found since then. This would indicate that fat tissue acts more as a storage compartment, when compared with plasma and breast milk. Anyhow, the significant reduction in the levels of PCDD/Fs in fat tissue is consistent with the decreasing trend in the dietary intake of PCDD/Fs observed in recent years. The intake of PCDD/Fs through food consumption for the adult population of Tarragona County was 210.1 pg I-TEQ/day in the baseline survey (1998) (Domingo et al., 1999), while in 2012 the dietary intake dropped to 33.1 pg WHO-TEQ/day (Domingo et al., 2012), being this intake similar to that previously found in 2006 (Martí-Cid et al., 2008). Although human exposure to PCDD/Fs may occur through a number of routes such as inhalation, dermal contact, and ingestion of soils and dust, it is well known that the diet is quantitatively the main exposure pathway for non-occupationally exposed individuals (Nadal et al., 2004; Passuello et al., 2010; Windal et al., 2010).

The current concentrations of PCDD/Fs in samples of adipose tissue from individuals of Tarragona County are within the range of those reported in the scientific literature. Takenaka et al. (2002) found mean PCDD/F concentrations of 49.0 pg WHO-TEQ/g fat in fat tissue of Japanese people, collected in 1999, being this level similar to the results of our baseline survey, conducted only one year before. In Turkey, Çok et al. (2007) observed that PCDD/F concentrations ranged from 3.2 to 19.7 pg WHO-TEQ/g fat (mean: 9.2 pg WHO-TEQ/g fat) in 23 adipose tissue samples collected from men in 2004. In turn, Shen et al. (2009) reported that the mean PCDD/F level in 24 adipose tissue samples collected in 2006 in the Zhejiang Province (China) was 19 pg TEQ/g fat

(range: 2.5-56 pg TEQ/g fat). In general terms, these levels are quite similar to those found in the present survey. Furthermore, our results indicate that the incineration of hazardous wastes did not result in any detectable exposure of PCDD/Fs to the population surrounding the facility, as indicated by the TEQ levels in adipose tissue. The PCDD/F congener profile showed that OCDD was the predominant congener, followed by 1,2,3,6,7,8-HxCDD and 1,2,3,4,6,7,8-HpCDD (Table 2). On the other hand, all samples showed considerably higher levels of dioxins (PCDDs) than furans (PCDFs), which is in agreement with the results of our previous studies (Schuhmacher et al., 1999b, 2004). Table 3 summarizes the PCDD/F concentrations in adipose tissue of each one of the sampling campaigns, classified according to gender. Similarly to previous surveys, in the current (2013) study women showed notable higher levels of PCDD/Fs than men (16.4 vs. 8.3 pg WHO-TEQ/g fat). However, the difference was not significant in any of the four studies.

Table 3. Concentrations of PCDD/Fs (in pg WHO-TEQ/g fat) in adipose tissue of individuals who had lived in Tarragona County (Catalonia, Spain), according to gender.

	1998	**2002**	**2007**	**2013**
Men	25.3 ± 9.4	7.2 ± 3.5	11.2 ± 8.1	8.3 ± 5.3
Women	45.7 ± 16.7	17.4 ± 16.1	23.8 ± 23.9	16.4 ± 15.9

Data given as mean ± standard deviation; n: number of samples

An increase of PCDD/F levels in adipose tissue with age was observed (R^2= 0.4389) not only in the current (2013) survey but also in previous (1998, 2002 and 2007) studies. The concentrations of PCDD/Fs in 60 samples of adipose tissue collected between 1998 and 2012, according to the age at the time of death, are depicted in Fig. 1. Additionally, in order to analyze the potential differences of the PCDD/F concentration in adipose tissue in the younger and older populations, samples were classified into two groups: people aged <55 years and ≥55 years. A separation point of 55 years old was selected because the average age of the individuals under study was 52.4 years old and the median was 53 years old. In 2013, PCDD/F concentrations of 5.85 and 17.40 pg WHO-TEQ/g fat were observed in the group of under and over 55 years, respectively, reaching this difference the level of statistical significance ($p<0.05$). Taking into account the global number of samples from the 4 campaigns (n=60), mean PCDD/F levels in adipose tissue of people who had died being <55 years and ≥55 years were 11.47 and 25.52 pg WHO-TEQ/g fat, respectively ($p<0.01$). In Korea, Moon et al. (2011) found a mean concentration of PCDD/Fs of 3.4 pg TEQ/g fat in human adipose tissue from

women (aged between 40 and 68). No correlations were observed between the age of the subject and the burdens of dioxin-like contaminants. Similarly, no significant correlations between age and PCDD/F levels in human adipose tissue were found in China (Shen et al., 2008). In contrast, some European investigators have detected a positive association between PCDD/F levels in adipose tissue and age, in 420 individuals living in southern Finland (Kiviranta et al., 2005), and in 20 women living in southern Spain (López-Espinosa et al., 2008). Although notable differences in the PCDD/F body burdens have been noted in different geographical areas, mainly linked to fish consumption (Nadal et al., 2004b), the reasons of this should be studied in more detail. Age is likely an indicator of past cumulative exposures. People born in earlier times experienced higher environmental PCDD/F levels and thus they carry a higher body burden through life. There is evidence from human and animal studies that blood dioxin half-lives, in the absence of weight change or fat redistribution, increase with age. However, this effect is relatively minor compared to the birth cohort effect of historical exposures (Lorber and Phillips, 2002).

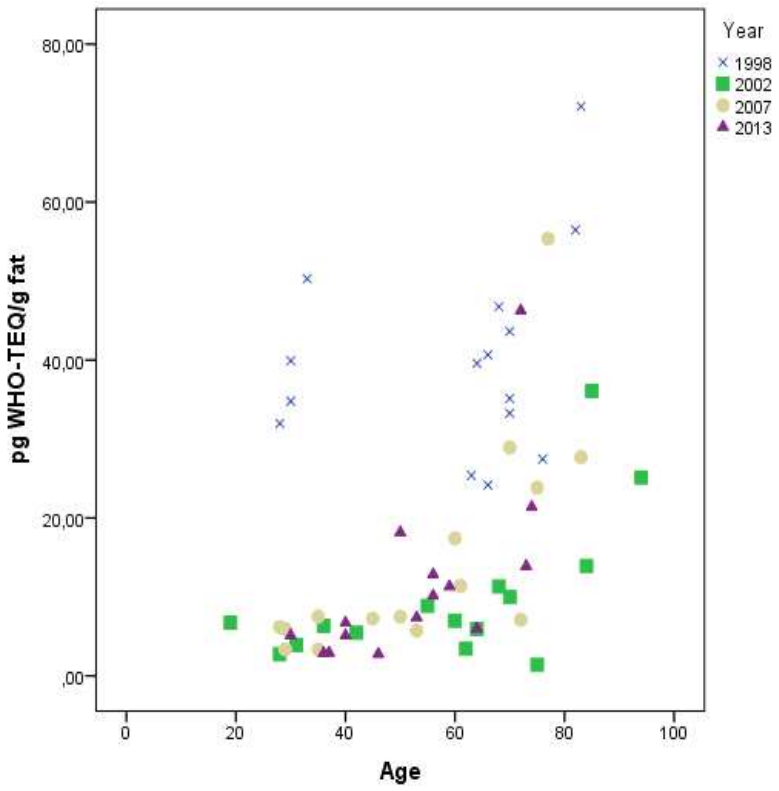


Fig. 1. PCDD/F concentrations in samples of adipose tissue from individuals of Tarragona County in 1998, 2002, 2007 and 2013.

The accumulation of PCDD/Fs according to gender has also been assessed in a number of investigations. However, most of them have focused on blood and plasma. Fromme et al. (2009) did not find any significant difference in the blood concentration of PCDD/Fs between men and women. However, they observed a significant increase in the WHO-TEQ levels with age. In the city of Mataró (Catalonia, Spain), Parera et al. (2013) recently found that PCDD/F levels in blood were higher in women than in men, showing both gender groups a slight reduction in comparison to precedent campaigns. Reis et al. (2007) also noted slightly higher PCDD/F levels in blood of Portuguese women, although differences in dioxin blood levels between males and females were not statistically significant. These authors also found that age was also associated with total TEQs in blood, with a significant correlation in a way that elder individuals owned higher PCDD/F values.

In recent years, the evaluation of several NHANES data sets have shown that a number of POPs are related to either body mass index (BMI) or waist circumference (Lee et al., 2007; Elobeid et al., 2010). BMI is physiologically related to the body's capacity to eliminate PCDD/Fs (Schildkraut et al., 1999). The elimination capacity of PCDD/Fs is indirectly proportional to the content of body fat, as individuals with less fat may eliminate 2,3,7,8-TCDD more easily (Emond et al., 2005; Collins et al., 2007). Since BMI tends to increase with age, it is difficult to study the effects of BMI independently of age. Collins et al. (2007) reported that age and BMI are both important factors for assessing background levels of 2,3,7,8-TCDD. Several studies indicate that body fat turnover is regulated at least, in part, by fat patterning, and that upper body fat turns over at a higher rate than peripheral fat located below the hips (Rodin, 1992). Therefore, measures other than BMI may provide further insight into the relationship between the amount and distribution of body fat and the capacity to store and eliminate PCDD/Fs. Although the amount of body fat has been also correlated with PCDD/F levels, it is rarely adjusted when comparing potentially exposed populations to a surveyed background (Landi et al., 1998). Pharmacokinetic properties of POPs can explain the observed relationships. POPs are stored in lipid reservoirs, while their concentrations change predictably with changes in adipose tissue volume. Levels in blood are proportional to blood lipid content, and weight gain increases the POP half-life (Thomaseth and Salvan, 1998). Lim et al. (2011) observed that weight gain over 10 years resulted in lower levels of polychlorinated biphenyls (PCBs) compared with weight stable or weight-loss conditions. However, the pharmacokinetics is complex, and relationships between POPs and a number of factors, such as BMI or weight change, are difficult to evaluate. Temporal changes depend on the magnitude and time of exposure, ongoing exposure, body fat mass, and changes in

fat mass during the time between exposure and the time of blood sampling, among others.

The pharmacokinetic characteristics of each PCDD/F congener determine the transport of chemicals, which pass from blood to the fat tissue. These characteristics include, for example, lipophilicity of the substance, molecular diameter, molecular weight, molar volume, and octanol-water partition coefficient (K_{ow}). Some constitutional descriptors such as the number and position of attached halogens and the number of hydrogen-bond acceptors, have also some contribution. The position of the halogen substitutes will determine the rigidity of the molecular structure and, consequently, its ability to pass from blood to other tissues (Mannetje et al., 2012). Recent studies suggest that different PCDD/F congeners have different partition coefficients that are dependent on the properties listed above (Needham et al., 2011). In addition, this ratio can be modified by the half-life of each PCDD/F congener. The ratio between the concentration of each PCDD/F congener in fat tissue and human plasma, expressed on a lipid basis, for the non-occupationally exposed subjects of Tarragona County (Spain) is shown in Table 4. All PCDD congeners showed fat:blood ratios >1, being 2,3,7,8-TCDD and OCDD those presenting the highest and lowest relationships (3.7 and 1.5, respectively). In turn, PCDFs showed a completely different pattern of the fat:blood ratio, with values under the unity for most congeners. The higher ratio was noted for 2,3,4,6,7,8-HxCDF (3.3), while 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, and 1,2,3,6,7,8-HxCDF showed values >1. The reasons behind these patterns need further investigations. These results have certain limitations because the samples of plasma and adipose tissue are not from the same individuals. Although studies did not differ in the timing of the sampling of both plasma and adipose tissue, being the mean age of subjects quite similar (52 and 50 years for donors of fat tissue and blood, respectively), different dietary habits could have altered these ratios.

Table 4. Fat: blood ratio of PCDD/Fs in subjects living in Tarragona County (pg/g lipid).

Congener	PCDD/Fs in fat tissue (pg/g lipid)[a]	PCDD/Fs in plasma (pg/g lipid)	Fat:blood (unitless)
2,3,7,8-TCDD	1.2	0.3	3.7
1,2,3,7,8-PeCDD	4.1	2.1	1.9
1,2,3,4,7,8-HxCDD	2.3	1.1	2.1
1,2,3,6,7,8-HxCDD	25.3	10.2	2.5
1,2,3,7,8,9-HxCDD	2.6	1.4	1.8
1,2,3,4,6,7,8-HpCDD	20.3	9	2.3
OCDD	113.3	75.8	1.5
2,3,7,8-TCDF	0.3	4.4	0.07
1,2,3,7,8-PeCDF	0.3	2.5	0.12
2,3,4,7,8-PeCDF	7.4	4.4	1.7
1,2,3,4,7,8-HxCDF	2.8	2.4	1.2
1,2,3,6,7,8-HxCDF	2.7	1.9	1.4
1,2,3,7,8,9-HxCDF	0.1	0.6	0.17
2,3,4,6,7,8-HxCDF	0.7	0.2	3.3
1,2,3,4,6,7,8-HpCDF	2.3	5.4	0.43
1,2,3,4,7,8,9-HpCDF	0.1	0.5	0.2
OCDF	0.3	6.5	0.05

[a] From Nadal et al. (2013)

3.2. Physiologically-based pharmacokinetic (PBPK) model

Physiologically-based pharmacokinetic (PBPK) models are mathematical representations of the human body where the tissues are considered well-stirred compartments linked by the blood flow (Nestorov, 2007). The final result is a set of ordinary differential equations that can be mathematically solved to predict the concentration of chemicals in human tissues. In recent years, PBPK models have been used in human health risk assessment to estimate the burdens of chemicals in human tissues, thus avoiding the analysis of this kind of samples (Chiu et al., 2007; Clewell and Clewell III, 2008). In the present study, a previous PBPK model (Maruyama et al., 2003; Fàbrega et al., 2014) was adapted to assess the concentration of PCDD/Fs in adipose tissue in four temporal scenarios: 1998, 2002, 2007 and 2013. The simulations were run for adult individuals living in the same area of study. Oral intake was assumed to be the only exposure pathway. Data about dietary intake of PCDD/Fs were obtained from previous studies conducted in the same area (Domingo et al., 1999; Bocio and Domingo, 2005; Martí-Cid et al., 2008; Domingo et al., 2012). Data of intakes and absorption rates were summarized in Table S1 (Annex 3). The characteristics and parameters of the model were previously described (Nadal et al., 2013). Briefly, 7 body compartments were

considered: blood, muscle, skin, richly perfused, adipose, kidney, and liver. The intake of PCDD/Fs, as well as other PBPK model parameters (e.g., volume, absorption, elimination and cardiac output of each tissue), were considered constant along time. The physical basis of the adipose tissue diffusion is related to the octanol:water partition coefficient (*K*ow). At steady state, the log *K*ow predicts the capacity of the chemical to diffuse into adipose tissue, being therefore accumulated in fat.

PCDD/F concentrations in the flow limited compartments (muscle, skin, richly perfused, fat, kidney and liver) were estimated by applying the following equation:

$$\frac{dCi}{dt} = \frac{Qi \times \left(Ca - \frac{Ci}{Ki:p} \right)}{Vi} \quad (1)$$

where Ci is the concentration of PCDD/Fs in the tissue i (pg/L), Qi is the blood flow in the tissue i (L/h), Ca is the arterial concentration (pg/L), Ki:p is the partition coefficient of tissue i, and Vi is the volume of the tissue i (L).

The PCDD/F content in the blood compartment was assessed by using the following equation:

$$\frac{dCblood}{dt} = \frac{\sum_i \left(\frac{Qi \times Ci}{Ki:p} \right) - \left(Cblood \times \sum_i Qi \right) + D \times Abs}{Vblood} \quad (2)$$

where Cblood is the concentration of PCDD/Fs in blood (pg/L), Qi is the blood flow in the tissue i (L/h), Ci is the concentration in the tissue *i* (pg/L), Ki:p is the partition coefficient of tissue *i*, D is the oral dose (pg/day), Abs is the gastrointestinal absorption (%), and Vblood is the volume of the tissue *i* (L).

Since liver was considered as an elimination organ, the predicted concentration of PCDD/Fs in this tissue was estimated by the following equation:

$$\frac{dCliver}{dt} = \frac{Qliver \times \left(Ca - \frac{Cliver}{Kl:p} \right) - Cliver \times Vliver \times K_1}{Vliver} \quad (3)$$

where Cliver is the concentration of PCDD/Fs in blood (pg/L), Qliver is the blood flow in the liver, Ca is the arterial concentration, Kl:p is the partition coefficient in the liver, K_1 is the elimination constant, partition coefficient (Kp) and Vliver is the volume in liver (L). Values of tissue volumes, cardiac output, absorption and elimination constants were

the same used in our previous models (Nadal et al., 2013), and were summarized in Table S2 and Table S3 (Annex 3).

A density of 0.92 g/mL in adipose tissue was used to recalculate the final concentration in pg/g tissue (Brown et al., 1997). The final system was a set ordinary differential equations which was handled by using Berkeley Madonna v.8.3.18. Stiff was considered as the method to solve the differential equations, while the step size (SD) in the simulations was set at 0.0001.

The measured and simulated concentrations of PCDD/Fs in fat tissue for the 1998 (baseline), 2002, 2007 and 2013 (current) surveys are summarized in Table 5. When comparing the modelled and experimental concentrations of PCDD/Fs in fat tissue, expressed as total WHO-TEQ, it can be observed that similar values were obtained in the four scenarios. However, experimental results are slightly higher than those simulated for all the surveys. Analyzing PCDD/F congeners individually, the simulated concentrations of all PCDDs, excepting 1,2,3,7,8,9-HxCDD, tended to suffer an underestimation with respect to those observed levels. In turn, the simulated levels of PCDFs, excepting 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, were slightly higher than those experimentally obtained. OCDF showed the most important difference between simulated and measured concentrations of PCDD/Fs, being 2-3

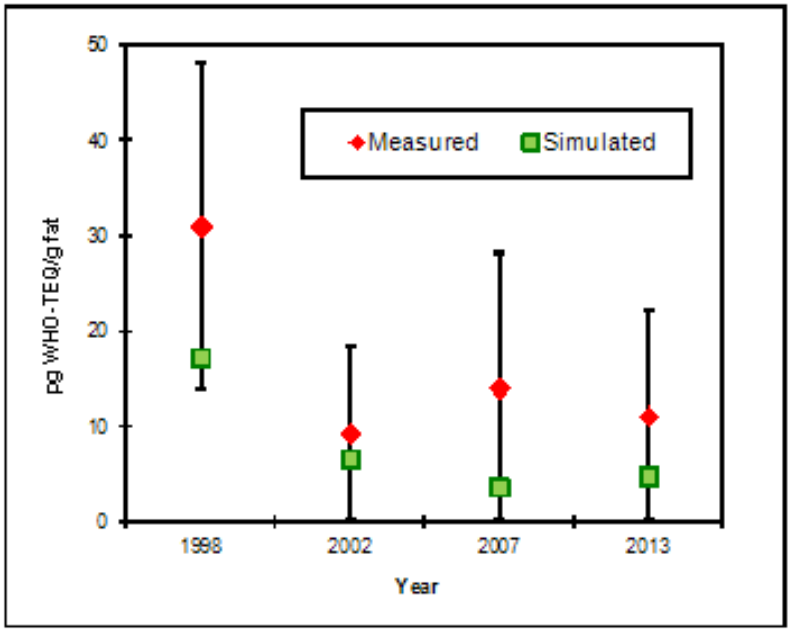


Fig. 2. Comparison between measured and simulated concentrations of PCDD/Fs in samples of adipose tissue collected in 1998, 2002, 2007 and 2013.

Table 5. Comparison between simulated (Sim) and measured (Exp) concentrations of PCDD/Fs found in adipose tissue of adults living near the HWI of Tarragona County (Spain).

	1998		2002		2007		2013	
	Sim	**Exp**	**Sim**	**Exp**	**Sim**	**Exp**	**Sim**	**Exp**
2,3,7,8-TCDD	3.40	4.13	0.81	1.39	0.48	1.68	0.88	1.24
1,2,3,7,8-PeCDD	3.91	11.37	2.25	3.73	1.68	5.28	1.62	4.11
1,2,3,4,7,8-HxCDD	0.89	5.61	0.74	2.78	0.35	3.30	0.22	2.26
1,2,3,6,7,8-HxCDD	10.22	59.40	3.29	19.20	2.03	28.10	0.87	25.35
1,2,3,7,8,9-HxCDD	9.07	8.12	4.37	2.08	2.19	3.55	5.32	2.63
1,2,3,4,6,7,8-	64.24	84.90	8.04	10.20	3.10	20.00	3.80	20.29
OCDD	114.52	477.50	7.85	53.60	2.67	151.90	3.53	113.27
2,3,7,8-TCDF	8.21	0.94	2.10	0.34	1.08	0.40	1.86	0.35
1,2,3,7,8-PeCDF	9.23	0.92	2.18	0.50	0.88	1.40	0.91	0.31
2,3,4,7,8-PeCDF	11.52	21.10	4.76	5.71	1.81	9.94	2.62	7.43
1,2,3,4,7,8-HxCDF	9.70	7.02	2.37	2.32	0.92	3.29	1.50	2.83
1,2,3,6,7,8-HxCDF	4.53	8.22	1.34	2.03	0.60	3.31	0.62	2.69
1,2,3,7,8,9-HxCDF	1.54	0.62	1.43	0.39	0.49	0.06	1.35	0.07
2,3,4,6,7,8-HxCDF	3.93	2.20	3.14	0.38	0.53	0.88	1.14	0.69
1,2,3,4,6,7,8-	45.72	4.81	5.98	2.06	4.99	2.99	9.63	2.34
1,2,3,4,7,8,9-	9.11	0.39	1.67	0.31	1.32	0.10	3.12	0.11
OCDF	376.86	0.72	9.59	2.59	40.67	0.49	21.78	0.31
WHO-TEQ	17.19	32.1	6.59	9.94	3.66	14.6	4.77	11.5

Concentrations are given in pg/g tissue.

orders of magnitude higher than the modelled results (21.78 vs. 0.31 pg/g fat). Notable dissimilarities between estimated and observed concentrations of OCDF in adipose tissue were also reported in previous campaigns (1998, 2002, and 2007). The transfer of POPs from the vascular environment to other biological tissues is highly influence by pharmacokinetic factors, such as tissue volume, anatomical localization, and blood flow rate. The default approach assumes that the tissue is flow limited. It means that the distribution of chemicals contained in blood across the well-stirred tissue compartment is fast and homogenous. Although this assumption is valid for the distribution of many xenobiotic chemicals into many tissues, because of their diffusion-limited characteristics, it appears to be incorrect for the movement of several highly lipophilic POPs across the adipose tissue (Levitt, 2010; La Merrill et al., 2013). In this case, the distribution of the chemicals is slower and may be incomplete. A comparison between measured and simulated concentrations of PCDD/Fs in samples of human adipose tissue collected in 1998, 2002, 2007 and 2013, is depicted in Fig. 2. Because food intake was assumed to be the only single exposure pathway, a significant reduction of fat concentrations of

PCDD/Fs was noted. This is in agreement with the significant decrease found in the dietary intake of PCDD/Fs (Domingo et al., 2012). An important uncertainty, represented by the high standard deviations, was observed in the experimental concentrations of PCDD/Fs. As abovementioned, age and BMI of individuals can affect PCDD/F accumulation in fat tissue. In addition, diverse dietary patterns may be also the reason of the differences found between individuals. Anyhow, simulated concentrations of PCDD/Fs in fat were within the range of experimental values, irrespectively of the sampling year. According to Berezhkovskiy (2011), the classical pharmacokinetic analysis of highly lipophilic POPs at low concentrations often leads to a substantial underestimation of the distribution volume during steady state (Vss) in obese people. Therefore, an accurate determination of Vss is required to assess the distribution and kinetics of environmental chemicals. In any case, further research to improve the understanding of the adipose tissue:blood partitioning mechanisms, and the differences according to the PCDD/F congener, is needed. Moreover, the histological and anatomical structure of different types of adipose tissue can influence their contribution to toxicokinetics (Sbarbati et al., 2010). Furthermore, age and BMI may be also important in epidemiology studies, where back-extrapolation from current PCDD/F levels is used. In order to improve the model, different partition ratios for each PCDD/F congener should be taken into account. Another issue for improvement is the inclusion of dynamic parameters of exposure, such as time varying lifetime exposure.

4. Conclusions

The PCDD/F concentrations in adipose tissue of 15 individuals who had been living for at least the last 10 years near a HWI located in Tarragona County (Spain), were here determined. A mean PCDD/F level of 11.5 pg WHO-TEQ/g fat was found, being significantly lower than the concentration observed in 1998 (32.1 pg WHO-TEQ/g fat), when the facility was still inactive (Schuhmacher et al., 1999b). Current values of PCDD/Fs in adipose tissue in Tarragona County are of the same order of magnitude than those recently observed in a number of industrialized countries. The important decrease, which was also noted in other biological monitors such as plasma and breast milk (Nadal et al., 2013; Schuhmacher et al., 2013), agrees well with the notable reduction in the dietary intake of PCDD/Fs recently found for the population living in the same area. Our findings confirm that, after 14 years of regular operations, air emissions of PCDD/Fs from the HWI do not mean a significant additional exposure to these organic pollutants for the population living near the facility.

On the other hand, our adapted PBPK model has demonstrated to be a reliable tool to predict the levels of PCDD/Fs in fat tissue, as it was also in plasma (Nadal et al., 2013). Although the application of the model should be of great interest to estimate the long-term accumulation of PCDD/Fs, not only in fat, but also in other biological tissues, an improvement of the PBPK model is required to obtain more accurate results.

References

Berezhkovskiy, L., 2011. On the accuracy of estimation of basic pharmacokinetic parameters by the traditional noncompartmental equations and the prediction of the steady-state volume of distribution in obese patients based upon data derived from normal subjects. Journal of Pharmacological Sciences 100, 2482–97.

Bocio, A., Domingo, J.L., 2005. Daily intake of polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans (PCDD/PCDFs) in foodstuffs consumed in Tarragona, Spain: a review of recent studies (2001-2003) on human PCDD/PCDF exposure through the diet. Environmental Research 97, 1-9.

Brown, R.P., Delp, M.D., Lindstedt, S.L., Rhomberg, L.R., Beliles, R.P., 1997. Physiological parameter values for physiologically based pharmacokinetic models. Toxicology & Industrial Health 13, 407-84.

Bunsan, S., Chen, W.Y., Chen, H.W., Chuang, Y.H., Grisdanurak, N., 2013. Modeling the dioxin emission of a municipal solid waste incinerator using neural networks. Chemosphere 92, 258-64.

Chiu, W.A., Barton, H.A., DeWoskin, R.S., Schlosser, P., Thompson, C.M., Sonawane, B., Lipscomb, J.C., Krishnan, K., 2007 Evaluation of physiologically based pharmacokinetic models for use in risk assessment. Journal of Applied Toxicology 27, 218-37.

Clewell, R.A., Clewell III, H.J., 2008. Development and specification of physiologically based pharmacokinetic models for use in risk assessment. Regulatory Toxicology & Pharmacology 50, 129-43.

Çok, İ., Donmez, M.K., Hakan Satıroğlu, M., Aydınuraz, B., Henkelmann, B., Kotalik, J., Schramm, K.W. 2007. Concentration of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and dioxin-like PCBs in human adipose tissue from Turkish men. Chemosphere 66, 1955-61.

Collins, J.J., Bodner, K., Burns, C.J., Budinsky, R.A., Lamparski, L.L., Wilken, M., Martin, G.D., Carson, M.L., 2007 Body mass index and serum chlorinated dibenzo-*p*-dioxin and dibenzofuran levels. Chemosphere 66, 1079-85.

Domingo, J.L., Perelló, G., Nadal, M., Schuhmacher, M., 2012 Dietary intake of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) by a population living in the vicinity of a hazardous waste incinerator. Assessment of the temporal trend. Environment International 50, 22-30.

Domingo, J.L., Schuhmacher, M., Granero, S., Llobet, J.M., 1999. PCDDs and PCDFs in food samples from Catalonia, Spain. An assessment of dietary intake. Chemosphere 38, 3517-28.

Elobeid, M., Padilla, M., Brock, D., Ruden, D., Allison, D., 2010. Endocrine disruptors and obesity: an examination of selected persistent organic pollutants in the NHANES 1999–2002 data. International Journal of Environmental Research & Public Health 7, 2988–3005.

Emond, C., Michalek, J.E., Birnbaum, L.S., DeVito, M.J., 2005. Comparison of the use of a physiologically based pharmacokinetic model and a classical

pharmacokinetic model for dioxin exposure assessments. Environmental Health Perspectives 113, 1666–8.

Estrellan, C.R., Iino, F., 2010. Toxic emissions from open burning. Chemosphere 80, 193-207.

Fàbrega, F., Kumar, V., Schuhmacher, M., Domingo, J.L., Nadal, M., 2014. PBPK modeling for PFOS and PFOA: Validation with human experimental data. Toxicology Letters S0378-4274, 00017-14.

Ferré-Huguet, N., Nadal, M., Schuhmacher, M., Domingo, J.L., 2006. Environmental impact and human health risks of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in the vicinity of a new hazardous waste incinerator: A case study. Environmental Sciences & Technology 40, 61-6.

Fromme, H., Albrecht, M., Boehmer, S., Büchner, K., Mayer, R., Liebl, B., Wittsiepe, J., Bolte, G., 2009. Intake and body burden of dioxin-like compounds in Germany: The INES study. Chemosphere 76, 1457-63.

Glorennec, P., Zmirou, D., Bard, D., 2005 Public health benefits of compliance with current E.U. emissions standards for municipal waste incinerators: A health risk assessment with the CalTox multimedia exposure model. Environment International 31, 693-701.

Hagmar, L., Wallin, E., Vessby, B., Jönsson, B.A.G., Bergman, Å., Rylander, L., 2006. Intra-individual variations and time trends 1991–2001 in human serum levels of PCB, DDE and hexachlorobenzene. Chemosphere 64, 1507-13.

Hu, S.W., ChangChien, G.P., Chan, C.C., 2004. PCDD/Fs levels in indoor environments and blood of workers of three municipal waste incinerators in Taiwan. Chemosphere 55, 611-20.

Kiviranta, H., Tuomisto, J.T., Tuomisto, J., Tukiainen, E., Vartiainen, T., 2005. Polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in the general population in Finland. Chemosphere 60, 854-69.

Kulkarni, P.S., Crespo, J.G., Afonso, C.A.M., 2008 Dioxins sources and current remediation technologies - A review. Environment International 34, 139-53.

La Merrill, M., Emond, C., Kim, M., Antignac, J., Le Bizec, B., Clément, K., Birnbaum, L.S., Barouki, R., 2013. Toxicological function of adipose tissue: focus on persistent organic pollutants. Environ Health Perspectives 121, 162-9.

Landi, M.T., Consonni, D., Patterson, D.G.J., Needham, L., Lucier, G., Brambilla, P., Cazzaniga, M.A., Mocarelli, P., Pesatori, A.C., Bertazzi, P.A., Caporaso, N.E., 1998. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin plasma levels in Seveso 20 years after the accident. Environ Health Perspectives 106, 273–7.

Lee, D., Lee, I., Porta, M., Steffes, M., Jacobs, D., 2007. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999–2002. Diabetologia 50, 1841-51.

Levitt, D.G., 2010. Relationship between the octanol/water partition coefficient and the diffusion limitation of the exchange between adipose and blood. BMC Clinical Pharmacology 10:1

Lim, J.S., Son, H.K., Park, S.K., Jacobs, D.J., Lee, D.H., 2011. Inverse associations between long-term weight change and serum concentrations of persistent organic pollutants. International Journal of Obesity 35, 744-7.

Liu, H.M., Lu, S.Y., Buekens, A.G., Chen, T., Li, X.D., Yan, J.H., Ma, X.L., Cen, K.F., 2012. Baseline soil levels of PCDD/Fs established prior to the construction of municipal solid waste incinerators in China. Chemosphere 86, 300-7.

Lopez-Espinosa, M.J., Kiviranta, H., Araque, P., Ruokojärvi, P., Molina-Molina, J.M., Fernandez, M.F., Vartiainen, T., Olea, N., 2008. Dioxins in adipose tissue of women in Southern Spain. Chemosphere 73, 967-71.

Lorber, M., Phillips, L., 2002. Infant exposure to dioxin-like compounds in breast milk. Environmental Health Perspectives 110, A325–32.

Mannetje, A., Coakley, J., Mueller, J.F., Harden, F., Toms, L.M., Douwes, J., 2012. Partitioning of persistent organic pollutants (POPs) between human serum and breast milk: A literature review. Chemosphere 89, 911-8.

Mari, M., Domingo, J.L., 2012 Toxic emissions from crematories: A review. Environment International 36, 131-7.

Mari, M., Díaz-Ferrero, J., Schuhmacher, M., Nadal, M., Domingo, J.L., 2013 Health risks of environmental exposure to PCDD/Fs near a hazardous waste incinerator in Catalonia, Spain. Journal of Risk Analysis and Crisis Response 3, 77-87.

Martí-Cid, R., Bocio, A., Domingo, J.L., 2008. Dietary exposure to PCDD/PCDFs by individuals living near a hazardous waste incinerator in Catalonia, Spain: Temporal trend. Chemosphere 70, 1588-95.

Maruyama, W., Yoshida, K., Tanaka, T., Nakanishi, J., 2003. Simulation of dioxin accumulation in human tissues and analysis of reproductive risk. Chemosphere 53, 301-13.

Mocarelli, P., Gerthoux, P.M., Patterson Jr., D.G., Milani, S., Limonta, G., Bertona, M., Signorini, S., Tramacere, P., Colombom, L., Crespi, C., Brambilla, P., Sarto, C., Carreri, V., Sampson, E.J., Turner, W.E., Needham, L.L., 2008. Dioxin exposure, from infancy through puberty, produces endocrine disruption and affects human semen quality. Environmental Health Perspectives 116, 70-7.

Moon, H.B., Lee, D.H., Lee, Y.S., Kannan, K., 2011. Concentrations and accumulation profiles of PCDDs, PCDFs and dioxin-like PCBs in adipose fat tissues of Korean women. Journal of Environmental Monitoring 13, 1096-101.

Nadal, M., Schuhmacher, M., Domingo, J.L., 2004a. Probabilistic human health risk of PCDD/F exposure: a socioeconomic assessment. Journal of Environmental Monitoring 6, 926-31.

Nadal, M., Espinosa, G., Schuhmacher, M., Domingo, J.L., 2004b. Patterns of PCDDs and PCDFs in human milk and food and their characterization by artificial neural networks. Chemosphere 54, 1375-82.

Nadal, M., Perelló, G., Schuhmacher, M., Cid, J., Domingo, J.L., 2008. Concentrations of PCDD/PCDFs in plasma of subjects living in the vicinity of a hazardous waste incinerator: Follow-up and modeling validation. Chemosphere 73, 901-906.

Nadal, M., Fàbrega, F., Schuhmacher, M., Domingo, J.L., 2013. PCDD/Fs in plasma of individuals living near a hazardous waste incinerator. A comparison of measured levels and estimated concentrations by PBPK modeling. Environmental Science and Technology 47, 5971-8.

Needham, L.L., Grandjean, P., Heinzow, B., Jørgensen, P.J., Nielsen, F., Patterson, D.G., Andreas, S., Turner, W.E., Weihe, P., 2011. Partition of environmental chemicals between maternal and fetal blood and tissues. Environmental Science and Technology 45, 1121-6.

Nestorov, I., 2007. Whole-body physiologically based pharmacokinetic models. Expert Opinion on Drug Metabolism and Toxicology 3, 235-49.

Ooi, T.C., Lu, L., 2011. Formation and mitigation of PCDD/Fs in iron ore sintering. Chemosphere 85, 291-9.

Parera, J., Serra-Prat, M., Palomera, E., Mattioli, L., Abalos, M., Rivera, J., Abad, E., 2013. Biological monitoring of PCDD/Fs and PCBs in the City of Mataró. A population-based cohort study (1995–2012). Science of the Total Environment 461–462, 612-7.

Passuello, A., Mari, M., Nadal, M., Schuhmacher, M., Domingo, J.L., 2010. POP accumulation in the food chain: Integrated risk model for sewage sludge application in agricultural soils. Environment International 36, 577-83.

Reis, M.F., Miguel, J.P., Sampaio, C., Aguiar, P., Melim, J.M., Päpke, O., 2007. Determinants of dioxins and furans in blood of non-occupationally exposed populations living near Portuguese solid waste incinerators. Chemosphere 67, S224-30.

Rodin, J., 1992. Determinants of body fat localization and its implications for health. Annals of Behavioral Medicine 14, 275-81.

Sbarbati, A., Accorsi, D., Benati, D., Marchetti, L., Orsini, G., Rigotti, G., Panettiere, P., 2010. Subcutaneous adipose tissue classification. European Journal of Histochemistry 54, e48.

Schildkraut, J.M., Demark-Wahnefried, W., DeVoto, E., Hughes, C., Laseter, J.L., Newman, B., 1999. Environmental contaminants and body fat distribution. Cancer, Epidemiology, Biomarkers and Prevention 8, 179-83.

Schuhmacher, M., Domingo, J.L., 2006. Long-term study of environmental levels of dioxins and furans in the vicinity of a municipal solid waste incinerator. Environment International 32, 397-404.

Schuhmacher, M., Domingo, J.L., Hagberg, J., Lindström, G., 2004. PCDD/F and non-*ortho* PCB concentrations in adipose tissue of individuals living in the vicinity of a hazardous waste incinerator. Chemosphere 57: 357-64.

Schuhmacher, M., Domingo, J.L., Llobet, J.M., Kiviranta, H., Vartiainen, T., 1999a. PCDD/F concentrations in milk of nonoccupationally exposed women living in southern Catalonia, Spain. Chemosphere 38, 995-1004.

Schuhmacher, M., Domingo, J.L., Llobet, J.M., Lindström, G., Wingfors, H., 1999b. Dioxin and dibenzofuran concentrations in adipose tissue of a general population from Tarragona, Spain. Chemosphere 38, 2475-87.

Schuhmacher, M., Domingo, J.L., Llobet, J.M., Lindström, G., Wingfors, H., 1999c. Dioxin and dibenzofuran concentrations in blood of a general population from Tarragona, Spain. Chemosphere 38, 1123-33.

Schuhmacher, M., Kiviranta, H., Ruokojärvi, P., Nadal, M., Domingo, J.L., 2013. Levels of PCDD/Fs, PCBs and PBDEs in breast milk of women living in the vicinity of a hazardous waste incinerator: Assessment of the temporal trend. Chemosphere 93, 1533-40.

Shen, H., Han, J., Tie, X., Xu, W., Ren, Y., Ye, C., 2009. Polychlorinated dibenzo-*p*-dioxins/furans and polychlorinated biphenyls in human adipose tissue from Zhejiang Province, China. Chemosphere 74, 384-8.

Takenaka, S., Todaka, T., Nakamura, M., Hori, T., Iida, T., Yamada, T., Hata, J-I., 2002. Polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and non-*ortho*, mono-*ortho* chlorine substituted biphenyls in Japanese human liver and adipose tissue. Chemosphere 49, 161-72.

Thomaseth, K., Salvan, A., 1998. Estimation of occupational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin using a minimal physiologic toxicokinetic model. Environmental Health Perspectives 106, 743–53.

Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2006. The 2005 World Health Organization reevaluation of human and mammalian Toxic Equivalency Factors for dioxins and dioxin-like compounds. Toxicological Sciences 93, 223-41.

Vilavert, L., Nadal, M., Mari, M., Schuhmacher, M., Domingo, J.L., 2010. Monitoring temporal trends in environmental levels of polychlorinated dibenzo-*p*-dioxins and dibenzofurans: results from a 10-year surveillance program of a hazardous waste incinerator. Archives of Environmental Contamination and Toxicology 59, 521-31.

White, S.S., Birnbaum, L.S., 2009. An overview of the effects of dioxins and dioxin-like compounds on vertebrates, as documented in human and ecological epidemiology. Journal of Environmental Science & Health, Part C. Environmental Carcinogenesis and Ecotoxicology Reviews 27, 197-211.

Windal, I., Vandevijvere, S., Maleki, M., Goscinny, S., Vinkx, C., Focant, J., Eppe, G., Van Loco, J., 2010. Dietary intake of PCDD/Fs and dioxin-like PCBs of the Belgian population. Chemosphere 79, 334-40.

Discussion of Chapters II and III

The PBPK model was firstly developed for PCDD/Fs, which show a relatively simple PK/PD behavior in the human body. PCDD/Fs are highly absorbed, while it was assumed they are not metabolized and the elimination mechanism is only through feces. PCDD/Fs were mainly chosen as a representative of classic POPs. The simulated results of PCDD/Fs in plasma and adipose tissue were very coincident with the experimental values, not only in terms of I-TEQ, but also in the profile of the 17 PCDD/F congeners. Measured mean level in plasma was 6.18 pg I-TEQ/g lipid, while modelled concentration was 7.9 pg I-TEQ/ g lipid. According to WHO (2010), a PBPK model may be considered as adequate if, on average, simulation results are inside the range within a factor of two of experimental data. Furthermore, for the first time adipose tissue has been validated demonstrating the reliability of PBPK to simulate fat. Due to their high octanol water partition coefficient (log Kow>4), adipose tissue is the main site of accumulation of PCDD/Fs. Hence, it is highly important to monitor this compartment. In adipose tissue, the simulated vs experimental levels of PCDD/Fs were also of the same order of magnitude (4.77 vs 11.15 pg I-TEQ/g lipid). Therefore, PBPK models have proven to be validated to estimate long-term concentrations of PCDD/Fs in human tissues.

The PBPK model was also applied to evaluate temporal trends of PCDD/Fs in the human body. The simulation was carried out in four temporal scenarios: 1998, 2002, 2007 and 2013. As a consequence of the reduction of PCDD/F emission sources, the levels of PCDD/Fs in human tissues have been decreasing over the last years in Tarragona County. In plasma, the concentration of PCDD/Fs were 27.0, 15.7, 9.36 and 6.18 pg I-TEQ/g lipid in 1998, 2002, 2007 and 2013. In adipose tissue, the concentrations were 32.1, 9.9, 14.6 and 11.5 pg WHO-TEQ/g fat, respectively, in the same campaigns.

According to our results, the use of PBPK models may notably reduce the cost of surveillance studies by minimizing the number of biological samples, something critical in the context of the current economic crisis. Moreover, the PBPK models are capable to make predictions of future scenarios of concentrations of PCDD/Fs in human tissues.

CHAPTER 4

PBPK MODELING FOR PFOS AND PFOA: VALIDATION WITH HUMAN EXPERIMENTAL DATA

Abstract

In recent years, because of the potential human toxicity, concern on perfluoroalkyl substances (PFASs) has increased notably with special attention to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). Unfortunately, there is currently an important knowledge gap on the burdens of these chemicals in most human tissues, as the reported studies have been mainly focused on plasma. In order to overcome these limitations, the use of physiologically-based pharmacokinetic (PBPK) models has been extended. The present study was aimed at testing an existing PBPK model for their predictability of PFOS and PFOA in a new case-study, and also to adapt it to estimate the PFAS content in human tissue compartments. Model validation was conducted by means of PFOA and PFOS concentrations in food and human drinking water from Tarragona County (Catalonia, Spain), and being the predicted results compared with those experimentally found in human tissues (blood, liver, kidney, liver and brain) of subjects from the same area of study. The use of human-derived partition coefficient (Pk) data was proven as more suitable for application to this PBPK model than rat-based Pk values. However, the uncertainty and variability of the data are still too high to get conclusive results. Consequently, further efforts should be carried out to reduce parametric uncertainty of PBPK models. More specifically, a deeper knowledge on the distribution of PFOA and PFOS within the human body should be obtained by enlarging the number of biological monitoring studies on PFASs.

1. Introduction

Perfluoroalkyl substances (PFASs) are a group of man-made substances, whose chemical structure is a carbon backbone, where the hydrogen has been substituted by fluorine. The carbon fluorine bound is among the strongest covalent bounds, conferring a high molecular stability. PFASs have widely used in consumer and industrial applications, including protective coatings for fabrics and carpets, paper coatings, insecticides, paints, cosmetics, and fire-fighting foams (Domingo, 2012). However, the properties making these chemicals useful, turns into environmental problems. Thus, PFASs show a high persistence and spreading capacity in the environment (Fujii et al., 2007). Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are among

the most widely spread PFASs, having been detected in a number of environmental matrices, including water, sediments, soils and biota (Post et al., 2009; Rumsby et al., 2009; Shi et al., 2012; Zareitalabad et al., 2013). Detectable concentrations of PFOA and PFOS have been found in food (Domingo et al., 2012a), while measurements in whole blood, plasma or serum samples from humans are also available from the scientific literature (Ehresman et al., 2007). Because of their low degradation, high bioaccumulation, potential toxicity and long-range transport capacity, both PFOS and PFOA are considered as persistent organic pollutants (POPs) (Chaemfa et al., 2010). In fact, PFOS was included as a POP under the Stockholm Convention in 2009, while PFOA remains a serious candidate to enter that list shortly. Very recently, PFOS was also identified as a priority hazardous substance according to the European Directive 2013/39/EU, in the field of water policy (European Commission, 2013). Consequently, regulatory agencies are paying considerable attention to the presence of PFOA and PFOS in the environment, as well as to the human health risks associated to their exposure.

Pharmacokinetic and pharmacodynamic characteristics of PFOA and PFOS have been studied in animals (Lau et al., 2007; Vanden Heuvel et al., 1991). These compounds are highly absorbed, not metabolized and poorly eliminated, being plasma, liver, kidney and lungs the main distribution tissues (Cui et al., 2009; Hundley et al., 2006). Both compounds have a high affinity to albumin, being therefore accumulated in plasma. Specific PFOA half-lives in a number of animal species are known. Elimination half-lives of PFOA, ranging 2–6 h, 17–19 days, and 30–21 days, have been estimated for rats, mice, and monkeys, respectively (Lau et al., 2007; Lau, 2012). Contrastingly, PFOA half-life in the human body is markedly higher, with an estimated value of 3.8 years. Regarding PFOS, half-lives have been established in 1-2 months, 4 months, and 4.8 years in rodents, monkeys, and humans, respectively (Chang et al., 2012). Although the reasons of these differences are not conclusive, it has been hypothesized that it can be due to a saturable process of resorption of PFASs from urine to plasma (Andersen et al., 2006). In addition to bioaccumulation, toxicity of PFOS and PFOA has been also characterized in animals (Lau et al., 2004; Stahl et al., 2011). A number of developmental effects, such as reduction of fetal weight, edema, cardiac abnormalities, and delayed ossification, as well as behavioral effects, have been reported following acute exposure to PFOS (Fuentes et al., 2006, 2007a,b). Subchronic and subacute toxicities induce hepatotoxicity, reduction of body weight, reduction of the levels of triglycerides and cholesterol in serum, liver hypertrophy and thyroid hormones reduction, while the neuroendocrine system seems also to be affected in rats exposed to PFOS (Austin et

al., 2003). Recently, PFOA exposure has been suggested to be associated with kidney and testicular cancer in human populations (Barry et al., 2013).

The time dependent concentration of a certain pollutant in human tissues can be predicted using physiologically-based pharmacokinetic (PBPK) models. These models consider the human body as a set of well stirred compartments linked by the blood flow. Physiological processes are represented by a set of ordinary differential equations describing the processes of administration, distribution, metabolism and elimination of a specific chemical (Loizou et al., 2008; Péry et al., 2013). The final result is a model that simulates the time course distribution of a substance in the human body (Nadal et al., 2013), which helps to quantify the relationship between measures of external exposure and internal dose. Detailed species-specific physiological, chemical, and biochemical parameters have been obtained. It allows not only extrapolating data to humans, but also assessing the possible sources of variability and uncertainty in model parameterization (Huizer et al., 2012). Due to their potential power, PBPK models have been largely used in pharmacological development and health risk assessment (Chiu et al., 2007; Clewell and Clewell III, 2008).

The objectives of the present study were the following: (1) to test an existing PBPK model for their predictability of PFOS and PFOA in a new case-study, and (2) to adapt the PBPK model to estimate the burdens in various human tissue compartments. The performance of this PBPK model, when using different partition coefficients (Pk), either from rats or humans, was studied in detail. In PBPK models, Pk is the main regulator parameter of the distribution of chemicals in the human body. Model validation was conducted using data on PFOS and PFOA levels in food and drinking water from Tarragona County (Tarragona, Spain). The model was calibrated and validated by using experimental data from autopsy tissues of subjects residing in the Tarragona County area.

2. Materials and Methods

A new PBPK model for PFOA and PFOS was developed based on a previously reported model (Loccisano et al., 2011, 2013). The key process adopted in the model is the kinetics of resorption by renal transporters in the filtrate compartment, where chemicals are reabsorbed back to plasma through a saturable process (Andersen et al., 2006; Tan et al., 2008). This resorption mechanism could be responsible for the high persistence of PFOA and PFOS in human blood, compared to the low persistence found in other animal species (e.g., rat, monkey). In addition to plasma, gut, liver, fat, kidney,

filtrate, and the remaining body compartments, the adapted PBPK model included lungs and brain. However, since it is not a potential site of absorption/accumulation for PFASs, skin was removed. The final compartmental structure of the adapted PBPK model is depicted in Fig. 1. The human tissues were selected due to their toxicokinetic relevance: plasma as a carrier tissue of PFASs in the human body, gut as an absorption site, kidney for its role in elimination (Barry et al., 2013), brain as target organ of PFASs neurotoxic effects (Mariussen, 2012), liver as an accumulative tissue for organic chemicals, lungs because they may exhibit immaturity after PFOS exposure (Grasty et al., 2005), and fat as a main site of accumulation in lipophilic tissues. The rest of the body was considered as an independent compartment to include PFASs remaining in other tissues of the human body. In plasma, more than 90% of PFOA and PFOS is bound to albumin, while <10% is free to move to other tissues (Han et al., 2003).

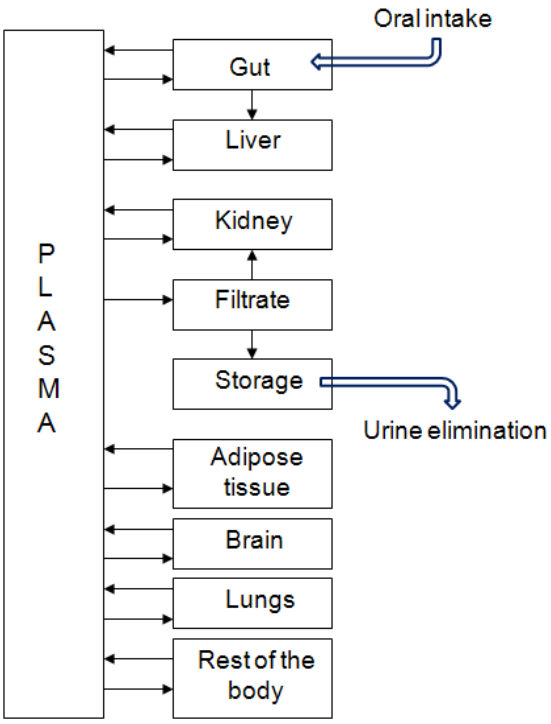


Fig. 1. Structure of the PBPK model for PFOA and PFOS.

The PBPK model was based on a series of differential equations. The expression to estimate the levels of PFOA and PFOS in non-elimination tissues (fat, brain, lungs, and rest of the body) was the following:

$$\frac{dCi}{dt} = \frac{Qi \times free \times (Ca - Ci/Ki : p)}{Vi} \quad (1)$$

where Ci is the cellular concentration in each tissue (pg/mL), Qi is the blood flow (mL/h), free means the free amount of PFASs in plasma (unitless), Ca is the arterial concentration (pg/mL), Ki:p is the partition coefficient (unitless), and Vi is the tissue volume (mL).

The cellular concentrations of PFOA and PFOS in gut were obtained by applying this equation:

$$\frac{dCg}{dt} = \frac{\left(Qg \times free \times \left(Ca - {Cg}/{Kg:p}\right) + Intake\right)}{Vg} \quad (2)$$

where Cg is the cellular concentration in gut (pg/mL), Qg is the blood flow to gut (mL/h), free means the free amount of PFASs in plasma (unitless), Ca is the arterial concentration (pg/mL), Kg:p is the gut partition coefficient (unitless), Intake is theoral daily intake (pg/h), and Vg is the gut volume (mL).

With respect to the liver compartment, estimated values of PFASs were estimated by means of the following expression:

$$\frac{dCl}{dt} = \frac{free \times \left(Ql \times Ca + Qg \times {Cg}/{Kg:p} - (Ql + Qg) \times \left({Cl}/{Kl:p}\right)\right)}{Vl} \quad (3)$$

where Cl is the cellular concentration in liver (pg/mL), free means the free amount of PFASs in plasma (unitless), Ql is the blood flow to liver (mL/h), Ca is the arterial concentration (pg/mL), Qg is the blood flow to gut (mL/h), Cg is the cellular concentration in gut (pg/mL), Kg:p is the gut partition coefficient (unitless), Kl:p is the liver partition coefficient (unitless), and Vl is the liver volume (mL).

For the kidney compartment the following equation was used:

$$\frac{dCk}{dt} = \frac{\left(Qk \times free \times \left(Ca - \frac{Ck}{Kk:p}\right) + \frac{Tm \times Cfil}{Kt + Cfil}\right)}{Vk} \quad (4)$$

where Ck is the cellular concentration in kidney (pg/mL), Qk is the blood flow to kidney (mL/h), free is the free amount of PFASs in plasma (unitless), Ca is the arterial concentration (pg/mL), Kk:p is the gut partition coefficient (unitless), Tm is the resorption maximum (pg/h), Cfil is the cellular concentration in filtrate (pg/mL), Kt is the affinity constant (ng/mL), and Vk is the kidney volume (mL).

Finally, PFAS concentrations in the filtrate compartment were simulated by applying this equation:

$$\frac{dCfil}{dt} = \frac{\left(Qfil \times \left(free \times Ca - Cfil \right) - \frac{Tm \times Cfil}{Kt + Cfil} \right)}{Vfil} \quad (5)$$

where Cfil is the cellular concentration in filtrate (pg/mL), Qfil is the blood flow to filtrate (mL/h), free is the free amount of PFASs in plasma (unitless), Ca is the arterial concentration (pg/mL), Tm is the resorption maximum (pg/h), Kt is the affinity constant (ng/mL), and Vfil is the filtrate volume (mL).

Flow limited PBPK equations were used in all tissues (Thompson et al., 2012). Physiological data of volumes and cardiac output, which were time-constant, were obtained from Brown et al. (1997) (Table 1). Plasma concentrations of PFOA and PFOS were adjusted by calibrating the elimination values to fit experimental values from a case-study (Ericson et al., 2007). Thus, our PBPK model was applied to estimate the burdens of PFOS and PFOA for a population living in Tarragona County (Catalonia, NE of Spain), from which data on human intake and body burdens were available (Domingo et al., 2012a,b; Ericson et al., 2007; Pérez et al., 2013). Human exposure to PFOA and PFOS was evaluated through two different pathways: water consumption and food intake. Water ingestion was calculated as the product of the concentration in human drinking water (2.40 and 1.81 ng/L for PFOA and PFOS, respectively) in Catalonia (Domingo et al., 2012b) and the most typical value of water daily intake (1.23 L/day), according to the US EPA (2011). Similarly, dietary exposure was estimated based on the mean PFAS concentration in 40 food items, which are representative of the Catalan diet, and the respective daily consumption by the general population (Domingo et al., 2012a). In agreement with previous findings (Ericson Jogsten et al., 2012; Fromme et al., 2009; Kim et al., 2013), food intake was found to be the most important contributive route to the exposure of PFOS and PFOA, with percentages of 97% and 98% of the total intake, respectively. Dietary exposure of PFOA and PFOS was estimated in 1.55 and 1.80 ng/kg body weight/day, respectively. Overall, the total daily intake of PFOA through water and food consumption was estimated to be 0.11 g/day for PFOA, while that of PFOS was found to be 0.13 g/day.

Table 1. Physiological parameters used in the PBPK model for PFOS and PFOA, and pharmacokinetic data.

	Volume	**Cardiac output**
Plasma	2.78	-
Fat	1.43	19.03
Brain	1.50	36.50
Lungs	1.00	10.61
Rest of the	61.59	64.27
Gut	1.14	56.47
Liver	1.64	58.97
Kidney	0.29	55.22
Filtrate	0.03	10.92
Total	71.4	312
	PFOA	**PFOS**
Tmc	147.4	86.0
Kt	0.116	0.0176
Free	0.03	0.03

Data taken from Brown et al. (1997) and Loccisano et al. (2011); Tmc: resorption maximum (µg/h); Kt: affinity constant (µg/L); Free: free fraction in plasma (unitless); Conversion from weight to volume was assumed to be 1.

For validation purposes, data on PFOA and PFOS in human tissues from people living in the area of study (Tarragona County) were used. Ericson et al. (2007) reported the levels of 13 PFASs, including PFOA and PFOS, in blood samples of 48 residents in that same area. The mean PFOS concentration in blood was 7.64 ng/mL, while PFOA mean level was 1.80 ng/mL. Recently, Pérez et al. (2013) analyzed the concentrations of 21 PFASs in 99 samples of autopsy tissues (brain, liver, lung, bone, and kidney) from subjects who had been living in Tarragona County (Catalonia, Spain). At the time of death, the mean age of subjects was 57 years, with minimum and maximum values of 28 and 86 years, respectively. A summary of the levels of PFOA and PFOS is shown in Fig. 2. Although PFASs have been largely monitored in human blood/plasma (Ehresman et al., 2007; Stahl et al., 2011), studies in other human tissues are certainly very limited, except some data on breast milk and liver (Kärrman et al., 2010; Lau et al., 2007; Yeung et al., 2013). To the best of our knowledge, to date only two studies have reported burdens of PFOS and PFOA in other human tissues. In Italy, Maestri et al. (2006) analyzed the concentrations of these compounds in samples of human liver, kidney, adipose tissue, brain, basal ganglia, hypophysis, thyroid, gonads, pancreas, lung, skeletal muscle, and blood.

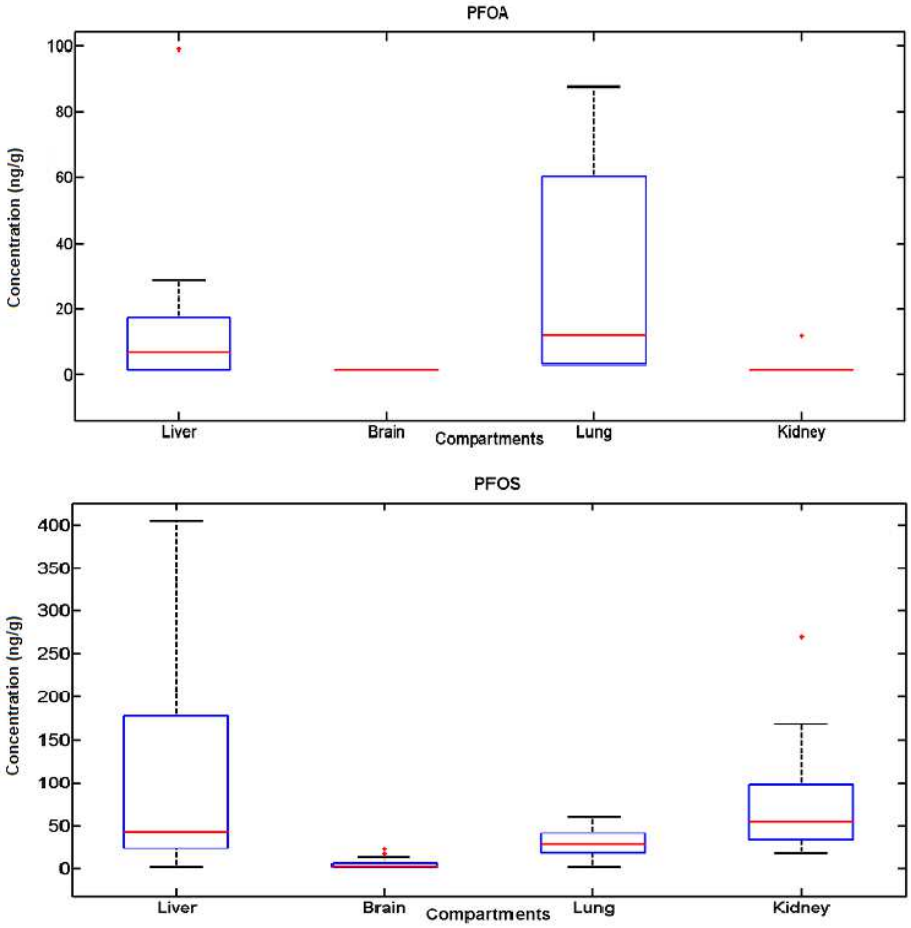


Fig. 2. Concentrations of PFOA and PFOS in autopsy tissues of 20 residents from Tarragona County (Catalonia, Spain). Data from Pérez et al., (2013).

In addition to the model validation, a particular study on the best Pk was conducted. Hence, the model was tested by using, as input data, Pk from studies conducted with either rats (Loccisano et al., 2011) or humans (Maestri et al., 2006). Data sets were compared to detect any improvement in the performance of both original and adapted PBPK models. In a flow limited equation, Pk is the main parameter governing the distribution of a chemical in the human body. However, this variable is usually obtained in animal experimentation studies without taking into account allometric scaling factors (Knaak et al., 1995), and assuming a steady state condition between tissue concentration and blood level. The final results by applying both data sets were compared with those previously observed in human tissues from people living in Tarragona County (Ericson et al., 2007; Pérez et al., 2013). The PBPK model was coded and simulated by using Berkeley Madona ™ v8.3.18 (University of California at Berkeley, USA).

3. Results and Discussion

3.1. Original PBPK model

In the first stage, the PBPK model developed by Loccisano et al. (2011) was used to predict PFOA and PFOS concentrations in human tissues for the current case-study. Parameterization data were taken from Loccisano et al. (2011), excepting those values regarding oral intake of food and drinking water, which were obtained from Domingo et al. (2012a). Simulation results were compared with experimental values regarding PFOA and PFOS in autopsy tissues from residents in the area of Tarragona (Table 2). Based on the model, the presence of PFOA and PFOS in plasma was mostly overestimated. The steady-state concentration of PFOA was estimated in 28.7 ng/g, while that of PFOS was 21.3 ng/g. Experimental values of PFOS and PFOA in plasma were reported to be 3.2 ± 1.2 and 13.6 ± 6.3 ng/g, respectively. Similarly, some disagreements were found for other human tissues when comparing modeled and monitored levels of both chemicals. In liver, the simulated PFOA concentration was 4.5-fold higher than the monitored mean value (13.6 ± 35.2 ng/g), while the predicted steady-state level of PFOS in liver was lower than empirical concentrations (79.6 vs. 102 ± 123 ng/g). Similar relationships were also detected in kidney, where simulated concentrations of PFOA and PFOS were 36.1 and 20.5 ng/g, respectively. By contrast, experimental mean levels of PFOA and PFOS in kidney were 2.0 ± 2.7 and 75.6 ± 61.2 pg/g. Consequently, the ratio of estimated: observed kidney PFOA concentration was 18, while that of PFOS was 0.27. According to the model outcomes, PFOA and PFOS concentrations in adipose tissue were 1.2 and 3.0 ng/g, respectively. Unfortunately, since no experimental values are available for this specific compartment, a comparison could not be carried out in adipose tissue. In general terms, the model simulation somehow overestimated the concentration for PFOA and PFOS, with the exception of PFOS in liver and kidney. Simulations results of PFOA in plasma, liver and kidney were >4-fold higher than the mean experimental values. However, predicted and empirical concentrations were of the same order of magnitude. Considering that analytical results were highly uncertain and variable, the results of the PBPK model should be considered as reasonably good.

Table 2. PFOS and PFOA concentration (in ng/g) found in human tissues. Experimental vs. simulated data after applying the original PBPK model.

	Tissue	Experimental			Simulated
		Mean±SD	Min-Max	LOD	
PFOA	Plasma	3.2±1.2	1.4-5.6	0.66	28.7
	Liver	13.6±35.2	<3-98.9	3.0	63.1
	Fat	n.a.	n.a.	n.a.	1.15
	Kidney	2.0±2.7	<3-11	3.0	36.1
PFOS	Plasma	13.6±6.3	1.4-28.9	0.09	21.3
	Liver	102±123	<3-405	3.0	79.6
	Fat	n.a.	n.a.	n.a.	3.01
	Kidney	75.6±61.2	<6-269	6.0	20.5

LOD: limit of detection; SD: standard deviation; n.a.: not available.

3.2. Adapted PBPK model

Because of the toxicological significance of PFOS and PFOA (Huet al., 2012; Sato et al., 2009; Yahia et al., 2010), brain and lung were also included in the current model (Fig. 1). New Pk values coming from human autopsy tissues (Maestri et al., 2006) were also applied and compared with Pk data from rats (Loccisano et al., 2011) (Table 3). As above mentioned, a constant oral intake of both PFOA and PFOS was considered. Descriptor parameters of the urinary elimination were calibrated by means of plasma concentrations (Ericson et al., 2007). Again, simulation results including the steady-state curve were compared with those recently reported in the same population group living in Tarragona County (Pérez et al.,2013). To understand the quality of experimental data, a statistical analysis was performed. Outliers (one for PFOA in liver and kidney, two for PFOS in brain, and one for PFOS in kidney) were eliminated from data treatment to avoid any potential distortion of the results. Predicted vs. observed concentrations of PFOA and PFOS, calculated by considering Pk values from human tissues are depicted in Fig. 3a and b, respectively. In the cases in which PFOA or PFOS levels were not detected in a sample, the concentration was assumed to be equal to the respective limit of detection (LOD). PFOA was not detected in brain, while it was only quantified in a single sample of kidney. Simulation results were clearly closer to empirical values when using Pk values from human autopsy tissues, rather than when applying Pk data coming from

experimental animal studies (Table 4). In general terms, simulated results were found inside the uncertainty range of experimental values, with the exception of PFOS in lung. This fact support the hypothesis that PFOS and PFOA are resorbed from urine back to plasma in a saturable process, as it was parameterized in the model. Although Pk is a key parameter in PBPK modelling, Pk values are usually obtained from studies in rodents. Our results highlight the importance to obtain Pk data from humans in order to estimate more accurately the body burdens of PFASs in particular, and chemical contaminants in general. For example, simulated liver concentrations for PFOA in steady-state were 7.03 and 3.33 ng/g for rat- and human-derived Pk values, respectively, while the measured PFOA concentration was 13.6 ± 35.2 ng/g. Liver PFOS levels were estimated to be 50.7 and 36.4 ng/g, taking into account the rat- or human- derived Pk datum, respectively (observed value: 102 ± 122.9 ng/g). In brain, mean PFOS content ranged between 0.16 and 3.48 ng/g, depending on the use of Pk values from animal and human studies, respectively. According to the experimental investigation, brain contained PFOS in a concentration of 4.9 ± 6.6 ng/g. Simulation concentrations in lungs, using both Pk values from rats and humans, were notably lower than those experimentally obtained for PFOA (0.47and 4.06 ng/g vs. 29.2 ± 32.2 ng/g) and PFOS (2.04 and 2.11 ng/g vs. 29.1 ± 16.8 ng/g). Important disagreements were also observed when comparing PFAS concentrations in kidney from the modeling and monitoring studies. Overall, the results from our adjusted model seemed to underestimate the real concentrations of PFOA and PFOS in the steady-state, contrasting with the original PBPK model (Loccisano et al., 2011). Notwithstanding, the good performance of the latter model was validated by comparing only measurements of PFOA and PFOS in human serum.

Table 3. Partition coefficients (Pk) used in the PBPK model.

	PFOA		**PFOS**	
	Human-based	**Rat-based**	**Human-based**	**Rat-based**
Liver	1.03	2.20	2.67	3.72
Fat	0.47	0.04	0.33	0.14
Brain	0.17	0.01	0.26	0.01
Lung	1.27	0.15	0.15	0.15
Kidney	1.17	1.05	1.26	0.80

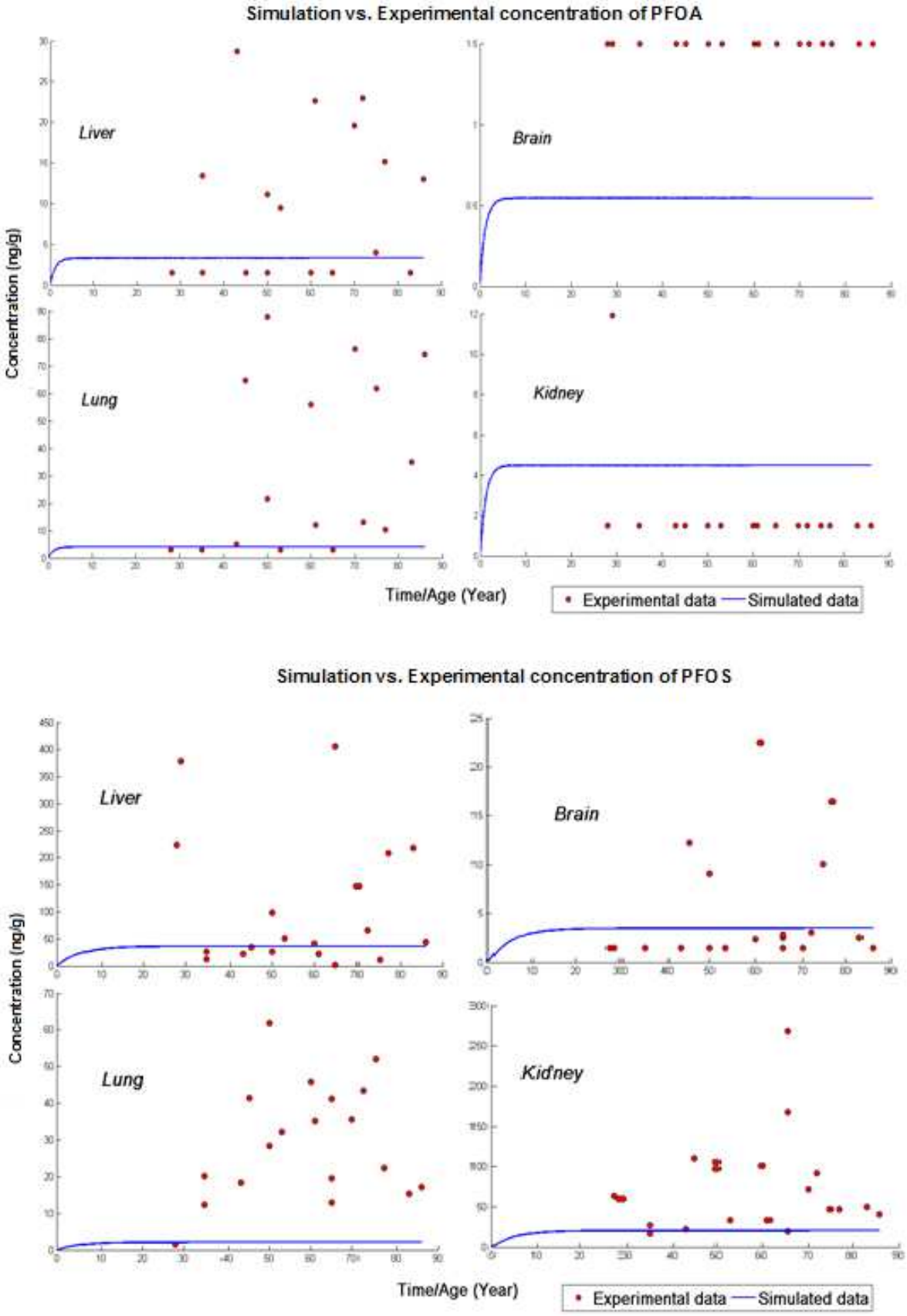


Fig. 3. Time course concentration of (a) PFOA and (b) PFOS in four human tissues from subjects who had been living in Tarragona County (Catalonia, Spain).

In the current study, validation was conducted by considering other tissue compartments, which increases the complexity in the model calibration. Although the use of data coming from studies with rodents or other animals (i.e., monkeys), is a common practice in PBPK modeling, the improvement in our model predictions confirms the World Health Organization (WHO) general guideline stating that the application of human data is always more desirable (WHO/IPCS,2010).

Table 4. Steady-state concentration (in ng/g) of PFOA and PFOS. Experimental vs. simulated data after applying the adapted PBPK model, considering rat- and human-derived Pk values.

	Tissue	Experimental		Simulated	
		Mean ± SD	Min-Max	Rat-based	Human-based
PFOA	Liver	13.6 ± 35.2	<3-98.9	7.03	3.33
	Brain	>1.5	<1.5	0.04	0.54
	Lung	29.2 ± 32.2	<6-87.9	0.47	4.06
	Kidney	2.0 ± 2.7	<3 -11.9	4.02	4.50
PFOS	Liver	102.3 ± 122.9	<3-405	50.7	36.4
	Brain	4.9 ± 6.6	<1.5-22.5	0.16	3.48
	Lung	29.1 ± 16.8	<3-61.8	2.04	2.11
	Kidney	75.6 ± 61.2	<6-269	13.1	20.5

SD: standard deviation.

The results obtained in the present work are in agreement with previous results where a resorption mechanism also presented a reasonably good matching in the plasma concentrations of PFOS and PFOA (Loccisano et al., 2011). Nonetheless, the high half-lives of both compounds cannot lay only on this fact, as protein binding maybe also a key process. In the present model, a high binding between PFAS and plasma proteins was taken into account, as suggested by Loccisano et al. (2011). However, the variation of the protein binding percentage can cause a variation in the plasma concentration. Therefore, further studies should demonstrate the weight of the protein binding and renal resorption in the long half-lives of some PFASs.

The significance of the model prediction when using two different partition coefficients was tested by performing a two-sample t-test. In both cases (PFOA and PFOS), the tests failed in rejecting the null hypothesis at the alpha significance level. It means that both data sets came from independent random samples of normal distributions, with equal means and equal, although unknown, variances. Further high P-values (0.92 and 0.95 for PFOA and PFOS, respectively) strongly validate the null

hypothesis. To test the significance of the results, the correlation coefficient (R), which measures the linearity of relationship between observed and predicted values, was calculated. Regarding PFOA, values of R = −0.12 and R = 0.44 were found for rat- and human- derived Kp data, respectively. On the other hand, regarding PFOS, R values were 0.88 and 0.95 considering rat- and human-Kp data, respectively. No significant difference was observed in the distribution mean of predicted values, clearly indicating that a better prediction may be achieved by using human partition coefficients.

4. Conclusions

In the current study, a previously developed PBPK model was modified by considering the main target tissues of toxicological relevance for PFOA and PFOS. In agreement with the original model (Loccisano et al., 2011), trends in the simulation results indicate that the urinary PFAS resorption-based PBPK model seems to be a reliable approach to explain the relatively longer half-life of PFOA and PFOS in human plasma.

Although the model had been successfully validated by using experimental data in human blood, good validation results were not achieved for other human tissues. Anyhow, current knowledge on the levels of PFASs in human tissues, other than blood/plasma, is very limited. Uncertainty and variability of experimental data, together with that limited knowledge, means an additional difficulty to analyze those data. Despite only blood is commonly used for PBPK modeling validation, the comparability with other human compartments would ensure the reliability of the model with respect to target tissues (WHO/IPCS, 2010). The present results clearly indicate the need to acquire more information concerning body burdens of PFASs in general and those of PFOA and PFOS in particular. Biological monitoring of these POPs is necessary, as they provide fundamental support for the development of PBPK models as well as other in-silico tools. The parameterization of the partition coefficient was also validated by comparing values derived from animal and human experimental studies. The use of human-derived Pk data was more suitable for application to this PBPK model than rat-based values. The model simulation assessed in the present study showed a huge uncertainty. Although results from PBPK modeling are usually uncertain (Barton et al., 2007), the characterization of variability and uncertainty in PBPK models is not a common practice. The uncertainty in PBPK models can be predicted by using Monte Carlo simulations, but also applying Bayesian inferences, or even fuzzy simulations (Gueorguieva et al., 2004). In order to assess the impact of the parameterization uncertainty in the model, a sensitivity analysis

was not performed. The reduction of uncertainty can be done by using more precise measurements of the parameterization data (Huizer et al., 2012), while the variability cannot be reduced, but only described. Therefore, the separate characterization of variability and uncertainty should be considered to address more appropriately this issue (Kumar et al., 2009). Finally, further research must be carried out to improve the performance of the PBPK model by introducing temporal dynamics of exposure concentration and physiological parameters for the long-term exposure to PFASs. Being the most sensitive known target organ for PFOA toxicity in animals (Post et al., 2012), the inclusion of the mammary gland within the PBPK model should be also considered in future studies. On the other hand, as humans are really exposed to multiple chemicals, future studies should be focused on assessing the suitability of PBPK modelling for the evaluation of mixtures of PFASs, instead of individual compounds, assuming that different PFASs show a relatively comparable toxicological profile (Borg et al., 2013).

References

Andersen, M.E., Clewell III, H.J., Tan, Y.M., Butenhoff, J.L., Olsen, G.W., 2006. Pharmacokinetic modeling of saturable, renal resorption of perfluoroalkylacids in monkeys-Probing the determinants of long plasma half-lives. Toxicology 227, 156-164.

Austin, M.E., Kasturi, B.S., Barber, M., Kannan, K., MohanKumar, P.S., MohanKumar, S.M.J., 2003. Neuroendocrine effects of perfluorooctane sulfonate in rats. Environmental Health Perspectives 111, 1485-1489.

Barry, V., Winquist, A., Steenland, K., 2013. Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environmental Health Perspectives, in press; doi: 10.1289/ehp.1306615

Barton, H.A., Chiu, W.A., Woodrow Setzer, R., Andersen, M.E., Bailer, A.J., Bois, F.Y., DeWoskin, R.S., Hays, S., Johanson, G., Jones, N., Loizou, G., MacPhail, R.C., Portier, C.J., Spendiff, M., Tan, Y.M., 2007. Characterizing uncertainty and variability in physiologically based pharmacokinetic models: State of the science and needs for research and implementation. Toxicological Sciences 99, 395-402.

Borg, D., Lund, B.O., Lindquist, N.G., Hakansson, H., 2013. Cumulative health risk assessment of 17 perfluoroalkylated and polyfluoroalkylated substances (PFASs) in the Swedish population. Environment International 59, 112-123.

Brown, R.P., Delp, M.D., Lindstedt, S.L., Rhomberg, L.R., Beliles, R.P., 1997. Physiological parameter values for physiologically based pharmacokinetic models. Toxicology and Industrial Health 13, 407-484.

Chaemfa, C., Barber, J.L., Huber, S., Breivik, K., Jones, K.C., 2010. Screening for PFOS and PFOA in European air using passive samplers. Journal of Environmental Monitoring 12, 1100-1109.

Chang, S.C., Noker, P.E., Gorman, G.S., Gibson, S.J., Hart, J.A., Ehresman. D.J., Butenhoff, J.L., 2012. Comparative pharmacokinetics of perfluorooctanesulfonate (PFOS) in rats, mice, and monkeys. Reproductive Toxicology 33 428-440.

Chiu, W.A., Barton, H.A., DeWoskin, R.S., Schlosser, P., Thompson, C.M., Sonawane, B., Lipscomb, J.C., Krishnan, K., 2007. Evaluation of physiologically based pharmacokinetic models for use in risk assessment. Journal of Applied Toxicology 27, 218-237.

Clewell, R.A., Clewell III, H.J., 2008. Development and specification of physiologically based pharmacokinetic models for use in risk assessment. Regulatory Toxicology and Pharmacology 50, 129-143.

Cui, L., Zhou, Q.F., Liao, C.Y., Fu, J.J., Jiang, G.B., 2009. Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis. Archives of Environmental Contamination & Toxicology 56, 338-349.

Domingo, J.L., 2012. Health risks of dietary exposure to perfluorinated compounds. Environment International 40, 187-195.

Domingo, J.L., Jogsten, I.E., Eriksson, U., Martorell, I., Perelló, G., Nadal, M., van Bavel, B., 2012a. Human dietary exposure to perfluoroalkyl substances in Catalonia, Spain. Temporal trend. Food Chemistry 135, 1575-1582.

Domingo, J.L., Ericson-Jogsten, I., Perelló, G., Nadal, M., van Bavel, B., Kärrman, A., 2012b. Human exposure to perfluorinated compounds in Catalonia, Spain: Contribution of drinking water and fish and shellfish. Journal of Agricultural & Food Chemistry 60, 4408-4415.

Ehresman, D.J., Froehlich, J.W., Olsen, G.W., Chang, S.C., Butenhoff, J.L., 2007. Comparison of human whole blood, plasma, and serum matrices for the determination of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and other fluorochemicals. Environmental Research 103, 176-184.

Ericson, I., Gómez, M., Nadal, M., van Bavel, B., Lindström, G., Domingo, J.L., 2007. Perfluorinated chemicals in blood of residents in Catalonia (Spain) in relation to age and gender: A pilot study. Environment International 33, 616-623.

Ericson Jogsten, I., Nadal, M., van Bavel, B., Lindström, G., Domingo, J.L., 2012. Per- and polyfluorinated compounds (PFCs) in house dust and indoor air in Catalonia, Spain: Implications for human exposure. Environment International 39, 172-180.

European Commission, 2013. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regarding priority substances in the field of water policy. Office Journal of the European Union L226, 1-17, 24

Fromme, H., Tittlemier, S.A., Völkel, W., Wilhelm, M., Twardella, D., 2009. Perfluorinated compounds – Exposure assessment for the general population in western countries. International Journal of Hygiene & Environmental Health 212, 239-270.

Fuentes, S., Colomina, M.T., Rodriguez, J., Vicens, P., Domingo, J.L., 2006. Interactions in developmental toxicology: Concurrent exposure to perfluorooctane sulfonate (PFOS) and stress in pregnant mice. Toxicology Letters 164, 81-89.

Fuentes, S., Colomina, M.T., Vicens, P., Domingo, J.L., 2007a. Influence of maternal restraint stress on the long-lasting effects induced by prenatal exposure to perfluorooctane sulfonate (PFOS) in mice. Toxicology Letters 171, 162-170.

Fuentes, S., Vicens, P., Colomina, M.T., Domingo, J.L., 2007b. Behavioral effects in adult mice exposed to perfluorooctane sulfonate (PFOS). Toxicology 242, 123-129.

Fujii, S., Polprasert, C., Tanaka, S., Lien, N.P.H., Qiu, Y., 2007. New POPs in the water environment: Distribution, bioaccumulation and treatment of perfluorinated compounds - A review paper. Journal of Water Supply: Research and Technology - AQUA 56, 313-326.

Gueorguieva, I.I., Nestorov, I.A., Rowland, M., 2004. Fuzzy simulation of pharmacokinetic models: case study of whole body physiologically based model of diazepam. Journal of Pharmacokinetics & Pharmacodynamics 31, 185-213.

Grasty, R.C., Bjork, J.A., Wallace, K.B., Lau, C.S., Rogers, S.M., 2005. Effects of prenatal perfluorooctane sulfonate (PFOS) exposure on lung maturation in the

perinatal rat. Birth Defects Research Part B: Developmental & Reproductive Toxicology 74, 405-416.

Han, X., Snow, T.A., Kemper, R.A., Jepson, G.W., 2003. Binding of perfluorooctanoic acid to rat and human plasma proteins. Chemical Research in Toxicology 16, 775-781.

Hu, Q., Franklin, J.N., Bryan, I., Morris, E., Wood, A., DeWitt, J.C., 2012. Does developmental exposure to perflurooctanoic acid (PFOA) induce immunopathologies commonly observed in neurodevelopmental disorders? Neurotoxicology 33, 1491-1498.

Huizer, D., Oldenkamp, R., Ragas, A.M.J., van Rooij, J.G.M., Huijbregts, M.A.J., 2012. Separating uncertainty and physiological variability in human PBPK modelling: The example of 2-propanol and its metabolite acetone. Toxicology Letters 214, 154-165.

Hundley, S.G., Sarrif, A.M., G.L., K., 2006. Absorption, distribution, and excretion of ammonium perfluorooctanoate (APFO) after oral administration to various species. Drug & Chemical Toxicology 29, 137-145.

Kärrman, A., Domingo, J.L., Llebaria, X., Nadal, M., Bigas, E., van Bavel, B., Lindström, G., 2010. Biomonitoring perfluorinated compounds in Catalonia, Spain: Concentrations and trends in human liver and milk samples. Environmental Science & Pollution Research 17, 750-758.

Kim, H.Y., Kim, S.K., Kang, D.M., Hwang, Y.S., Oh, J.E., 2013. The relationships between sixteen perfluorinated compound concentrations in blood serum and food, and other parameters, in the general population of South Korea with proportionate stratified sampling method. Science of the Total Environment 470-471, 1390-1400.

Knaak, J.B., Al-Bayati, M.A., Raabe, O.G., 1995. Development of partition coefficients, Vmax and Km values, and allometric relationships. Toxicology Letters 79, 87-98.

Kumar V., Mari, M., Schuhmacher, M., Domingo, J.L., 2009. Partitioning total variance in risk assessment: Application to a municipal solid waste incinerator. Environmental Modelling & Software 24, 247-261.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: A review of monitoring and toxicological findings. Toxicological Sciences 99, 366-394.

Lau, C., Butenhoff, J.L., Rogers, J.M., 2004. The developmental toxicity of perfluoroalkyl acids and their derivatives. Toxicology & Applied Pharmacology 198, 231-241.

Lau. C., 2012. Perfluoroalkyl acids: recent research highlights. Reproductive Toxicology 33 405-409.

Loccisano, A.E., Campbell, J.L., Andersen, M.E., Clewell, H.J., 2011. Evaluation and prediction of pharmacokinetics of PFOA and PFOS in the monkey and human using a PBPK model. Regulatory Toxicology & Pharmacology 59, 157-175.

Loccisano, A.E., Longnecker, M.P., Campbell, J.L., Andersen, M.E., Clewell, H.J., 2013. Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages. Journal of Toxicology and Environmental Health - Part A 76, 25-57.

Loizou, G., Spendiff, M., Barton, H.A., Bessems, J., Bois, F.Y., d'Yvoire, M.B., Buist, H., Clewell III, H.J., Meek, B., Gundert-Remy, U., Goerlitz, G., Schmitt, W., 2008. Development of good modelling practice for physiologically based pharmacokinetic models for use in risk assessment: The first steps. Regulatory Toxicology and Pharmacology 50, 400-411.

Maestri, L., Negri, S., Ferrari, M., Ghittori, S., Fabris, F., Danesino, P., Imbriani, M., 2006. Determination of perfluorooctanoic acid and perfluorooctanesulfonate in human tissues by liquid chromatography/single quadrupole mass spectrometry. Rapid Communications in Mass Spectrometry 20, 2728-2734.

Mariussen, E., 2012. Neurotoxic effects of perfluoroalkylated compounds: Mechanisms of action and environmental relevance. Archives of Toxicology 86, 1349-1367.

Nadal, M., Fàbrega, F., Schuhmacher, M., Domingo, J.L., 2013. PCDD/Fs in plasma of individuals living near a hazardous waste incinerator. a comparison of measured levels and estimated concentrations by PBPK modeling. Environmental Science & Technology 47, 5971-5978.

Pérez, F., Nadal, M., Navarro-Ortega, A., Fàbrega, F., Domingo, J.L., Farré, M., Barceló, D., 2013. Accumulation of perfluoroalkyl substances in human tissues. Environment International 59: 354-362.

Péry, A.R.R., Brochot, C., Zeman, F.A., Mombelli, E., Desmots, S., Pavan, M., Fioravanzo, E., Zaldívar, J.M., 2013. Prediction of dose-hepatotoxic response in humans based on toxicokinetic/toxicodynamic modeling with or without *in vivo* data: A case study with acetaminophen. Toxicology Letters 220, 26-34.

Post, G.B., Louis, J.B., Cooper, K.R., Boros-Russo, B.J., Lippincott, R.L., 2009. Occurrence and potential significance of perfluorooctanoic acid (PFOA) detected in New Jersey public drinking water systems. Environmental Science & Technology 43, 4547-4554.

Post, G.B., Cohn, P.D., Cooper, K.R., 2012. Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: A critical review of recent literature. Environmental Research 116, 93-117.

Rumsby, P.C., McLaughlin, C.L., Hall, T., 2009. Perfluorooctane sulphonate and perfluorooctanoic acid in drinking and environmental waters. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 367, 4119-4136.

Sato, I., Kawamoto, K., Nishikawa, Y., Tsuda, S., Yoshida, M., Yaegashi, K., Saito, N., Liu, W., Jin, Y., 2009. Neurotoxicity of perfluorooctane sulfonate (PFOS) in rats and mice after single oral exposure. Journal of Toxicological Sciences 34, 569-574.

Shi, Y., Pan, Y., Wang, J., Cai, Y., 2012. Distribution of perfluorinated compounds in water, sediment, biota and floating plants in Baiyangdian Lake, China. Journal of Environmental Monitoring 14, 636-642.

Stahl, T., Mattern, D., Brunn, H., 2011. Toxicology of perfluorinated compounds. Environmental Sciences Europe 23, 38.

Tan, Y.M., Clewell III, H.J., Andersen, M.E., 2008. Time dependencies in perfluorooctyl acids disposition in rat and monkeys: A kinetic analysis. Toxicology Letters 177, 38-47.

Thompson, M.D., Beard, D.A., Wu, F., 2012. Use of partition coefficients in flow-limited physiologically-based pharmacokinetic modeling. Journal of Pharmacokinetics & Pharmacodynamics 39, 313-327.

US EPA., 2011. Exposure Factors Handbook. US Environmental Protection Agency. EPA/600/R-09/052F. Washington, DC, USA.

Vanden Heuvel, J.P., Kuslikis, B.I., Van Rafelghem, M.J., Peterson, R.E., 1991. Tissue distribution, metabolism, and elimination of perfluorooctanoic acid in male and female rats. Journal of Biochemical Toxicology 6, 83-92.

WHO/IPCS, 2010. Characterization and application of physiologically based pharmacokinetic models in risk assessment. IPCS harmonization project document No. 9, Geneva, Switzerland.

Yahia, D., El-Nasser, M.A., Abedel-Latif, M., Tsukuba, C., Yoshida, M., Sato, I., Tsuda, S., 2010. Effects of perfluorooctanoic acid (PFOA) exposure to pregnant mice on reproduction. Journal of Toxicological Sciences 35, 527-533.

Yeung, L.W.Y., Guruge, K.S., Taniyasu, S., Yamashita, N., Angus, P.W., Herath, C.B., 2013. Profiles of perfluoroalkyl substances in the liver and serum of patients with liver cancer and cirrhosis in Australia. Ecotoxicology & Environmental Safety 96, 139-146.

Zareitalabad, P., Siemens, J., Hamer, M., Amelung, W., 2013. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in surface waters, sediments, soils and wastewater – A review on concentrations and distribution coefficients. Chemosphere 91, 725-732.

CHAPTER 5

PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING OF PERFLUOROALKYL SUBSTANCES (PFASs) IN THE HUMAN BODY

Abstract

Nowadays, there are limited data on the levels of perfluoroalkyl substances (PFASs) other than perfluorooctane sulfonic acid (PFOS) and pefluorooctanoic acid (PFOA) in the human body. Moreover, most of this information has been extracted from the biological monitoring of plasma, while the occurrence of PFASs in other human tissues is not well studied. *In silico* research has emerged as an effective complement to biomonitoring, being physiologically-based pharmacokinetic (PBPK) modeling a reliable method. The objective of the present study was to develop a generic PBPK model to assess the concentration of PFASs in human tissues, based on an existing model previously validated for PFOS and PFOA. Experimental data on PFAS concentrations in human tissues from individuals in Tarragona County (NE of Spain) were used to estimate the values of some distribution and elimination parameters needed for the simulation. No significant correlations were found between these parameters and the PFAS chain length. The model was finally validated for 5 PFASs by using data of intake and plasma concentrations from residents in Andøya Island (Northern Norway).

1. Introduction

In recent years, perfluoroalkyl substances (PFASs), a group of organic compounds, have attracted an important social and scientific attention. PFASs are chemically characterized for the total or partial replacement of hydrogen atoms with fluorine, except those hydrogen atoms whose substitution affects the nature of any functional group. Common PFASs include perfluoroalkyl carboxylic acids (PFCAs), with a carboxylic acid (-COOH) in the extreme of the carbon chain, and perfluoroalkyl sulfonic acids (PFSAs), with a sulfonic acid ($-SO_3H$) in that extreme of the molecule (Buck et al., 2011). Because of their resistance to thermic and chemical degradation, PFASs have been widely used in a number of industrial applications. Since PFASs are highly persistent in environment, bioaccumulative in living organisms, potentially toxic, as well as subjected to long-range atmospheric transport capacity, these compounds have been labeled as persistent organic pollutants (POPs) (Wang et al., 2009). Furthermore, PFASs have been detected worldwide in a wide range of environmental and biological samples

(Yoo et al., 2009; Stahl et al., 2010; Domingo, 2012; Boiteux et al., 2012; Custer et al., 2014; Vorkamp et al., 2014).

Due to their high covalent carbon fluorine bond, PFASs are not metabolized, being poorly eliminated in the human body (Lau et al., 2004; Lau, 2012). PFASs are mainly distributed to well-perfussed tissues such as plasma, liver and kidney. Half-lives in human blood have been estimated in 5.4 years for perfluorooctane sulfonic acid (PFOS), between 2.3 and 3.8 years for pefluorooctanoic acid (PFOA), and 8.5 years for perfluorohexane sulfonic acid (PFHxS) (Lau, 2012). In contrast, shorter half-lives: 32, 30 and 3 days, have been estimated for perfluorohexanoic acid (PFHxA), perfluorobutane sulfonic acid (PFBS) and perfluorobutanoic acid (PFBA), respectively (Olsen et al., 2009; Lau, 2012; Russell et al., 2013). Some PFASs show a notably capacity to bind to human proteins, such as those in plasma and liver. Hence, liver PFASs may interact with fatty acid binding proteins (FABPs), making easier its displacement (Zhang et al., 2013). In plasma, PFASs are strongly bound to serum albumin, with a bound fraction >90 % for rats and humans (Han et al., 2003). Different PFASs seem to elucidate similar toxicological properties regardless their molecular structure, being liver the target tissue (Yeung et al., 2013). Despite the lack of toxicological studies, most PFASs elucidate hepatoxicity and reproductive toxicity as well as toxic effects on body weight, development, cholesterol, thyroid hormone and immunotoxicity (Fuentes et al., 2006, 2007; Lau et al., 2007; Stahl et al., 2011).

The U.S. company 3M was the primary global manufacturer of PFASs. The POP properties of PFOS and PFOA, the two most largely studied PFASs, began to raise an important concern for the regulatory agencies in the 1990s. Consequently, 3M completely phased out the production of PFOS in 2002 (Wang et al., 2009; Taniyasu et al., 2013). In 2009, PFOS was included in the list of POPs under the Stockholm Convention, while PFOA is a serious candidate to enter the same list. After ceasing PFOS production, its concentration in human tissues and environmental matrices has been progressively reducing (Armitage et al., 2006; Furdui et al., 2008; Olsen et al., 2008). In 2006, the U.S. EPA created the PFOA 2010/2015 Stewardship program aimed at reducing the 95% of the PFOA product releases and product content in 2010, as well as completely eliminate the production of PFOA in 2015 in western countries (U.S. EPA, 2014). Due to these regulatory restrictions, the producers of PFASs started to search alternatives to PFOS and PFOA. The company 3M has been developing a new line of products based in PFBS chemistry (Renner, 2006). In turn, other companies were partnered in the Fluoro Council, aimed at developing a new line of products based on the perfluorohexanoic acid (PFHxA) chemistry (Buck et al., 2011). Due to the higher

environmental degradation, quicker elimination rates in humans and low toxicity of PFBS and PFHxA, PFOA has been progressively replaced in industrial applications (Olsen et al., 2009; Russell et al., 2013). However, although PFOS and PFOA have been extensively studied, there is an important lack of pharmacokinetic studies of PFASs other than PFOS and PFOA. Furthermore, investigations on PFAS mixtures are very scarce, limited to few approaches (Borg et al., 2013).

Similarly to other substances, the use of experimental animals has been recurrent to identify the main toxic endpoints of PFASs and their distribution in the body (Inoue et al., 2012; Kowalczyk et al., 2012). Unfortunately, human data are particularly scarce because of the limitation to obtain tissue samples. Furthermore, alternatives to animal testing are being promoted at international level to avoid or minimize the use of live animals, being *in silico* tools one of the major promising alternatives (Benfenati et al., 2010). Consequently, the development of physiologically-based pharmacokinetic (PBPK) models has progressively increased in recent years, being these not only applied to drugs, but also to environmental toxicants. PBPK models are mathematical representations of the human body, whose attempt is to describe the time course distribution of chemicals (Nestorov, 2007). PBPK models, which are often used in human health risk assessment (U.S. EPA 2006; WHO/IPCS 2010), may be used to elucidate the pharmacokinetics and pharmacodynamics (PK/PD) of chemicals in the human body (Medinsky, 1995; Wang et al., 2013). Recently, various PBPK models have been developed to estimate the distribution of PFOS and PFOA within the human body (Andersen et al., 2006; Tan et al., 2008; Loccisano et al., 2011, 2013; Fàbrega et al., 2014). It has been suggested that the most important mechanism of removal of PFOS and PFOA in the human body is urinary elimination, with a renal resorption mechanism in the filtrate compartment (Andersen et al., 2006; Tan et al., 2008). The free fraction of PFOS and PFOA is also an important parameter to determine the distribution of PFOS and PFOA. However, there still exist some gaps and notable uncertainties around the pharmacokinetics and pharmacodynamics of PFASs different from PFOS and PFOA.

The objective of the present study was to develop a generic PBPK model to assess the concentration of 11 PFASs in human tissues. A previous model (Fàbrega et al., 2014), validated for PFOS and PFOA, was here adjusted to other PFASs. Experimental data on PFAS concentrations in human tissues from individuals in Tarragona County (NE of Spain) were used to estimate the values of some distribution and elimination parameters needed for the simulation. The correlations with the chain length of each respective PFAS were also studied. Afterwards, the model was validated by using data of intake and plasma concentration from Andøya Island (Northern Norway).

2. Materials and Methods

2.1. PBPK model for PFASs

Based on previously obtained data for PFOS and PFOA (Fàbrega et al., 2014), a PBPK model to estimate the concentrations of 9 additional PFASs in different human tissues was here developed. Overall, up to 11 PFASs with different chain length were considered for the PBPK modeling (Table 1). The tissues studied in the present model were selected according to their pharmacokinetic relevance in the distribution and elimination of PFASs. Plasma, gut, liver, fat, kidney, filtrate, bone marrow, brain, lungs were selected as compartments of interest, while the rest of the body was integrated into an additional single compartment. The conceptual structure of the PBPK model is depicted in Fig. 1. The intake of chemicals was considered to occur only by food and water consumption through the gut. Kidney was selected because it is the organ where elimination takes place (Han et al., 2012). Liver, bone marrow, kidney, lungs and brain were chosen because quantifiable concentrations of PFASs have been found in autopsy tissues (Pérez et al., 2013). Finally, plasma was used as a descriptor of the systemic circulation. The PBPK model was based on flow-limited equations (Thompson and Beard, 2011).

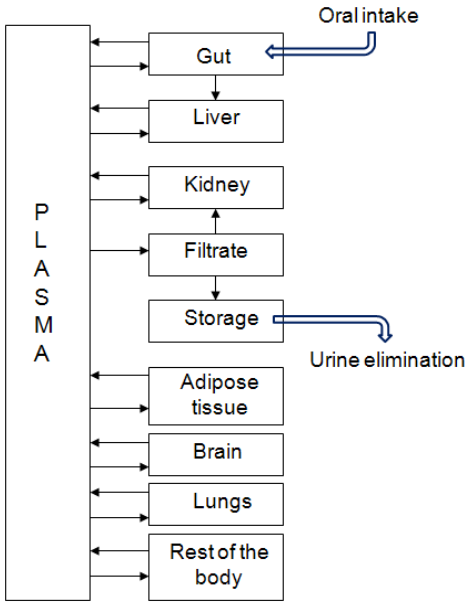
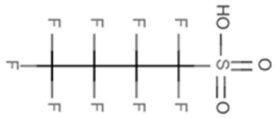
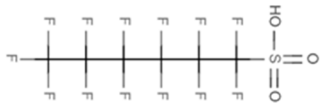
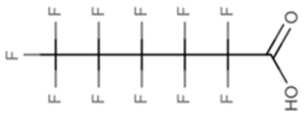
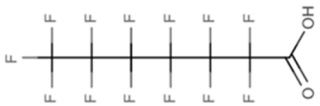
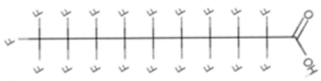
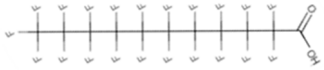


Fig. 1. Structure of the PBPK model

Table 1. Structure of the selected PFASs

Compound name	Abbreviation	CAS	Structure	Molecular weight
Perfluorobutane sulfonic acid	PFBS	375-73-5		300.1
Perfluorohexane sulfonic acid	PFHxS	355-46-4		400.1
Perfluorooctane sulfonic acid	PFOS	1763-23-1		500.1
Perfluorodecane sulfonic acid	PFDS	335-77-3		600.2
Perfluorohexanoic acid	PFHxA	307-24-4		314.1
Perfluoroheptanoic acid	PFHpA	375-85-9		364.1
Pefluorooctanoic acid	PFOA	335-67-1		414.1
Perfluorononanoic acid	PFNA	375-95-1		464.1
Perfluorodecanoic acid	PFDA	335-76-2		514.1
Perfluoroundecanoic acid	PFUnDA	2058-94-8		564.1
Perfluorotetradecanoic acid	PFTeDA	376-06-7		714.1

The concentrations of PFASs in non-eliminating tissues (bone marrow, fat, brain, lungs, and rest of the body) were estimated through the following mathematical equation:

$$\frac{dCi}{dt} = \frac{Qi \times free \times (Ca - Ci / Ki:p)}{Vi} \quad (1)$$

where Ci is the concentration in tissue i (pg/mL), Qi is the blood flow to the tissue i (mL/h), free is the unbound fraction in plasma, Ca is the arterial concentration (pg/mL), Ki:p is the partition coefficient, and Vi is the volume of tissue i.

PFAS concentration in gut was calculated by means of the following expression:

$$\frac{dCg}{dt} = \frac{Qg \times free \times (Ca - Cg / Kg:p) + Intake}{Vg} \quad (2)$$

where Cg is the concentration in gut (pg/mL), Qg is the blood flow to gut (mL/h), free is the unbound fraction in plasma, Ca is the arterial concentration (pg/mL), Kg:p is the partition coefficient, Intake is the hourly ingestion of PFASs (pg/h), and Vg is the volume in gut compartment.

For the liver compartment, the concentrations of PFASs were estimated by means of this equation:

$$\frac{dCl}{dt} = \frac{free \times (Ql \times Ca + Qg \times Cg / Kg:p - (Ql + Qg) \times (Cl / Kl:p))}{Vl} \quad (3)$$

where Cl is the concentration in liver (pg/mL), free is the unbound fraction in plasma, Ql is the blood flow to liver (mL/h), Ca is the arterial concentration (pg/mL), Qg is the blood flow to gut (mL/h), Cg is the concentration in gut (pg/mL), Kg:p is the partition coefficient in gut, Kl:p is the partition coefficient in liver, and Vl is the volume in liver compartment.

For the kidney compartment, the used equation was:

$$\frac{dCk}{dt} = \frac{(Qk \times free \times (Ca - Ck / Kk:p) + \frac{Tm \times Cfil}{Kt + Cfil}}{Vk} \quad (4)$$

where Ck is the concentration in kidney (pg/mL), Qk is the blood flow to the kidney (mL/h), free is the unbound fraction in plasma, Ca is the arterial concentration (pg/mL), Kk:p is the partition coefficient in kidney, Tm is the resorption maximum (pg/h), Kt is the affinity constant (pg/mL), and Vk is the volume of kidney.

Finally, the levels of PFASs in the filtrate compartment were estimated by means of the following mathematical expression:

$$\frac{dCfil}{dt} = \frac{Qfil \times (free \times Ca - Cfil) - \frac{Tm \times Cfil}{Kt + Cfil}}{Vfil} \quad (5)$$

where Cfil is the concentration in filtrate compartment (pg/mL), Qfil is the blood flow to filtrate (mL/h), free is the unbound fraction in plasma, Ca is the arterial concentration (pg/mL), Tm is the resorption maximum (pg/h), Kt is the affinity constant (ng/mL), and Vfil is the volume of filtrate compartment.

All the variables, including volumes, cardiac output, partition coefficient (Pk), intake and elimination parameters, were considered to be constant along time. Data on time volumes and cardiac output were taken from Brown et al., (1997). Due to their high absorption rates observed in rats, PFASs were assumed to be completely absorbed (D'Eon and Mabury, 2011; Hundley et al., 2006). In turn, since PFASs are poorly metabolized in animals, metabolism was considered negligible (Ophaug and Singer, 1980; Hundley et al. 2006). Although other exposure pathways, such as air inhalation and dermal absorption, might have a relevant contribution to the total intake of PFASs (D'Eon and Mabury., 2011; Ericson et al., 2012; Nadal and Domingo, 2014), water intake and food consumption were considered as the only routes of PFAS entrance to the human body. For confirmation purposes, values of dust ingestion of PFASs from the population living in the same area of study (Ericson et al., 2012) were compared with the dietary and water intake of PFASs, being the former considerably lower. In addition, since PFASs were not detected in air samples, air inhalation was considered negligible (Ericson et al., 2012). Calculations were performed for the adult population of Tarragona County. Water intake was assumed to be 1.022 L/day, while the mean body weight was set at 70 kg. The daily intake of PFASs through food and water for the population of Tarragona County (Table 2), was obtained elsewhere (Ericson et al., 2009; Domingo et al., 2012). A constant intake was considered for the lifespan of the individuals under study. The mean lifetime of the individuals was set to 80 years.

Table 2. Dust ingestion, water and food intake of PFASs in Tarragona County (Catalonia, Spain) (ng/day). Total exposure was assessed by considering only water intake and food consumption

	Dust[a]	Water[b]	Food[c]	Total intake
PFBS	0.05	1.64 (25.0)	4.90 (75.0)	6.54
PFHxS	0.05	0.49 (10.5)	4.20 (89.5)	4.69
PFOS	0.18	2.23 (1.4)	160.30	162.53
PFDS	0.00	n.d.	1.40 (100)	1.40
PFHxA	0.07	1.06 (3.1)	33.60 (96.9)	34.66
PFHpA	0.08	1.33 (0.5)	261.10	262.43
PFOA	0.48	2.95 (0.8)	385 (99.2)	387.95
PFNA	0.34	1.51 (1.5)	97.30 (98.5)	98.81
PFDA	0.49	0.57 (3.3)	16.80 (96.7)	17.37
PFUnDA	0.17	0.38 (0.4)	95.20 (99.6)	95.58
PFTDA	0.04	n.d.	32.90 (100)	32.90

n.d.: Non detected. [a]Ericson et al., (2012); [b]Ericson et al., (2009); [c]Domingo et al., (2012). Between parentheses, percentage of water and food intake, respect to total exposure.

The distribution of PFASs in the human body was described by the Pk, which is defined as the concentration of a chemical in a specific tissue in relation to its concentration in blood. To calculate Pk, the concentration of each PFAS found in autopsy tissues from subjects of Tarragona County was divided by the mean blood level of the same compound found in samples of donors from the same area of study (Ericson et al., 2007; Pérez et al., 2013). Pks were assessed for liver, bone marrow, brain, lung and kidney. For the remaining tissues (adipose, gut, filtrate and rest of the body), a constant value of the Pks, coming from data on PFOA in rats, was used (Kudo et al., 2007).

The elimination parameters of the PBPK model (Tm and Kt) and the free fraction of PFASs in plasma were also assessed for the case-study of Tarragona County. Data on the body burdens of PFASs in plasma, as well as those in a number of tissues (liver, bone, kidney, lungs, and brain) were available from previous investigations (Ericson et al., 2007; Pérez et al., 2013). PFASs were considered to be bound to serum albumin in a fraction of 97%, while the remaining fraction (3%) was available for accumulation in other tissues and elimination through urine (Han et al., 2003; Chen and Guo, 2009; Bischel et al., 2010). In order to assess the elimination parameters (Tm, Kt) and free fraction, the model was coded and parameterized, being the simulated plasma concentration fitted with experimental values of PFASs (Ericson et al., 2007). Values of Tm, Kt and free fraction constants were here obtained. The model was coded by using

Berkeley Madonna™ v 8.3.18, using a Stiff method to solve the differential equations (Macey et al., 2009).

2.2. Validation of the PBPK model

The validation step consisted on executing the PBPK model for another population. Adults living in Andøya Island (Norway), for which data on intake and plasma concentrations of PFHxS, PFOS, PFHpA, PFOA and PFNA were available (Rylander et al., 2009; Haug et al., 2010), were chosen as case-study. To obtain minimum and maximum values, the PBPK simulation was conducted for the whole range of elimination and distribution parameters. As previously demonstrated (Huizer et al., 2012) and confirmed by sensitivity analysis (unpublished results), Tm, Kt and free fraction were the variables with the highest uncertainty in the PBPK model. Minimum and maximum values for Tm and Kt were set as 0.3 times the coefficient of variation (CV) (Allen et al., 1996). In turn, 0.1% and 3% were considered as the minimum and maximum percentages of the free fraction, respectively (Huizer et al., 2012). The daily intake of food and water consumption by the adult Norwegian population, was set at 1.2, 18, 8.2, 31 and 9.5 ng/day for PFHxS, PFOS, PFHpA, PFOA and PFNA, respectively (Haug et al., 2010). Levels of PFASs in plasma were taken from a biological monitoring study of 44 women and 16 men living in the coastal population of Northern Norway, which was performed in September 2005 (Rylander et al., 2009). Mean plasma concentrations of PFHxS, PFOS, PFOA and PFNA were 1.8, 33, 4.4 and 0.95 ng/mL, respectively. Although PFHpA was not detected, the limit of detection (0.26 ng/mL) was used to validate the model. Finally, model validation was based on the comparison of experimental data on PFASs in plasma and the range of values predicted by the PBPK model. Since the human body is a system subjected to a number of biological processes, PBPK models averaging within a factor of 2 in comparison to experimental data may be considered as validated (WHO/IPCS 2010).

3. Results and Discussion

3.1. PBPK models for PFASs

A PBPK model was developed to estimate the concentration of 11 PFASs in human tissues. To simulate the internal distribution of PFASs, values of some physicochemical (Pks) and biochemical (Tm, Kt, and free fraction) parameters were needed. Pks were estimated by using data on PFAS concentration in plasma and

autopsy tissues from individuals of Tarragona County, Spain (Ericson et al., 2007; Pérez et al., 2013). The partition coefficients of 11 PFASs are summarized in Table 3. Pk values ranged from 0.001 for PFDS, PFDA and PFTeA in liver, as well as PFTeA in bone marrow, to 201.6 for PFHxA in brain. The correlation between the carbon chain length and those physicochemical and biochemical parameters of each respective PFAS was also studied. No significant correlations were noted between the PFAS chain length and Pk (Fig. 2). However, available experimental information is relatively scarce, since PFAS concentrations from only 20 individuals had been obtained.

Table 3. Pks (unitless) of 11 PFASs obtained by using data on autopsy tissues

	Liver	**Bone Marrow**	**Brain**	**Lung**	**Kidney**
PFBS	1.38	4.91	0.74	27.31	12.27
PFHxS	0.72	0.28	0.50	1.27	3.27
PFOS	7.48	0.11	0.36	2.13	5.54
PFDS	0.001	3.28	0.58	5.99	9.46
PFHxA	128.80	39.87	201.60	56.11	6.27
PFHpA	47.82	110.71	1.94	24.98	11.20
PFOA	4.23	18.73	0.37	9.08	0.62
PFNA	1.65	2.66	37.80	19.47	36.02
PFDA	0.001	0.28	43.68	31.92	11.57
PFUnDA	0.002	0.25	14.82	4.61	11.69
PFTeDA	0.001	0.001	63.13	24.95	15.78

Data from Pérez et al., (2013).

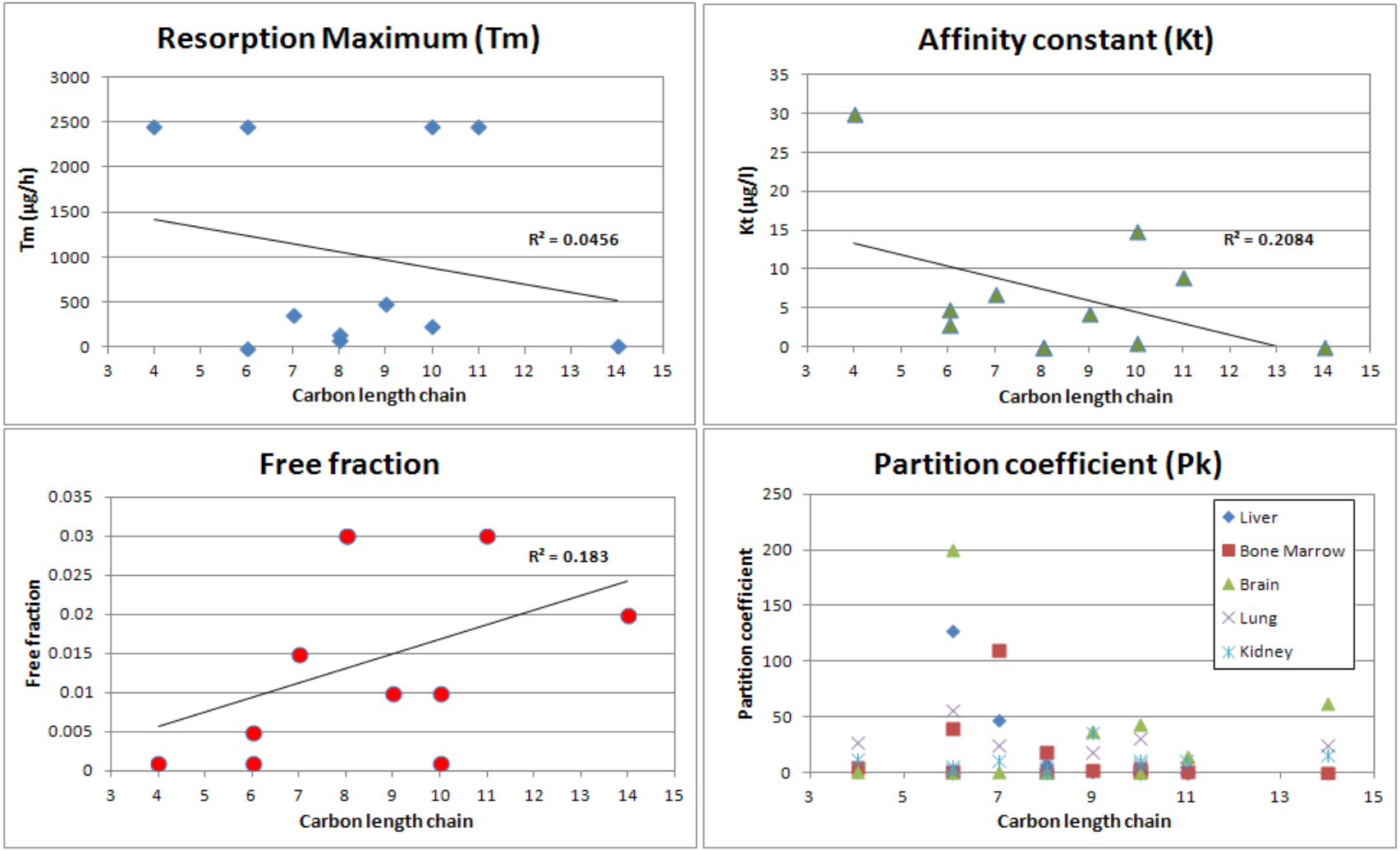


Fig. 2. Correlation between PFAS chain length and a number of parameters: Tm, Kt, free fraction and Pk.

Based on the results of animal studies (Andersen et al., 2006; Tan et al., 2008), the elimination of PFOS and PFOA is mainly urinary, with a resorption mechanism in the filtrate compartment. Once in the urine, the chemicals are resorbed back to the plasma in a saturable mechanism, driven by resorption maximum (Tm) and affinity constant (Kt). Tm and Kt are analogous constants to the kinetics of the Michaelis-Menten reactions for the enzymatic reactions. This mechanism was expected to be the responsible of the high half-lives of PFOS and PFOA in human blood. However, it has not been proved yet to be involved in the elimination of shorter chain PFASs. In the present study, a resorption mechanism was assumed for all the compounds, including C4 and C6 shorter chain PFASs (Han et al., 2012). The elimination constants (Tm and Kt), and the value of free fraction of PFASs in plasma, were calibrated by running multiple simulations of the PBPK model until fitting the simulation results with experimentally obtained PFAS concentrations in plasma. The elimination constants resulting from the PBPK modelling are summarized in Table 4. The final results for Tm ranged from 6.1 to 2456.3 μg/h, Kt ranged from 0.018 to 30 μg/L, and free fraction ratios ranged from 0.001 to 0.03. Significant correlations between the carbon chain length and any of these constants were not found ($p>0.05$) (Fig. 2). The model was able to simulate the pharmacokinetic behavior of PFASs with a shorter chain than PFOS and PFOS, such as PFBA or PFBS, whose half-lives in blood are considerably lower. To the best of our knowledge, this is the first attempt to develop in humans a PBPK model for PFASs other than PFOS and PFOA. According to the present PBPK model, the urinary elimination, the resorption mechanism and the strong protein binding are the hallmark of the absorption, distribution, metabolism, and excretion (ADME) of PFASs in the human body. Although other mechanisms of elimination may play an important role (Harada et al., 2007), data are currently too scarce to accurately evaluate their influence. Moreover, this is a generic model that should be improved by including more refined individual PK data of each individual chemical compound. Anyhow, it is undoubtedly of great interest as information on the body burdens of PFASs, other than plasma, is not available.

Table 4. Elimination constants coming from PBPK model simulation

	Tm (µg/h)	Kt (µg/L)	Free
PFBS	2456.3	30	0.001
PFHxS	2456.3	3	0.005
PFOS	86.0	0.018	0.030
PFDS	2456.3	15	0.001
PFHxA	6.1	5	0.001
PFHpA	368.4	7	0.015
PFOA	147.4	0.116	0.030
PFNA	491.3	4.5	0.010
PFDA	245.6	0.6	0.010
PFUnDA	2456.3	9	0.030
PFTeDA	24.6	0.070	0.020

Tm: resorption maximum (µg/h/); Kt: affinity constant (µg/L); Free: free fraction in plasma (unitless)

The estimated steady-state concentration of PFASs in target tissues is shown in Table 5. The simulations followed a trend with a nearly lineal part at the beginning, reaching a plateau after 20-30 years. The result of the simulations found in tissues depends on the Pk, as well as on the elimination constants and the daily intake. In liver, the highest concentrations corresponded to PFOS, PFHxA, PFHpA, PFOA and PFNA, with values ranging from 1.32 to 127.6 ng/g (PFNA and PFOS, respectively). In contrast, the minimum values of other long-chain PFASs (PFDS, PFDA, PFTeDA, and PFUnDA) were also found in liver (0.0002, 0.0005, 0.0005, and 0.0016 ng/g, respectively). This fact is surprising, as liver is usually considered the main target organ where PFASs are accumulated. Furthermore, there are no evidences of low concentrations of long chain PFASs in liver (Karrman et al., 2010). PFOA was the predominant compound in bone marrow and lung (205.30 and 99.70 ng/g, respectively). Unexpectedly, the highest concentration of PFHxA (39.19 ng/g) was estimated in brain. Since PFHxA is rapidly cleared from biota, with elimination half-lives of 0.5-1.5 months in humans (Russell et al., 2013), high levels of this compound should not be found in the human body. Kidney showed a similar profile of PFASs to those in plasma and liver, being PFOS the main contributor (113.40 ng/g).

Table 5. Simulated concentration of PFASs in target tissues of individuals from Tarragona County, Spain.

	Plasma	Liver	Bone Marrow	Brain	Lung	Kidney
PFBS	0.65	0.55	1.92	0.29	10.70	5.70
PFHxS	6.36	0.38	0.15	0.26	0.67	2.07
PFOS	17.06	127.6	1.88	6.14	36.40	113.40
PFDS	0.52	0.0002	0.53	0.09	0.98	1.85
PFHxA	0.89	27.0	7.76	39.19	10.92	1.27
PFHpA	0.70	34.5	79.25	1.39	17.86	9.40
PFOA	10.97	46.5	205.30	4.06	99.70	8.19
PFNA	0.79	1.32	2.08	29.60	15.20	33.40
PFDA	0.54	0.0005	0.14	21.30	15.55	6.75
PFUnDA	0.61	0.0016	0.15	8.97	2.79	8.50
PFTeDA	0.39	0.0005	0.01	25.20	9.95	7.50

Units: ng/g

For model parameterization, data on PFAS concentrations in blood and other human tissues from two independent studies, performed in Tarragona County in 2007 and 2008, were used (Ericson et al., 2007; Pérez et al., 2013). The number of analyzed autopsy samples was limited to 20, therefore contributing to a higher uncertainty of the PBPK model results. There is a lack of reliable experimental data for their use in model parameterization. Thus, limited available data may contribute to higher uncertainty in the PBPK model due to temporal variability (different years of sampling) and physiological variability (age group differences) associated with the experimental data. Moreover, sources of variability, like changes of body weight and cardiac output along time, which were not taken into account, should be explored in further investigations.

3.2. Validation of the PBPK model

For validation purposes, the current PBPK model was applied in a case-study in Norway, where information about dietary intake and plasma concentration of a number of PFASs was available (Haug et al., 2010; Rylander et al., 2009). The concentrations of PFHxS, PFOS, PFHpA, PFOA, and PFNA were assessed in 21 samples of selected foods and beverages, including meat, fish, vegetables, fruits, eggs, milk, cereals, bread and drinking water (Haug et al. 2010). The samples were purchased between 2008 and 2009 in Norway, and the daily consumption of foodstuffs for the adult population was assessed by using statistical data. The total intake of PFASs for the adult Norwegian population was estimated in 1.2, 18, 8.2, 31 and 9.5 ng/day for PFHxS, PFOS, PFHpA,

PFOA and PFNA, respectively. In turn, plasma concentrations of PFASs for the Norwegian population were also obtained from the scientific literature (Rylander et al., 2009). Samples from 44 men and 16 women (aged 26-60) living in Andøya Island (Northern Norway) were collected, and the PFAS content was determined. Mean concentrations of PFHxS, PFOS, PFOA and PFNA in plasma were 1.1, 29, 3.9 and 0.81 ng/mL plasma, respectively. In turn, PFHpA was not detected in any sample. The experimental concentrations were compared with the results of the PBPK simulation. The ranges of experimental concentrations were reported for all the compounds, with the exception of PFHpA, which was not detected (Rylander et al., 2009). Since the limit of detection (LOD) for PFHpA was provided (0.26 ng/g), this value was used to validate the model. The minimum and maximum values for the simulation were assessed by running the model with the minimum and maximum values of Tm, Kt, and free fraction. These have been identified as the most uncertain parameters of the PBPK model (Allen et al., 1996; Huizer et al., 2012), being also identified as the most sensitive (unpublished results). The comparison of the simulated and experimental concentrations of 5 different PFASs in plasma from people living in Norway is depicted in Fig. 3. Excepting for PFHpA, which was undetected in plasma, the experimental concentrations of PFASs were slightly higher than the modeled results, therefore underestimating the internal dose of PFASs. This fact could be linked to a potential interaction among PFASs, as well as between these and other chemicals present in blood. In the past, PBPK modeling has been increasingly used to assess the pharmacokinetics of chemical mixtures (El-Masri et al., 2004; Dennison et al., 2004). These approaches are based on the comparison of the levels of simulated mixture responses with those anticipated from the individual response addition. The main objective is to determine the interaction threshold, which is defined as the combined total dose of chemicals at which interactions become significant in terms of joint toxicity of a mixture (Mumtaz et al., 2012). More specifically, complex interactive effects of PFOS and PFOA in zebrafish embryos have been observed, being additive, synergistic or antagonistic according to the mixture ratios of individual chemicals (Ding et al., 2013). Anyhow, since the range of experimental data fell within the range of simulation results, the PBPK model was confirmed to be validated and applicable to PFHxS, PFOS, PFHpA, PFOA and PFNA. However, further investigations are still needed in order to incorporate more refined PK/PD data in the model, as well as to reduce its uncertainty.

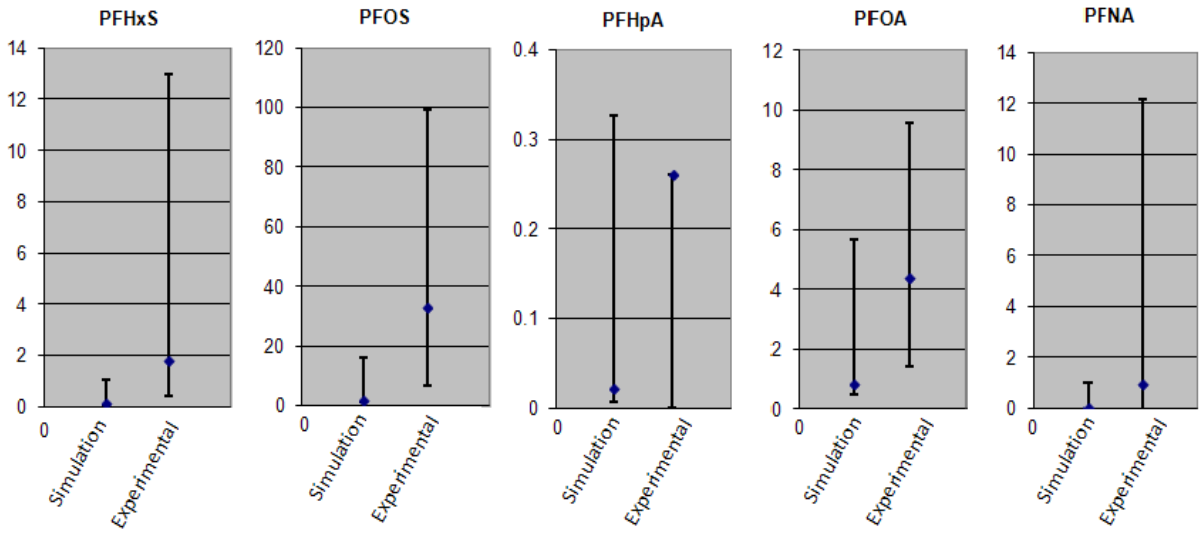


Fig. 3. Experimental and simulated concentrations of PFASs (ng/mL) in plasma from people living in Andøya Island (Norway).

4. Conclusions

A PBPK model was here developed and validated to simulate the body burdens of 9 PFASs, additionally to PFOS and PFOA. The model structure was characterized by owning a mechanism of urinary elimination, a mechanism of resorption of the chemicals from urine to plasma, and a strong protein binding for all the compounds. This generic PBPK model was able of simulating compounds with important pharmacokinetic differences, including half-lives in plasma (Lau et al., 2012). Our findings support that the regulation of both short- and long-chain PFASs is done by the abovementioned PK mechanism. Although further evidence is still necessary, this is a first and successful attempt to describe and simulate the PK of PFASs by using these mechanisms. Furthermore, the PBPK model was validated for 5 of the compounds by means of a case-study in Norway. The relatively small differences between the experimental and the modeled results are a good indicator of the reliability of the model. However, some biochemical and mathematical aspects, such as the involvement of other elimination mechanisms and the uncertainty of parameterization data, deserve further research. Moreover, because of the scarcity of PK data for PFASs, other than PFOS and PFOA, differences among the compounds could not be studied in depth by PBPK modeling. Since the generation of quantitative time-course data sets is essential for the validation of PBPK models, an increase of well-conducted human biomonitoring investigations should be also enhanced to assure the validity of these models and their suitability for estimating PFAS body burdens.

References

Allen, B.C., Covington, T.R., Clewell, H.J., 1996. Investigation of the impact of pharmacokinetic variability and uncertainty on risks predicted with a pharmacokinetic model for chloroform. Toxicology 111, 289-303

Andersen, M.E., Clewell III, H.J., Tan, Y.M., Butenhoff, J.L., Olsen, G.W., 2006. Pharmacokinetic modeling of saturable, renal resorption of perfluoroalkylacids in monkeys-Probing the determinants of long plasma half-lives. Toxicology 227, 156-164

Armitage, J., Cousins, I.T., Buck, R.C., Prevedouros, K., Russell, M.H., MacLeod, M., Korzeniowski, S.H., 2006. Modeling global-scale fate and transport of perfluorooctanoate emitted from direct sources. Environmental Sciences & Technology 40, 6969-6975

Benfenati, E., Gini, G., Hoffmann, S., Luttik, R., 2010. Comparing *in vivo*, *in vitro* and *in silico* methods and integrated strategies for chemical assessment: Problems and prospects. Alternatives to Laboratory Animals 38, 153-166

Bischel, H.N., Macmanus-Spencer, L.A., Luthy, R.G., 2010. Noncovalent interactions of long-chain perfluoroalkyl acids with serum albumin. Environmental Sciences & Technology 44, 5263-5269

Boiteux, V., Dauchy, X., Rosin, C., Munoz, J.F., 2012. National screening study on 10 perfluorinated compounds in raw and treated tap water in France. Archives of Environmental Contamination & Toxicology 63, 1-12

Borg, D., Lund, B.O., Lindquist, N.G., Hakansson, H., 2013. Cumulative health risk assessment of 17 perfluoroalkylated and polyfluoroalkylated substances (PFASs) in the Swedish population. Environment International 59, 112-123

Brown, R.P., Delp, M.D., Lindstedt, S.L., Rhomberg, L.R., Beliles, R.P., 1997. Physiological parameter values for physiologically based pharmacokinetic models. Toxicology & Industrial Health 13, 407-484

Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousin, I.T., de Voog, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen S.P., 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integrated Environmental Assessment & Management 7, 513-541

Chen, Y.M., Guo, L.H., 2009. Fluorescence study on site-specific binding of perfluoroalkyl acids to human serum albumin. Archives of Toxicology 83, 255-261

Custer, C.M., Custer, T.W., Dummer, P.M., Etterson, M.A., Thogmartin, W.E., Wu, Q., Kannan, K., Trowbridge, A., McKann, P.C., 2014. Exposure and effects of perfluoroalkyl substances in tree swallows nesting in Minnesota and Wisconsin, USA. Archives of Environmental Contamination & Toxicology 66, 120-138

D'Eon, J.C., Mabury, S.A., 2011. Is indirect exposure a significant contributor to the burden of perfluorinated acids observed in humans? Environmental Science & Technology 45, 7974-7984

Dennison, J.E., Andersen, M.E., Dobrev, I.D., Mumtaz, M.M., Yang, R.S., 2004. PBPK modeling of complex hydrocarbon mixtures: gasoline. Environmental Toxicology & Pharmacology 16, 107-119

Ding, G., Zhang, J., Chen, Y., Wang, L., Wang, M., Xiong, D., Sung, Y., 2013. Combined effects of PFOS and PFOA on zebrafish (*Danio rerio*) embryos. Archives of Environmental Contamination & Toxicology 64, 668-675

Domingo, J.L., 2012. Health risks of dietary exposure to perfluorinated compounds. Environment International 40, 187-195

Domingo, J.L., Jogsten, I.E., Eriksson, U., Martorell, I., Perello, G., Nadal, M., van Bavel, B., 2012. Human dietary exposure to perfluoroalkyl substances in Catalonia, Spain. Temporal trend. Food Chemistry 135, 1575-1582

El-Masri, H.A., Mumtaz, M.M., Yushak, M.L., 2004. Application of physiologically-based pharmacokinetic modeling to investigate the toxicological interaction between chlorpyrifos and parathion in the rat. Environmental Toxicology & Pharmacology 16, 57-71

Ericson, I., Domingo, J.L., Nadal, M., Bigas, E., Llebaria, X., van Bavel, B., Lindstrom, G., 2009. Levels of perfluorinated chemicals in municipal drinking water from Catalonia, Spain: Public health implications. Archives of Environmental Contamination & Toxicology 57, 631-638

Ericson, I., Gómez, M., Nadal, M., van Bavel, B., Lindström, G., Domingo, J.L., 2007. Perfluorinated chemicals in blood of residents in Catalonia (Spain) in relation to age and gender: A pilot study. Environment International 33, 616-623

Ericson, J.I., Nadal, M., van Bavel, B., Lindstrom, G., Domingo, J.L., 2012. Per- and polyfluorinated compounds (PFCs) in house dust and indoor air in Catalonia, Spain: implications for human exposure. Environment International 39, 172-180

Fàbrega, F., Kumar, V., Schuhmacher, M., Domingo, J.L., Nadal, M., 2014. PBPK modelling for PFOS and PFOA: Validation with human experimental data. Toxicology Letters, S0378-4274, 00017-4.

Fuentes, S., Colomina, M.T., Vicens, P., Franco-Pons, N., Domingo, J.L., 2007. Concurrent exposure to perfluorooctane sulfonate and restraint stress during pregnancy in mice: effects on postnatal development and behaviour of the offspring. Toxicological Sciences 98, 589-598

Fuentes, S., Colomina, M.T., Rodriguez, J., Vicens, P., Domingo, J.L., 2006. Interactions in developmental toxicology: concurrent exposure to perfluorooctane sulfonate (PFOS) and stress in pregnant mice. Toxicology Letters 164, 81-89

Furdui, V.I., Helm, P.A., Crozier, P.W., Lucaciu, C., Reiner, E.I., Marvin, C.H., Whittle, D.M., Mabury, S.A., Tomy, G.T., 2008. Temporal trends of perfluoroalkyl compounds with isomer analysis in Lake Trout from Lake Ontario (1979−2004). Environmental Science & Technology 42, 4739-4744

Han, X., Nabb, D.L., Russell, M.H., Kennedy, G.L., Rickard, R.W., 2012. Renal elimination of perfluorocarboxylates (PFCAs). Chemical Research in Toxicology 25, 35-46

Han, X., Snow, T.A., Kemper, R.A., Jepson, G.W., 2003. Binding of perfluorooctanoic acid to rat and human plasma proteins. Chemical Research in Toxicology 16, 775-781

Harada, K.H., Hashida, S., Kaneko, T., Takenaka, K., Minata, M., Inoue, K., Saito, N., Koizumi, A., 2007 Biliary excretion and cerebrospinal fluid partition of perfluorooctanoate and perfluorooctane sulfonate in humans. Environmental Toxicology & Pharmacology 24, 134-139

Haug, L.S., Salihovic, S., Jogsten, I.E., Thomsen, C., van Bavel, B., Lindstrom, G., Becher, G., 2010. Levels in food and beverages and daily intake of perfluorinated compounds in Norway. Chemosphere 80, 1137-1143

Huizer, D., Oldenkamp, R., Ragas, A.M., van Rooij, J.G., Huijbregts, M.A., 2012. Separating uncertainty and physiological variability in human PBPK modelling: The example of 2-propanol and its metabolite acetone. Toxicology Letters 214, 154-165

Hundley, S.G., Sarrif, A.M., Kennedy, G.L., 2006. Absorption, distribution, and excretion of ammonium perfluorooctanoate (APFO) after oral administration to various species. Drug & Chemical Toxicology 29, 137-145

Inoue, Y., Hashizume, N., Yakata, N., Murakami, H., Suzuki, Y., Kikushima, E., Otsuka, M., 2012. Unique physicochemical properties of perfluorinated compounds and their bioconcentration in common carp *Cyprinus carpio* L. Archives of Environmental Contamination & Toxicology 62,672-680

Karrman, A., Domingo, J.L., Llebaria, X., Nadal, M., Bigas, E., van Bavel, B., Lindstrom, G., 2010. Biomonitoring perfluorinated compounds in Catalonia, Spain:

concentrations and trends in human liver and milk samples. Environmental Science & Pollution Research International 17, 750-758

Kowalczyk, J., Ehlers, S., Fürst, P., Schafft, H., Lahrssen-Wiederholt, M., 2012. Transfer of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) from contaminated feed into milk and meat of sheep: pilot study. Archives of Environmental Contamination & Toxicology 63, 288-298.

Kudo, N., Sakai, A., Mitsumoto, A., Hibino, Y., Tsuda, T., Kawashima, Y., 2007. Tissue distribution and hepatic subcellular distribution of perfluorooctanoic acid at low dose are different from those at high dose in rats. Biological & Pharmaceutical Bulletin 30, 1535-1540

Lau, C., 2012. Perfluoroalkyl acids: Recent research highlights. Reproductive Toxicology 33, 405-409

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicological Sciences 99, 366-394

Lau, C., Butenhoff, J.L., Rogers, J.M., 2004. The developmental toxicity of perfluoroalkyl acids and their derivatives. Toxicological & Applied Pharmacology 198, 231-241

Loccisano, A.E., Campbell Jr, J.L., Andersen, M.E., Clewell III, H.J., 2011. Evaluation and prediction of pharmacokinetics of PFOA and PFOS in the monkey and human using a PBPK model. Regulatory Toxicology & Pharmacology 59, 157-175

Loccisano, A.E., Longnecker, M.P., Campbell Jr, J.L., Andersen, M.E., Clewell III, H.J., 2013. Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages. Journal of Toxicology & Environmental Health A 76, 25-57

Macey, R., Oster, G., Zahnley, T., 2009. Berkeley-Madonna. University of California, Department of Cellular and Molecular Biology. Berkeley, CA, USA.

Medinsky, M.A., 1995. The application of physiologically based pharmacokinetic/ pharmacodynamic (PBPK/PD) modeling to understanding the mechanism of action of hazardous stances. Toxicology Letters 79, 185-191

Mumtaz, M., Fisher, J., Blount, B., Ruiz, P., 2012., Application of physiologically-based pharmacokinetic modeling in chemical risk assessment. Journal of Toxicology 904603

Nadal, M., Domingo, J.L., 2014. Indoor dust levels of perfluoroalkyl substances (PFASs) and the role of ingestion as an exposure pathway: A review. Current Organic Chemistry, in press.

Olsen, G.W., Chang, S.C., Noker, P.E., Gorman, G.S., Ehresman, D.J., Lieder, P.H., Butenhoff, J.L., 2009. A comparison or the pharmacokinetics of perfluorobutanesulfonate (PFBS) in rats, monkey and humans. Toxicology 256, 65-74

Olsen, G.W., Mair, D.C., Church, T.R., Ellefson, M.E., Reagen, W.K., Boyd, T.M., Herron, R.M., Medhdizadehkashi, Z., Nobiletti, J.B., Rios, J.A., Butenhoff, J. L., Zobel, L.R., 2008. Decline in perfluorooctanesulfonate and other polyfluoroalkyl chemicals in American Red Cross adult blood donors, 2000-2006. Environmental Science & Technology 42, 4989-4995

Ophaug, R.H., Singer, L., 1980. Metabolic Handling of perfluorooctanoic acid in rats. Experimental Biology & Medicine 163, 19-23

Pérez, F., Nadal, M., Navarro-Ortega, A., Fàbrega, F., Domingo, J.L., Barceló, D., Farré, M., 2013. Accumulation of perfluoroalkyl substances in human tissues. Environmental International 59, 354-362

Renner, R., 2006. The long and the short of perfluorinated replacements. Environmental Science & Technology 40, 12-13

Russell, M.H., Nilsson, H., Buck, R.C., 2013. Elimination kinetics of perfluorohexanoic acid in humans and comparison with mouse, rat and monkey. Chemosphere 93, 2419-2425

Rylander, C., Brustad, M., Falk, H., Sandanger, T.M., 2009. Dietary predictors and plasma concentrations of perfluorinated compounds in a coastal population from Northern Norway. Journal of Environmental & Public Health 2009, 268219

Stahl, T., Mattern, D., Brunn, H., 2011. Toxicology of perfluorinated compounds. Environmental Sciences Europe 23:38

Stahl, T., Falk, S., Failing, K., Berger, J., Georgii, S., Brunn, H., 2012. Perfluorooctanoic acid and perfluorooctane sulfonate in liver and muscle tissue from wild boar in Hesse, Germany. Archives of Environmental Contamination & Toxicology 62, 696-703

Tan, Y.M., Clewell III, H.J., Andersen, M.E., 2008. Time dependencies in perfluorooctylacids disposition in rat and monkeys: A kinetic analysis. Toxicology Letters 177, 38-47

Taniyasu, S., Senthilkumar, K., Yamazaki, E., Yeung, L.W., Guruge, K.S., Kannan, K., 2013. Perfluoroalkyl substances in the blood of wild rats and mice from 47 prefectures in Japan: use of samples from nationwide specimen bank. Archives of Environmental Contamination and Toxicology 65, 149-170

Thompson, M.D., Beard, D.A., 2011. Development of appropriate equations for physiologically based pharmacokinetic modeling of permeability-limited and flow-limited transport. Journal of Pharmacokinetics & Pharmacodynamics 38, 405-421

U.S.EPA, 2006. Approaches for the application of physiologically based pharmacokinetic (PBPK) models and supporting data in risk assessment (final report). U.S. Environmental Protection Agency, Washington, D.C., EPA/600/R-05/043F.

U.S.EPA, 2014. 2010/1015 PFOA Stewardship program, http://www.epa.gov/oppt/pfoa/pubs/stewardship/index.html. Accessed 03-06-2014

Vorkamp, K., Nielsen, F., Kyhl, H.B., Husby, S., Nielsen, L.B., Barington, T., Andersson, A.M., Jensen, T.K., 2014. Polybrominated diphenyl ethers and perfluoroalkyl substances in serum of pregnant women: Levels, correlations, and potential health implications. Archives of Environmental Contamination and Toxicology, in press, doi:10.1007/s00244-013-9988-z

Wang, J., Xia, S., Xue, W., Wang, D., Sai, Y., Liu, L., Liu, X., 2013. A semi-physiologically-based pharmacokinetic model characterizing mechanism-based auto-inhibition to predict stereoselective pharmacokinetics of verapamil and its metabolite norverapamil in human. European Journal of Pharmaceutical Sciences 50, 290-302

Wang, T., Wang, Y., Liao, C., Cai, Y., Jiang, G., 2009. Perspectives on the Inclusion of perfluorooctane sulfonate into the Stockholm Convention on Persistent Organic Pollutants. Environmental Sciences & Technology 43, 5171-5175

WHO/IPCS, 2010. Characterization and application of physiologically based pharmacokinetic models in risk assessment. IPCS harmonization project document No. 9, Geneva, Switzerland.

Yeung, L.W., Guruge, K.S., Taniyasu, S., Yamashita, N., Angus, P.W., Herath, C.B., 2013. Profiles of perfluoroalkyl substances in the liver and serum of patients with liver cancer and cirrhosis in Australia. Ecotoxicology & Environmental Safety 96, 139-146

Yoo, H., Yamashita, N., Taniyasu, S., Lee, K.T., Jones, P.D., Newsted, J.L., Khim, J.S., Giesy, J.P., 2009. Perfluoroalkyl acids in marine organisms from Lake Shihwa, Korea. Archives of Environmental Contamination & Toxicology 57, 552-560

Zhang, L., Ren, X.M., Guo, L.H., 2013. Structure-based investigation on the interaction of perfluorinated compounds with human liver fatty acid binding protein. Environmental Sciences & Technology 47, 11293-11301.

Discussion of Chapters IV and V

After the development of the PBPK model for PCDD/Fs, the model was adapted to a different group of POPs: PFASs. PFOS and PFOA, two of the most well known PFASs, were originally selected. The elimination mechanism was changed, and the role of the protein binding to plasma albumin was added. The mechanism of elimination was considered to occur by urine elimination with a saturable resorption mechanism from urine back to the plasma. In liver, the target tissue of PFOS and PFOA, the simulated concentration of PFOA was 3.33 ng/g, while the experimental value was 13.6±35.2 ng/g. For PFOS, the simulated and experimental concentrations were 36.4 ng/g and 102.3±122.9 ng/g, respectively. Due to the coincidence of the simulated concentrations within the range of measured levels of PFOS and PFOA, the model was considered as validated.

A comparison of Pks values obtained by using data from rats and humans, was conducted. As expected, the use of Pks coming from human data simulated better the concentration in human tissues. This clearly indicates the need to acquire more information regarding the body burdens of PFOS and PFOA to improve the accuracy of Pks. Although PBPK model validation is commonly carried out by using only blood data, this should be extended to other human tissues to ensure its reliability, with special emphasis on target tissues.

Due to the regulatory restrictions for PFOS and PFOA, industrial producers are searching alternatives and developing new lines of products with lighter PFASs, such as PFBS or PFHxA. Thus, the PBPK model was extended to 9 PFASs more. For the first time, PFASs in human tissues other than PFOS and PFOA were simulated by using PBPK models. Although the mechanism of elimination of PFASs other than PFOS and PFOA is not well known, the PBPK model was capable to simulate PFASs through urinary elimination via resorption mechanism. This is not the proof of the resorption of all the PFASs but it is a successful first attempt to simulate PFASs using this mechanism. Notwithstanding, further evidence is still necessary. PBPK modeling demonstrates to be a reliable tool to elucidate the mechanism of elimination of different environmental pollutants, as well as other PK processes. Moreover, the parametric data of elimination (Tm and Kt), free fraction and Pk were also assessed using the PBPK model. Significant correlations between the carbon chain length of the PFASs and the elimination parameters were not found. However, this should be further explored, as these variables should be better calibrated using more experimental data. The number of analyzed

autopsy samples was limited to 20, therefore contributing to a higher uncertainty of the PBPK model results. Limited available data may contribute to a higher uncertainty in the PBPK model due to temporal variability (different years of sampling) and physiological variability (age group differences) associated with the experimental data.

For validation purposes, the extended PBPK model was applied in a case-study in Andøya Island (Northern Norway), where information about dietary intake and plasma concentration was available for PFHxS, PFOS, PFHpA, PFOA and PFNA. The experimental concentrations were compared with the results of the PBPK simulation. The model was considered as validated because experimental concentrations were within the range of the PBPK simulation results. The relatively small differences between the experimental and the modeled results are a good indicator of the reliability of the model.

CHAPTER 6

INFLUENCE OF THE UNCERTAINTY IN THE VALIDATION OF PBPK MODELS: A CASE-STUDY FOR PFOS AND PFOA.

Abstract

Physiologically-based pharmacokinetic (PBPK) models are mathematical representations of the human body that aims to describe the time course distribution of chemicals in human tissues. PBPK models are developed using experimental data which may have an error associated with the measurements. The uncertainty associated with the parametric data used in PBPK modeling is huge, thus affecting the process and reliability of model validation. The objective of the present work was to assess the parametric uncertainty associated to a PBPK model developed for perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), and validated by using experimental data from people living in Tarragona County (NE of Spain). A sensitivity analysis was performed to understand the degree of influence of input parameters on the final outcomes. Data of partition coefficients (Pks), elimination constants (Tm and Kt) and unbound fraction in plasma (Free) were obtained from autopsy individuals in the area of Tarragona, while data on human intake of PFOS and PFOA were also compiled from previous studies in the same area. The uncertainty of the PBPK models were assessed by propagating the parametric uncertainty using Latin Hypercube Sampling (LHS) technique, and the uncertainty bound with mean concentrations of PFOS and PFOA in different tissue compartments, was estimated. Finally, the simulation results were compared with the experimental data and Student's *t*-test was executed to assess the validity of the model. Significant differences were found when comparing the visual and statistical validation of the PBPK model.

1. Introduction

Physiologically-based pharmacokinetic (PBPK) models aim to simulate the time course concentration of chemical compounds in the human body (Nestorov, 2007). In PBPK modeling, tissues are considered compartments linked by the blood flow, and the time course concentration of chemicals are described by mathematical equations that can be solved computationally (Thompson and Beard, 2011, 2012). The complexity of the PBPK models depends on the administration, distribution, metabolism and elimination (ADME) properties of the chemicals in the human body. Although, the first

PBPK model was developed in 1937, it was impossible to compute the equations for the model resolution (Teorell, 1937). Later, in the 1970s, the development of computational tools allowed the resolution and the development of the PBPK modeling (Bischoff et al., 1970; Jones and Rowland-Yeo, 2013). Nevertheless, the scarcity of pharmacokinetic and pharmacodynamic (PK/PD) data and the implementation of the *in vivo* and *in silico* tools to estimate PK/PD data, have delayed the extensive use of PBPK models until the last decade (Mumtaz et al., 2012; Rowland et al., 2011). Nowadays, PBPK modeling is extensively used in drug development and human health risk assessment (Chiu et al., 2007; Rowland et al., 2011).

PBPK modeling involves the use of input parameters that were generated by experimental assays. Consequently these variables own some variability and error associated with the measurements. The variability is the inter-individual differences in the anatomical and physiological characteristics among individuals, and cannot be eliminated but only reduced (Kumar et al., 2009). On the other hand, the uncertainty is due to the lack of knowledge in the parameter estimation, being this reducible (Bois et al., 2010a). To estimate the model variability and uncertainty several approaches have been used in PPBK modeling such as Monte-Carlo simulation, Fuzzy simulations and Bayesian Markov chain Monte Carlo (MCMC) (Bois et al., 2010b; Gueorguieva et al., 2005; Hack, 2006; Sweeney et al., 2001; Woodruff et al., 1992). In the past, some studies characterized the differences between uncertainty and variability in PBPK models (Chiu et al., 2009; Huizer et al., 2012; Kumar et al., 2009; Mörk et al., 2009). The variability and uncertainty of input parameters affect the time course concentration, being dependent on the sensitivity of the parameters. A sensitivity analysis (SA) provides a quantitative assessment of the degree of influence of the input parameters on model results and have been used in PBPK models in the past (Loizou and McNally, 2010; McNally et al., 2011). Uncertainty plays a key role in the validation of the PBPK models. Validation is the process of evaluation of the model with the reliability and relevance of a particular approach (Chiu et al., 2007; WHO., 2010). PBPK models can be validated in many ways, and until now no single method has been accepted by the regulatory agencies. In general, the simulations whose results are within a factor of 2 of the experimental results may be considered as valid (WHO., 2010). The most common approach is the visual comparison of the experimental data with the simulated results (Chiu et al., 2007). This process of validation is highly affected by the structural and parametric error of the PBPK models. Instead, any model can be proven to be absolutely valid, the process of validation may be meaningless if the uncertainty of the PBPK model is excessively high. When high values of uncertainty are found in a PBPK model, any

experimental value may fall within the range of the model simulation, and thus the model can be easily considered valid. To improve the reliability of the PBPK models, a proper study of the model uncertainty is necessary for an accurate validation of the model (WHO., 2010). Until nowadays, the uncertainty in PBPK modeling has been poorly studied (WHO., 2010), and therefore case the process of model validation may be doubtful.

The objective of the present work was to study the uncertainty associated to PBPK modeling parameterization, and the subsequent validation of the PBPK model. A previous PBPK model developed for perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) in Tarragona County was used (Fàbrega et al., 2014). In a first step, a sensitivity analysis was performed, and the most sensitive parameters were selected for the uncertainty analysis. In the second step, the uncertainty of the PBPK models were assessed by propagating the parametric uncertainty using Latin Hypercube Sampling (LHS) technique and uncertainty bound along with mean concentrations of PFOS and PFOA in different tissue compartments. Finally, the uncertainty of PBPK model parameterization was compared with the experimental values from the area under study and a statistical analysis was performed to validate the result.

2. Materials and Methods

2.1. PBPK model for PFOS and PFOA

In a previous study, a PBPK model was developed for two perfluoroalkyl substances (PFASs): PFOS and PFOA (Fàbrega et al., 2014). PFOS and PFOA are well absorbed via oral route, not metabolized in human body, and poorly eliminated. The mechanism of elimination for PFOS and PFOA has been proved to be mainly through urine elimination, with a resorption mechanism: once in the urine PFOS and PFOA are resorbed back from urine to plasma. (Andersen et al., 2006; Loccisano et al., 2011; Tan et al., 2008). Moreover, these compounds strongly bind to serum albumin, and only a small fraction is available to move to tissues and be eliminated (Bischel et al., 2010; Chen and Guo, 2009; Han et al., 2003). Model structure, parameterization data and equations were previously described in Fàbrega et al. (2014). In summary, the model structure includes plasma, gut, liver, kidney, filtrate, storage, fat, brain, lungs and rest of the body. Structure of the PBPK model is depicted in Figure 1. The model was carried out for a case study in Tarragona County (NE of Spain). The intake was considered to occur through ingestion of food and water (Domingo et al., 2012a; Domingo et al., 2012b). The key parameter for the distribution is the partition coefficient (Pk), defined as

the concentration of a chemical in a tissue over its concentration in blood. Pks were assessed by using data of blood donors and autopsy tissues (Ericson et al., 2007; Pérez et al., 2013). Elimination was assumed to be through a resorption mechanism in kidney. The elimination constants are resorption maximum (Tm) and affinity constant (Kt), being previously assessed using data in human tissues (Fàbrega et al., 2014).Tm and Kt are analogous parameters to the maximum velocity (Vmax) and Michaelis-Menten constant (K_M) in the Michaelis-Menten kinetics. Finally, PFOS and PFOA are strongly bound to plasma albumin. The remaining unbound fraction was called *Free fraction*, and is one of the key parameters in the distribution of PFOS and PFOA in PBPK modeling.

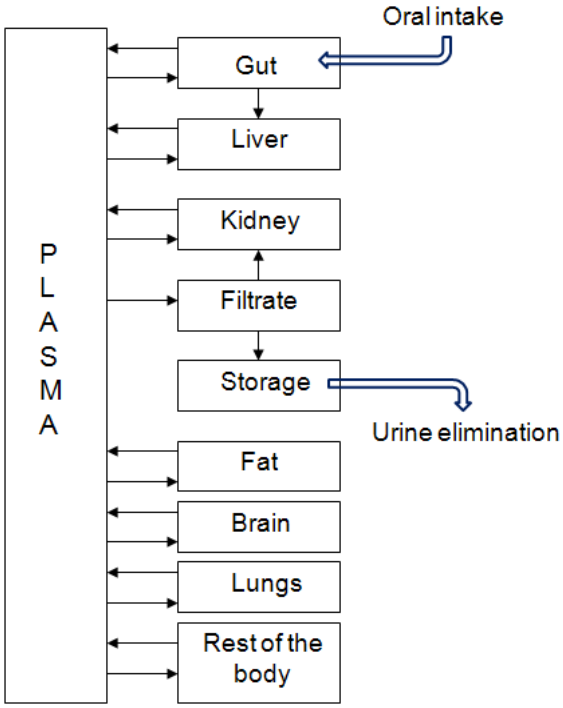


Fig. 1. Structure of the PBPK model.

2.2. Case-study

The PBPK model for PFOS and PFOA was applied in a case study in Tarragona County (NE of Spain) (Fàbrega et al., 2014), where data of human intake (Domingo et al., 2012a; Domingo et al., 2012b) and human autopsy tissues were available (Ericson et al., 2007; Pérez et al., 2013). Data from the general population with no contact with industrial production or manufacture of PFASs (Ericsson et al., 2007; Pérez et al., 2013) were used. The ingestion of food was assessed by using data from Domingo et al. (2012b), data for all the population cohorts. For PFOS, the mean concentration in food

was 2.26 ng/kg body weight/day, and the minimum and maximum concentrations were 1.57 and 4.48 ng/kg body weight/day, respectively. For PFOA, the mean concentration in food was 4.68 ng/ kg body weight/day, and the minimum and maximum concentrations were 0.83 and 19 ng/ kg body weight/day, respectively. The estimated concentrations in water were 1.8 ng/L and 2.4 ng/L for PFOS and PFOA, respectively (Domingo et al., 2012a). Finally, considering a body weight of 70 kg and a water consumption of 1.23 L day (US EPA, 2011), the total intake of PFOS and PFOA in the adults of Tarragona County was estimated in 0.16 μg/day and 0.33 μg/day (Fàbrega et al., 2014). The minimum intake was assessed in the range 0.06-0.11 μg/day, and the maximum intake was considered to be within 0.32 and 1.33 μg/day, for PFOS and PFOA, respectively.

The concentration of PFOS and PFOA in human tissues has been poorly analyzed in the past (Karrman et al., 2010; Maestri et al., 2006; Pérez et al., 2013). In Tarragona County, data of PFOS and PFOA were available for blood, liver, brain lung and kidney. These were used for validation purposes. PFOS and PFOA concentrations in blood were previously assessed for 48 blood donors, where the average age of the blood donors was 40 years old, with a range between 20 and 60 years old (Ericson et al., 2007). For PFOS, blood level was 7.64±3.54 ng/mL, while PFOA concentration was 1.80±0.66 ng/mL. Similarly, the concentration of PFOS and PFOA were previously assessed in liver, brain, lung, bone marrow and kidney in 20 autopsies of individuals in Tarragona County (Pérez et al., 2013). The average concentration of PFOA was 13.6±35.2, 29.2±32.2 and 2.00±2.70 ng/g wet weight for liver, lung and kidney, respectively. In brain, PFOA was under its respective detection limit (DL), being the mean concentration 1.50 ng/g wet weight (½ of the DL). For PFOS the concentrations found in human tissues were 102±123, 4.90±6.60, 29.1±16.9 and 75.6±61.2 ng/g wet weight for liver, brain, lung and kidney, respectively. At the time of death, the average age of the individuals was 57 years old, with a range of ages between 28 and 86 years old.

2.3. Sensitive analysis (SA)

A SA for the PBPK model parameterization was performed to study the parameters with the most important contribution to the final outcomes (Evans and Eklund, 2001). SA was performed using the method of sensitivity index (SI), which is based on the absolute value of the difference between the maximum and minimum value over the maximum value (Hamby, 1994):

$$SI = \left| \frac{D\max - D\min}{D\max} \right| \quad (1)$$

To assess the SA in the PBPK model, the range of the parameters under study were used. PBPK parameters included body weight (BW), plasma volume, liver volume, brain volume, lung volume, kidney volume, cardiac output to liver, cardiac output to brain, cardiac output to lungs, cardiac output to kidney, Tm, Kt, Free fraction and Intake. Moreover, the partition coefficient (Pk) to gut, liver, kidney, fat, brain, lungs and rest of the body were also included. Physiological data and their ranges, came from Huizer et al. (2012) and Brown et al. (1997). The partition coefficients (Pk) of the different organs were previously assessed using experimental data found in human tissues (Fàbrega et al., 2014; Maestri et al., 2006). The mean and uncertainty range (minimum – maximum) for the parametric data are summarized in table 1. Data of Tm, Kt and Free fraction comes from our previous PBPK model (Fàbrega et al., 2014). For sensibility analysis of the different parameters a coefficient of variation (CV) of 0.3 was used (Allen et al., 1996; Brochot et al., 2007; Sweeney et al., 2001). The parametric uncertainty of intake was assessed for different population cohorts (Domingo et al., 2012b). Although, the intake has usually both uncertainty-variability components, we only assumed that a significant part of uncertainty comes from data treatment.

Table 1. Mean, range and sensitivity of the parameters used in the PBPK model, ranked according their sensitivity.

PFOS				PFOA			
Parameter	**Mean**	**Range**	**Sensitivity**	**Parameter**	**Mean**	**Range**	**Sensitivity**
Tmc	3.50	0.617-17.2	22.7	Free	0.03	9.46e-7-70.9	185696
Q. kidney	0.17	0.118-0.265	3.82	Kt	0.116	1.12e-4-39.8	585
Free	0.03	9.45e-7-70.9	1	Q. kidney	0.177	0.118-0.265	4.01
Kt	0.018	3.30e-7-56.5	0.999	Intake	0.331	0.061-1.33	0.952
Pk Rest body	0.2	0.0007-17.9	0.781	Tm	6.0	1.46-20.9	0.929
Intake	0.161	0.112-0.316	0.624	Pk Rest body	0.12	0.0002-40.2	0.664
Pk fat	0.033	0.004-15.0	0.438	BW	71.4	64.7-79.1	0.161
BW	71.4	64.7-79.1	0.158	Pk fat	0.467	0.008-16.2	0.055
Pk gut	0.57	0.013-17.7	0.073	Pk gut	0.05	9.97e-6-33.7	0.025
Pk brain	0.255	0.002-16.8	0.017	Pk liver	1.03	0.077-14.1	0.002
Pk lung	0.155	0.0003-24.8	0.017	Pk brain	0.17	0.0005-20.8	0.0008
Pk liver	2.67	0.494-13.6	0.007	Pk kidney	1.17	0.096-11.5	0.0006
Pk kidney	1.26	0.105-14.7	0.003	Volume plasma	0.04	0.026-0.059	0.0004
Volume brain	0.021	0.014-0.032	0.001	Volume lung	0.014	0.009-0.021	0.0003
Q. brain	0.117	0.078-0.176	0.0008	Volum liver	0.023	0.015-0.034	0.0003
Volum liver	0.023	0.015-0.034	0.0007	Pk lung	1.27	0.105-13.72	0.0002
Q. liver	0.189	0.126-0.283	0.0005	Q. liver	0.189	0.126-0.283	0.0002
Q. lung	0.034	0.023-0.051	0.0002	Volum kidney	0.004	0.003-0.006	0.0002
Volume plasma	0.04	0.026-0.059	0.0002	Q. brain	0.117	0.078-0.176	0.0002
Volum kidney	0.004	0.003-0.006	0.0001	Q. lung	0.034	0.023-0.051	0.0002
Volume lung	0.014	0.009-0.021	0.0001	Volume brain	0.021	0.014-0.032	0.0001

BW: Body weight (kg); Tm: Resorption maximum (μg/h); Kt: Affinity constant (μg/L); Free: free fraction (unitless); Pk: partition coefficient (unitless); Intake (μg/day). Cardiac output to tissues and tissue volumes are given in the fraction of total cardiac output and total volume.

2.4. Uncertainty analysis

The uncertainty of the PBPK model was assessed for those parameters with a higher contribution according to the SA. In a first step, the Latin Hypercube Sampling (LHS) method was used. LHS is a stratified sampling method to reduce the number of runs necessary for a Monte Carlo (MC) simulation and to achieve a distribution with a reasonable calculation (McKay et al., 1979). In LHS, the range of each variable is divided into N intervals, where N is the number of iterations of MC simulation. Then, a random value is obtained for each segment following a uniform distribution. After that, the variables are randomly grouped for each MC simulation. The outcomes were used to simulate the minimum and maximum concentration of PFOS and PFOA in the human tissues. The final results were the simulation of the concentration of PFOS and PFOA for plasma, liver, brain lung and kidney. The minimum and maximum simulation bands were used to validate the experimental results by comparing the experimental data with the simulation results obtained in the PBPK model (Chiu et al., 2007).

2.5. Statistical analysis

The *t*-test was applied to the results obtained for the PBPK model to assess the validity of the model simulation. *T*-test is used to determine if two populations means are equal (Snedecor and Cochran, 1989). The statistic test was assessed using:

$$t = \frac{\bar{x} + \bar{y}}{\sqrt{\frac{s_x^{\,2}}{n} + \frac{s_y^{\,2}}{m}}} \tag{2}$$

where x and y are the means, s_x and s_y are the standard deviation, and “n” and “m” are the size of the sample. When the variances of the data samples are different, the statistical test under the null hypothesis has Student’s distribution, and the sample standard deviation is replaced by the pooled standard deviation:

$$s = \sqrt{\frac{(n-1)s_x^{\,2} + (m-1)s_y^{\,2}}{n+m-2}} \tag{3}$$

To test the significance, a level of significance calls alpha (α) need to be set. Alpha explains how the extreme observed results must be in order to reject the null hypothesis of the *t*-test. Alpha is associated to the confidence level of the *t*-test. Commonly, alpha has a value between 0.05 to 0.01, indicating a 95% and 99% of level of confidence,

respectively. On the other hand, p-value is the probability that the observed statistic occurred by chance alone. To test the significance α is compared with p-value to accept or reject the null hypothesis.

3. Results and Discussion

3.1 Sensitivity analysis (SA)

SA was performed to identify how input uncertainty impacts the final outcomes. SA outcomes were ranked and summarized in Table 1. For PFOS, the highest values obtained in the SA were Tm, followed by cardiac output to kidney (Q. kidney), Free, Kt, Pk for the rest of the body and the oral intake. For PFOA, the main parameters according to the SA was for Free fraction, followed by Kt, Q. kidney, oral intake, Tm and Pk for the rest of the body. In general, cardiac output and volumes to tissues showed the smallest values of SA, with the only exception of Q. kidney. The reason of the high SA for Q. kidney may be that kidney is the elimination tissue, and elimination parameters, whose uncertainty is important, give a higher degree of influence to this parameter. SA in Q. kidney was not studied because it is a physiological parameter with natural variability, and the present study was limited to parametric uncertainty (Huizer et al., 2012). Similarly, the results of the SA for Pks were among the lower results with the exception of Pk for rest of the body that was considerably big. The most plausible explanation for the high value in the rest of the body is that this compartment is a lumped tissue that receives all the uncertainty of the other tissues together (Thompson and Beard, 2012). Using the result of SA, the study of the uncertainty was focused on the most sensitive parameters, namely Tm, Kt, Free and intake. For PFOA, more than the 99.9% of the contribution comes from these four parameters, whereas for PFOS, the contribution percentage for the selected parameters was 82.6%.

3.2. Uncertainty assessment of parametric data

The mean, minimum and maximum concentrations of PFOS and PFOA in plasma, liver, brain, lung and kidney were simulated, and compared with experimental data (Figures 2, 3, 4, 5 and 6). The parametric samples of the LHS were used to simulate the concentrations of PFOS and PFOA in human tissues. In many cases, the result was a set of simulations that were not converging. For those set of unconverged simulation, the values of Tm, Kt, Free and intake were adjusted by calibrating the value of the parameter to obtain the minimum and maximum results where the simulation trend converged. Table

2 summarizes the parametric data obtained for the Tm, Kt, Free and Intake for the PBPK model (Fàbrega et al., 2014). This process of calibration may underestimate the final value for the true range of uncertainty but allows to obtain simulations that converges with close aproximation to the true range of uncertainty. In the next paragraph, the behaviour of the parameters studied is discussed.

Table 2. Values of Tm, Kt and Free fraction used in the PBPK model to assess the mean, minimum and maximum concentration of PFOS and PFOA.

	PFOS			**PFOA**		
	Mean	**Minimum**	**maximum**	**Mean**	**Minimum**	**Maximum**
Tm	86	17.4	2456	147	35.9	245
Kt	0.02	24.9	0.2	0.11	41.5	0.04
Free	0.03	1.0	0.02	0.03	1.0	0.01
Intake	0.17	0.11	0.32	0.33	0.06	1.33

Tm: Resorption maximum (μg/h); Kt: Affinity constant (μg/L); Free: free fraction (unitless); Intake (μg/day).

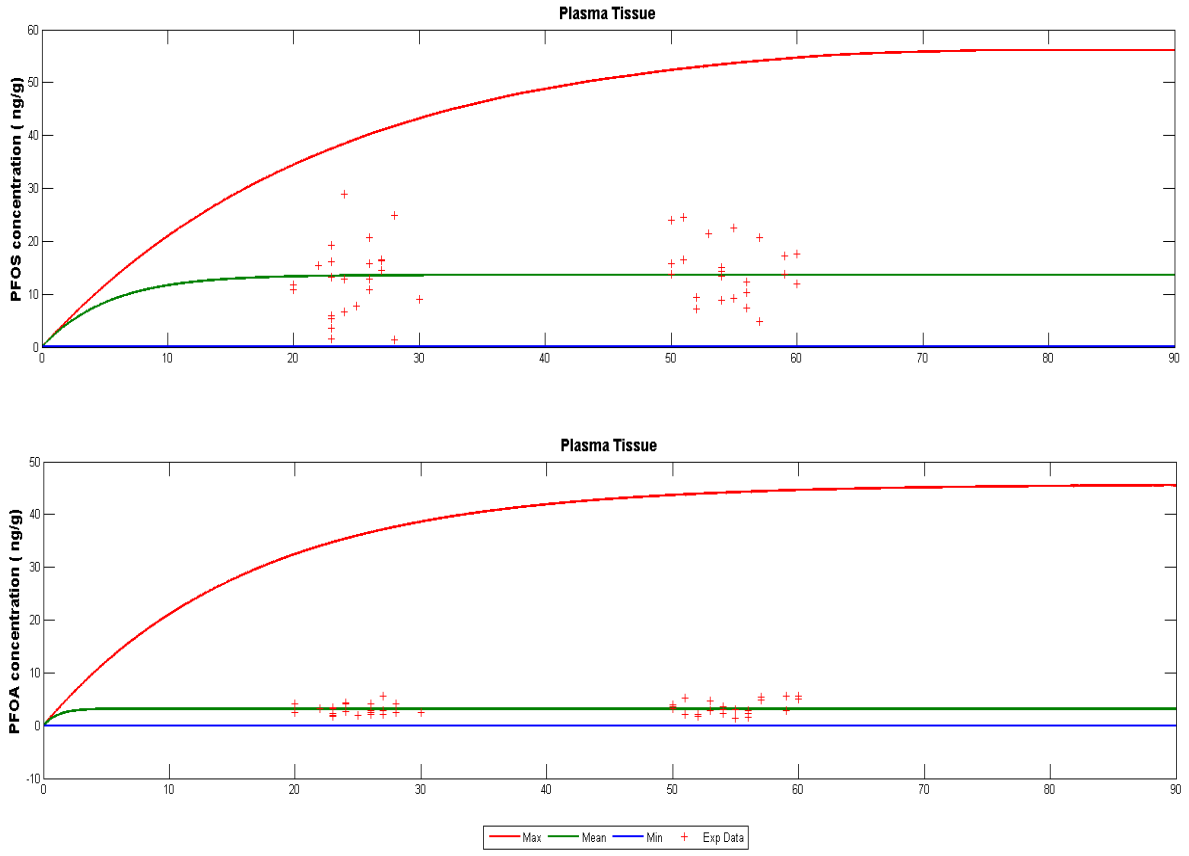


Fig. 2. Time course concentration of PFOS and PFOA in plasma. Simulated concentrations (mean, minimum and maximum) vs measured concentrations of PFOS and PFOA.

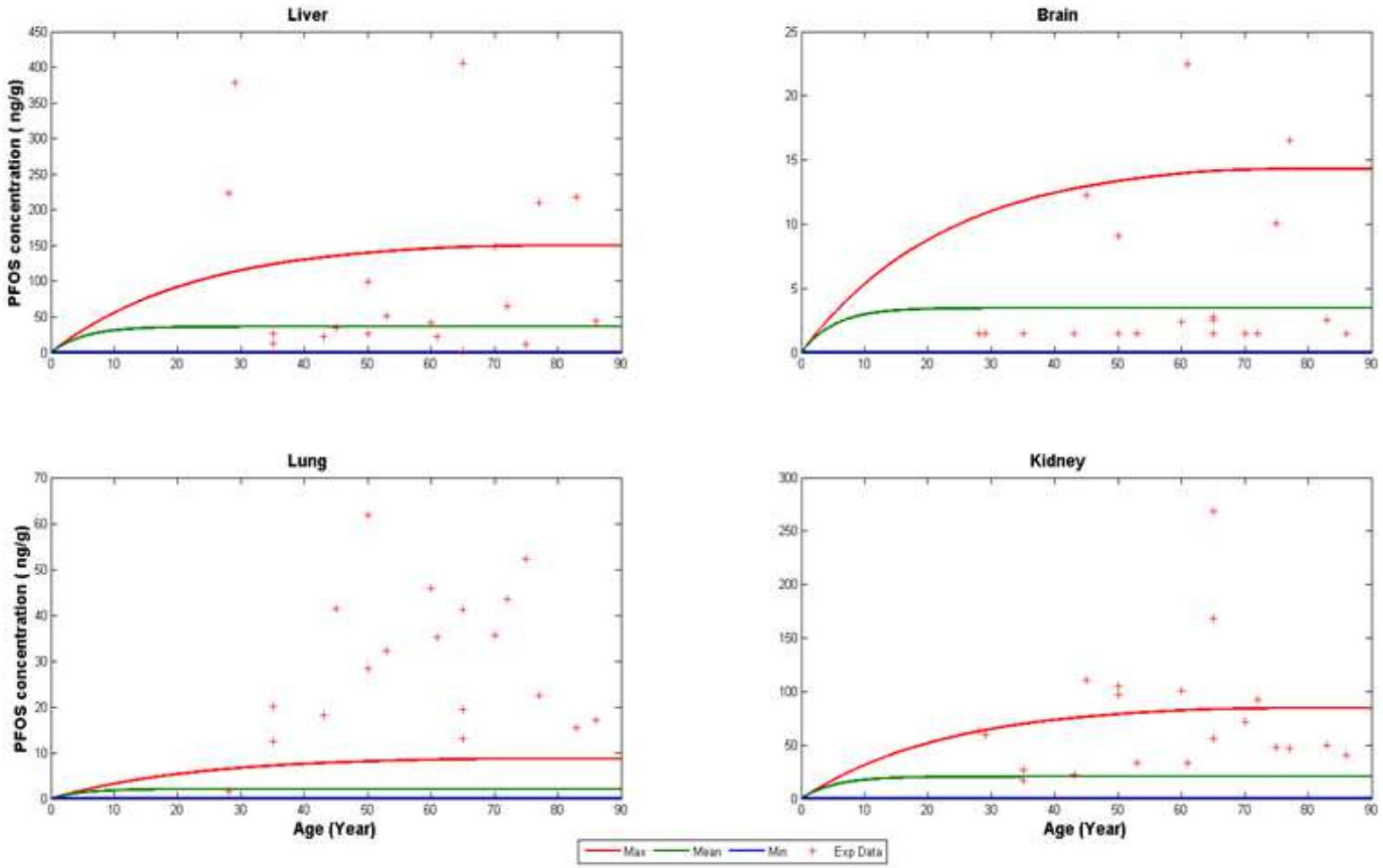


Fig. 3. Time course concentration of PFOS in liver, brain, lung and kidney. Simulated concentrations (mean, minimum and maximum) vs measured concentrations of PFOS.

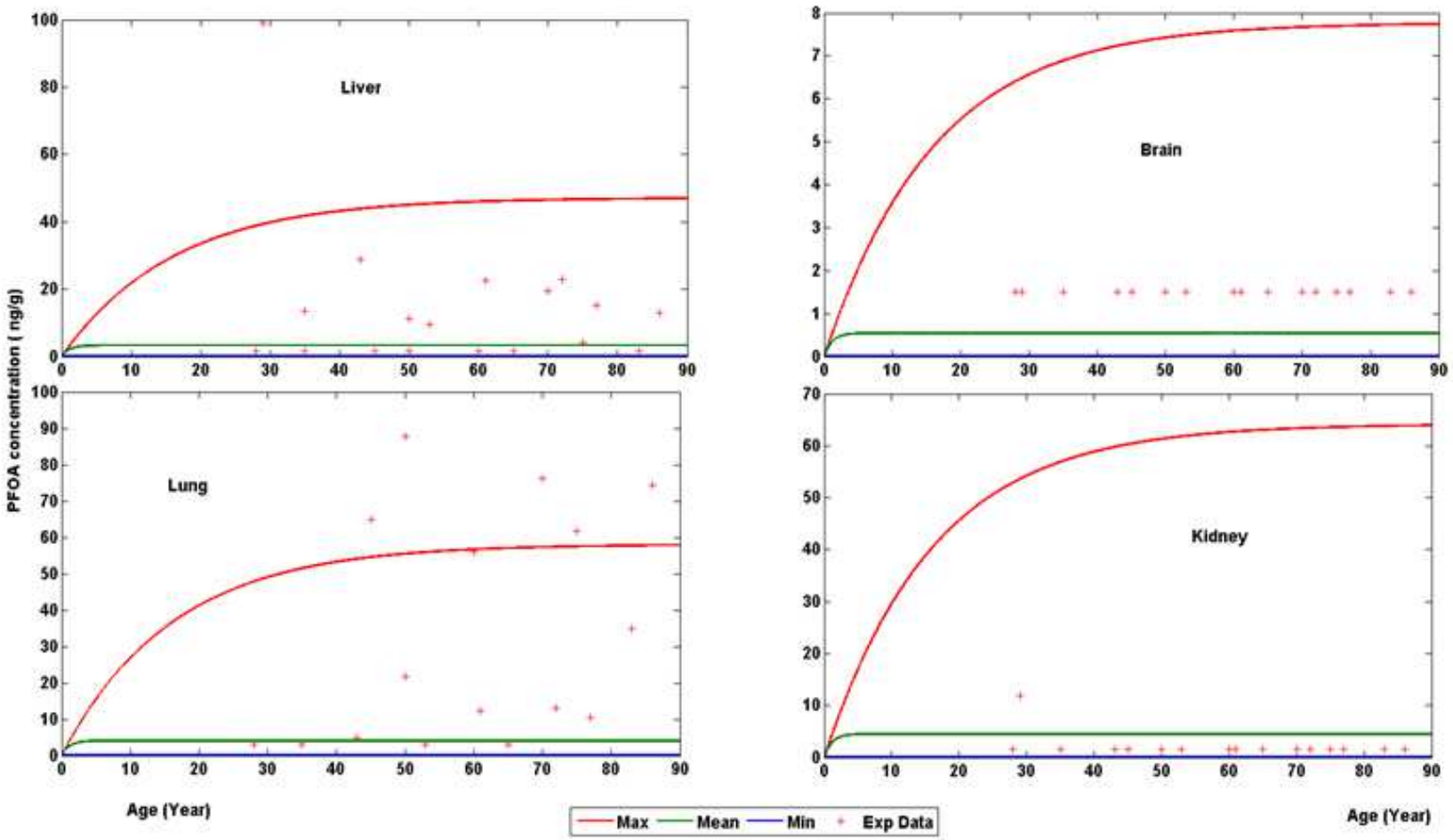


Fig. 4. Time course concentration of PFOA in liver, brain, lung and kidney. Simulated concentrations (mean, minimum and maximum) vs measured concentrations of PFOA.

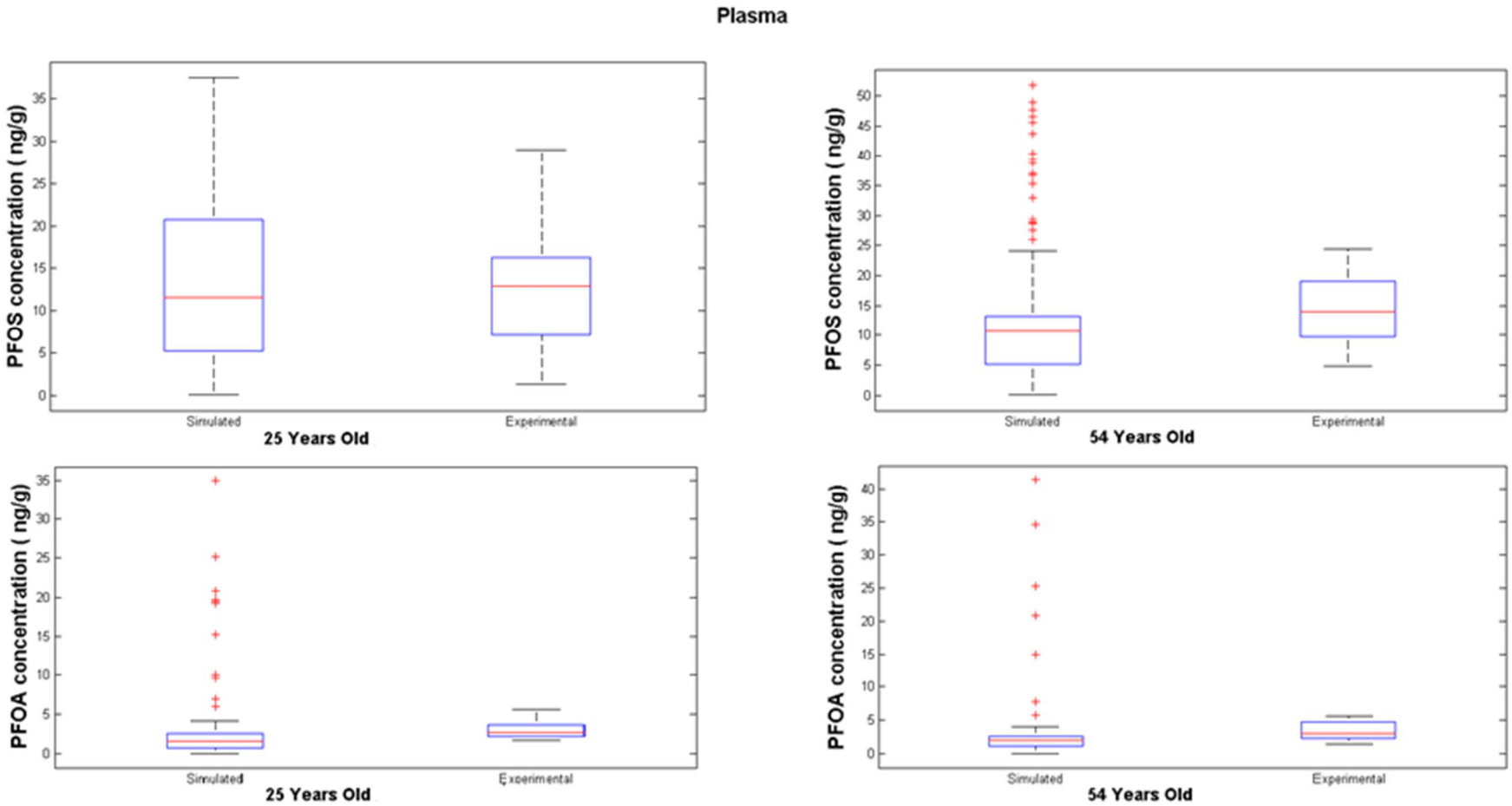


Fig. 5. Simulated vs experimental concentrations of PFOS and PFOA in plasma from two population groups, whose mean age is 25 and 54 years old.

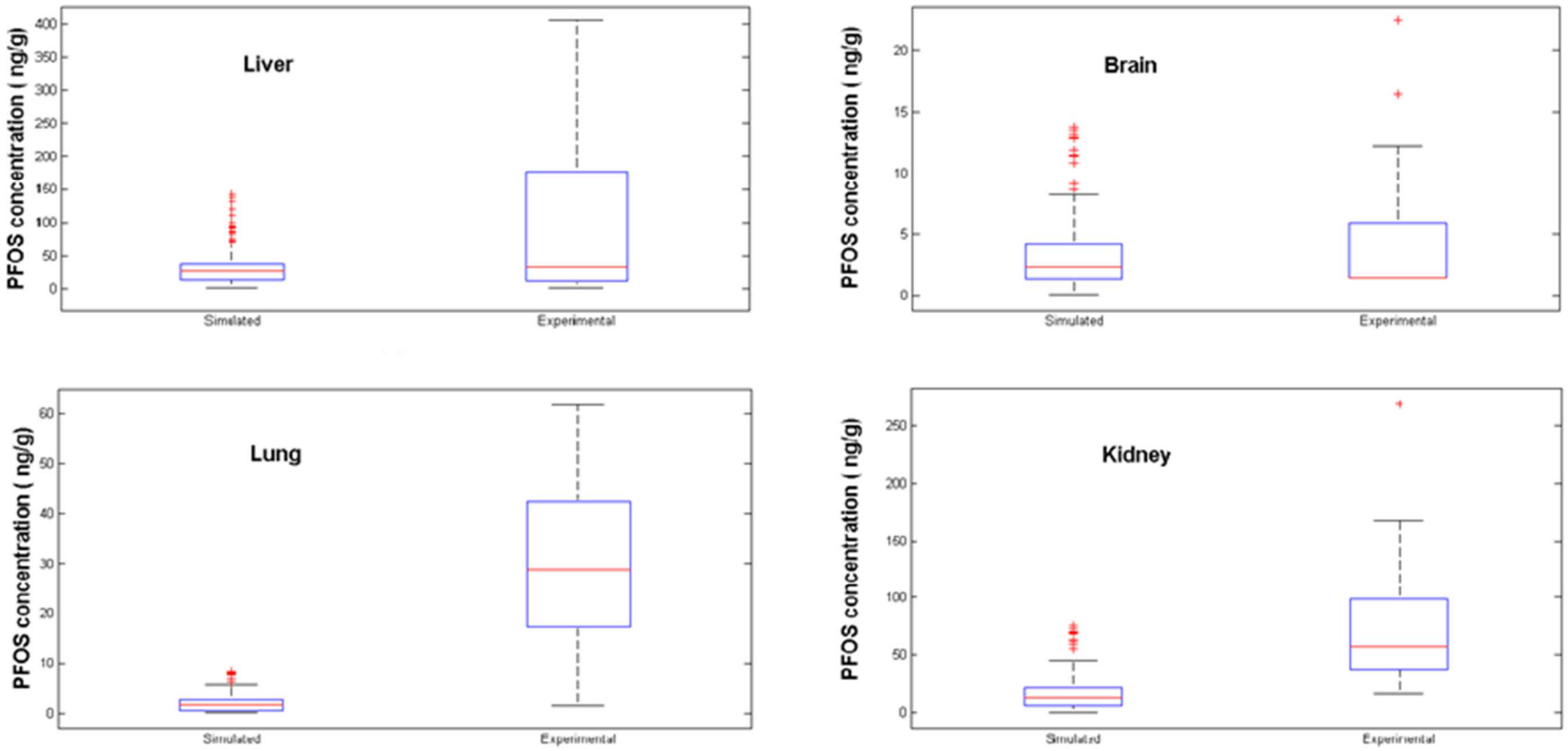


Fig. 6. Simulated vs experimental concentrations of PFOS in liver, brain, lung and kidney.

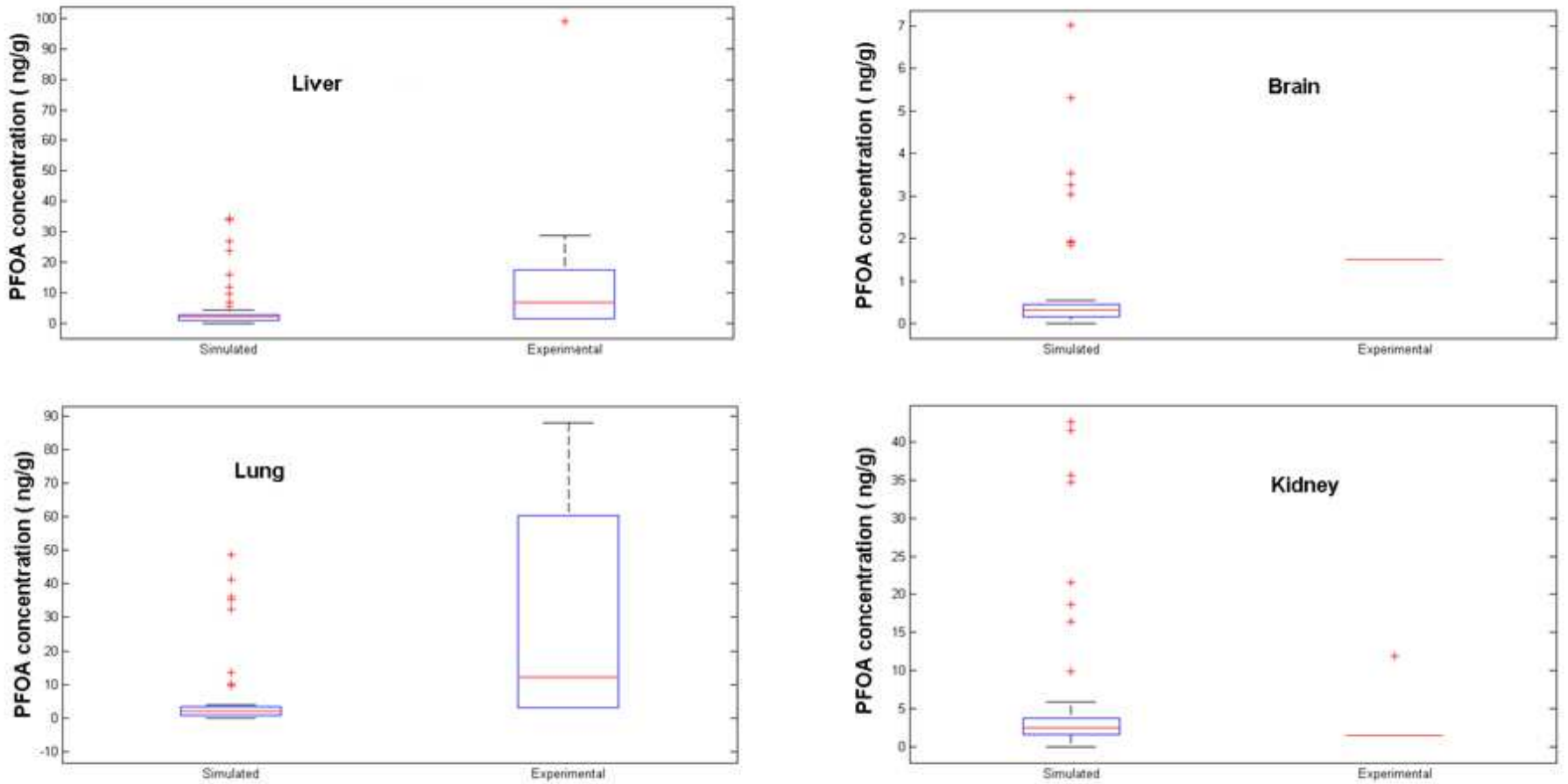


Fig. 7. Simulated vs experimental concentration of PFOA in liver, brain, lung and kidney.

Regarding the elimination process, the behaviour of the parameters Tm and Kt is analogous to the metabolic reaction of the Michaelis-Menten (Kou et al., 2005). Tm is the maximum amount of PFOS or PFOA that can be resorbed back from the urine to plasma compartment, being analogous to the maximum velocity of reaction (Vmax) in the Michaelis-Menten reactions. In turn, Kt is obtained when Vmax is equal to Vmax/2, and it is analogous to the Michaelis-Menten constant (Km). When Tm increases, the final values of concentration in tissues also increase. In contrast, when Kt increases the concentration in tissues decreases, because the increasing of Kt generates a decreasing of the slope of the trend, and subsequently the final result when the simulation converges. For Free fraction, the increasing of the parameter generates a decreasing of the final outcomes because the amount of free fraction is proportional to the tissue concentration. For the intake, the bigger the value the bigger the final concentration found in human tissues, because a higher amount of chemicals enter the human body.

3.3. Model validation

3.3.1 Visual validation

The most usual process of model validation in PBPK models is the visual comparison of simulation results and experimental values (Chiu et al., 2007). The simulation of the mean, minimum and maximum was conducted in plasma, liver, brain, lung and kidney for PFOS and PFOA, and compared with the experimental values found in Tarragona County (Ericson et al., 2007; Pérez et al., 2013). The comparison of experimental data and the simulation trends is depicted in Figures 2, 3 and 4. The simulations were run for 90 years that is the maximum age of the experimental data available. All the simulations trends followed a hyperbolic profile similar to the Michaelis-Menten saturation curve (Kou et al., 2005), that reach the convergence when the age of the population is close to 60 years old. The results for the most of the simulations were that the simulation trend was in between the data of experimental results. The differences between the minimum and maximum values of the experimental results were statistically significant. For PFOS, the minimum and maximum concentrations in the steady state ranged 3.0-405, 1.5-23.0, 3.0-61.8 and 6.0-269 ng/g in liver, brain, lung and kidney, respectively. For PFOA, the minimum and maximum concentration in the steady state ranged 3.0-99, 6.0-88 and 3.0-12 for liver, brain and kidney, respectively. The ranges found in experimental data imply that the range of simulation results would be bigger than the experimental ranges and thus a visual validation can be established. For plasma, all the experimental results were between the maximum and minimum for PFOS

and PFOA (Figure 2 and 5). The most part of the experimental results found in plasma were close to the median. For PFOA, the distribution of the experimental results in plasma was closer to the median in comparison to PFOS, where the dispersion was higher. In liver, all the experimental PFOA values were in the range of minimum and maximum concentration. In contrast, few outliers were found for PFOA, being out of the simulation range. In brain, all the experimental data were in the range for PFOA, but in PFOS two experimental points were out of simulation range. In lung, for PFOA five experimental points were out of the range and for PFOS all the experimental points were out of the range with only one exception. Finally, in kidney, for PFOA all the experimental points were in the range, but for PFOS seven experimental points were out of the range. Over all, visual inspections establish that the simulations results for PFOA were better than the simulations results for PFOS, thus validating the PBPK model.

Furthermore, the box plot of experimental data and simulation results (in the steady state) is depicted in Figures 6 and 7. Two population groups according to age were studied: adults from 20 to 30 years old (average: 25 years old), and people aging from 50 to 60 years old (average: 54 years old). Many outliers were found in the simulation box plot, but the simulated and experimental box plot were very coincident in the ranges. Regarding the other tissues, the box plot showed a bigger dispersion of the results in the experimental tissue in comparison to the simulation. The most notably dispersion was in lungs and kidney, for PFOS, and in lung, for PFOA. The analysis of the box plot is in agreement with the simulation trends and improves the visual analysis of the results.

However, the validation of the model depends also on the range of the simulation results. In a first step, the simulations generated by the LHS were not converging, and the concentration of PFOS and PFOA were out of the range. Afterwards, the values of Tm, Kt and Free were recalibrated to get the convergence of the simulations, obtaining its minimum and maximum values (Table 2). The values of Tm, Kt and Free were lower than those generated with the LHS and, therefore, the final simulations of PFOS and PFOA may be underestimated. Lung and kidney were the tissues with more experimental points out of the range, and the model may be considered useless to simulate PFOS in these of PFOS tissues. This fact evidences the necessity to include the uncertainty of the parameters for the evaluation of the validity of the PBPK models. The validity of the model in lungs, for PFOS and PFOA, and kidney, for PFOS, should be reconsidered.

Concentration of PFOS and PFOA in tissues depends highly on Pk values. The Pks were previously assessed using data found in human tissues and plasma (Fàbrega

et al., 2014; Maestri et al., 2006). Whereas the simulated concentration in plasma was fitted with the experimental values for both PFOS and in PFOA, the simulation concentration found in some tissues were far from the experimental value. This fact demonstrated that these Pks should be better estimated using better experimental data. Mathematical algorithms used to provide a good estimation tool for the assessment of Pks in environmental contaminants, but for PFOS and PFOA the behaviour of these compounds makes it difficult to estimate due to the ambiphilic structure of PFASs (Peyret et al., 2010). Thus, the only way to estimate the Pks in PFOS and PFOA is the use of experimental data. Due to the relatively good fitting between simulation and experimental results in plasma, we can assume that one of the main factors responsible of the results obtained in tissues others than plasma were the PKs, besides of the parametric uncertainty.

3.3.2. Statistical validation

To assure the statistical validity of the final results, a student's *t*-test was performed. Data are summarized in Tables 3 and 4. Plasma levels corresponding to two age groups were compared (25 and 45 years old). Probabilities (p values) ranged from 0.69 to 0.86 for PFOS and PFOS, assuming equal variance. In consequence, there was no significant difference between experimental and simulated results. Assuming unequal variance, p values ranged from 0.53 to 0.73, and no significant differences were proved between experimental and simulation results. In other tissues, the validity of the model depends on the α value. For α=1% the results had significant differences for PFOA in brain and lung, and for PFOS in lung and kidney. Assuming α=5% significant differences were observed in liver, brain and lung in PFOA and in liver, lung and kidney in PFOS. The PBPK model is validated to simulate plasma, but in other tissues the model do not successfully simulates the concentration of PFOS and PFOA. The process of model validation differed depending on the fact of using visual analysis or statistical analysis for model validation. For visual analysis, all the simulation should be considered as a valid, since most experimental values fell in simulation ranges. In contrast, statistical analysis test statistical significance of two groups of data with clear set of criteria and avoid human biases in judgement. Althought, visual analysis are predominant in PBPK modeling, statistical analysis should be incorporated in the model validation to avoid biases in the visual validation.

Table 3: Two sample *t*- test of simulated and experimental concentration of PFOS and PFOA in plasma tissue for different age groups, assuming equal and unequal variances.

Age Group		Equal variances			Unequal variances		
		$H_{\alpha = 1\%}$	$H_{\alpha = 5\%}$	P	$H_{\alpha = 1\%}$	$H_{\alpha = 5\%}$	P
PFOS	25 years old	0	0	0.69	0	0	0.61
	54 years old	0	0	0.69	0	0	0.53
PFOA	25 years old	0	0	0.86	0	0	0.72
	54 years old	0	0	0.85	0	0	0.73

Table 4: Two sample *t*- test of simulated and experimental concentration of PFOS and PFOA.

	PFOS			PFOA		
Tissue	$H_{\alpha = 1\%}$	$H_{\alpha = 5\%}$	P	$H_{\alpha = 1\%}$	$H_{\alpha = 5\%}$	P
Liver	0	1	0.027	0	1	0.05
Brain	0	0	0.34	1	1	1.56E-15
Lung	1	1	5.42E-07	1	1	0.003
Kidney	1	1	5.23E-04	0	0	0.162

4. Conclusions

The study of the uncertainty depends on the parametric data and the structure of the model. Thus, the study of the parametric uncertainty is model-dependent. Consequently, the conclusions are also model-dependent and cannot be extrapolated to all the PBPK models. The result of this study may help PBPK modelers to identify the most uncertain parameters and the influence of the uncertainty in PBPK modelling. The parameters studied were Tm, Kt, free and intake, since they had the highest contribution according to the SA. In fact, the SA contribution onto the variance of these four parameters was 82.6, for PFOS, and 99.9%, for PFOA. A greater confidence in uncertainty and variability analyses would result if parameter distributions and correlations among parameters etc. are based on the best available biological understanding of the systems. Therefore, the real uncertainty associated to the PBPK parameters may be underestimated. The validation of the PBPK model was performed visually and using statistical analysis (*t*-test). For plasma the model outcomes were considered as a validated for PFOS and PFOA, either by using visual analysis or the *t*-test. In contrast, the visual validation of the PBPK model appeared to be suitable for the most part of the scenarios, but the statistical analysis showed that for many scenarios was not valid. The tissue simulations were not considered as valid for lungs and kidney in PFOS, and for brain and lungs in PFOA, according to the student's *t*-test. Statistical

analysis should to be included in the validation process of PBPK modeling to avoid biases in the visual validation. Pk is the responsible of the distribution of PFOS and PFOA to tissues other than plasma. To adjust the simulations, more accurate Pks values should be found by using better experimental data.

References

Allen, B.C., Covington, T.R., Clewell, H.J., 1996. Investigation of the impact of pharmacokinetic variability and uncertainty on risks predicted with a pharmacokinetic model for chloroform. Toxicology 111, 289-303.

Andersen, M.E., Clewell III, H.J., Tan, Y.M., Butenhoff, J.L.. Olsen, G.W., 2006. Pharmacokinetic modeling of saturable, renal resorption of perfluoroalkylacids in monkeys--probing the determinants of long plasma half-lives. Toxicology 227, 156-164.

Bischel, H.N., Macmanus-Spencer, L.A.. Luthy, R.G., 2010. Noncovalent interactions of long-chain perfluoroalkyl acids with serum albumin. Environmental Sciences & Technology 44, 5263-5269.

Bischoff, K.B., Dedrick, R.L.. Zaharko, D.S., 1970. Preliminary model for methotrexate pharmacokinetics. Journal of Pharmaceutical Sciences 59, 149-154.

Bois, F.Y., Jamei, M.. Clewell, H.J., 2010a. PBPK modelling of inter-individual variability in the pharmacokinetics of environmental chemicals. Toxicology 278, 256-267.

Bois, F.Y., Jamei, M.. Clewell, H.J., 2010b. PBPK modelling of inter-individual variability in the pharmacokinetics of environmental chemicals. Toxicology 278, 256-267.

Brochot, C., Smith, T.J.. Bois, F.Y., 2007. Development of a physiologically based toxicokinetic model for butadiene and four major metabolites in humans: global sensitivity analysis for experimental design issues. Chemico-Biological Interactions 167, 168-183.

Brown, R.P., Delp, M.D., Lindstedt, S.L., Rhomberg, L.R.. Beliles, R.P., 1997. Physiological parameter values for physiologically based pharmacokinetic models. Toxicological & Industrial Health 13, 407-484.

Chen, Y.-M., Guo, L.-H., 2009. Fluorescence study on site-specific binding of perfluoroalkyl acids to human serum albumin. Archives of Toxicology 83, 255-261.

Chiu, W.A., Barton, H.A., DeWoskin, R.S., Schlosser, P., Thompson, C.M., Sonawane, B., Lipscomb, J.C., Krishnan, K., 2007. Evaluation of physiologically based pharmacokinetic models for use in risk assessment. Journal of Applied Toxicology 27, 218-237.

Chiu, W.A., Okino, M.S., Evans, M.V., 2009. Characterizing uncertainty and population variability in the toxicokinetics of trichloroethylene and metabolites in mice, rats, and humans using an updated database, physiologically based pharmacokinetic (PBPK) model, and Bayesian approach. Toxicology & Applied Pharmacology 241, 36-60.

Domingo, J.L., Ericson-Jogsten, I., Perello, G., Nadal, M., Van, B.B., Karrman, A., 2012a. Human exposure to perfluorinated compounds in Catalonia, Spain: contribution of drinking water and fish and shellfish. Journal of Agricultural & Food Chemistry 60, 4408-4415.

Domingo, J.L., Jogsten, I.E., Eriksson, U., Martorell, I., Perello, G., Nadal, M., Bavel, B., 2012b. Human dietary exposure to perfluoroalkyl substances in Catalonia, Spain. Temporal trend. Food Chemistry 135, 1575-1582.

Ericson, I., Gómez, M., Nadal, M., van Bavel, B., Lindström, G.. Domingo, J.L., 2007. Perfluorinated chemicals in blood of residents in Catalonia (Spain) in relation to age and gender: A pilot study. Environment International 33, 616-623.

Ericson, J.I., Nadal, M., van, B.B., Lindstrom, G., Domingo, J.L., 2012. Per- and polyfluorinated compounds (PFCs) in house dust and indoor air in Catalonia, Spain: implications for human exposure. Environment International 39, 172-180.

Evans, M.V., Eklund, C.R., 2001. A graphical application of sensitivity analysis for gas uptake experiments using chloroform as an example. Toxicology Methods 11, 285-297.

Fàbrega, F., Kumar, V., Schuhmacher, M., Domingo, J.L.. Nadal, M., 2014. PBPK modeling for PFOS and PFOA: Validation with human experimental data. Toxicology Letters S0378-4274, 00017-14.

Gueorguieva, I., Nestorov, I.A., Aarons, L., Rowland, M., 2005. Uncertainty analysis in pharmacokinetics and pharmacodynamics: Application to naratriptan. Pharmacological Research 22, 1614-1626.

Hack, C.E., 2006. Bayesian analysis of physiologically based toxicokinetic and toxicodynamic models. Toxicology 221, 241-248.

Hamby, D.M., 1994. A review of techniques for parameter sensitivity analysis of environmental models. Environmental Monitoring & Assessment 32, 135-154.

Han, X., Snow, T.A., Kemper, R.A.. Jepson, G.W., 2003. Binding of perfluorooctanoic acid to rat and human plasma proteins. Chemical Research in Toxicology 16, 775-781.

Huizer, D., Oldenkamp, R., Ragas, A.M., van, R.J.G.. Huijbregts, M.A., 2012. Separating uncertainty and physiological variability in human PBPK modelling: The example of 2-propanol and its metabolite acetone. Toxicology Letters 214, 154-165.

Jones, H.M., Rowland-Yeo, K., 2013. Basic Concepts in Physiologically Based Pharmacokinetic Modeling in Drug Discovery and Development. CPT: Pharmacometrics and Systems Pharmacology 2, e63.

Karrman, A., Domingo, J.L., Llebaria, X., Nadal, M., Bigas, E., van, B.B., Lindstrom, G., 2010. Biomonitoring perfluorinated compounds in Catalonia, Spain: concentrations and trends in human liver and milk samples. Environmental Sciences & Pollution Research International 17, 750-758.

Kou, S.C., Cherayil, B.J., Min, W., English, B.P., Xie, X.S., 2005. Single-Molecule Michaelis−Menten Equations. The Journal of Physical Chemistry B 109, 19068-19081.

Kumar, V., Mari, M., Schuhmacher, M., Domingo, J.L., 2009. Partitioning total variance in risk assessment: Application to a municipal solid waste incinerator. Environmental Modeling & Software 24, 247-261.

Loccisano, A.E., Campbell Jr, J.L., Andersen, M.E.. Clewell III, H.J., 2011. Evaluation and prediction of pharmacokinetics of PFOA and PFOS in the monkey and human using a PBPK model. Regulatory Toxicology & Pharmacology 59, 157-175.

Loizou, G., McNally, K., 2010. Sensitivity analysis of PBPK models. Toxicology Letters 196, S98-S98.

Maestri, L., Negri, S., Ferrari, M., Ghittori, S., Fabris, F., Danesino, P., Imbriani, M., 2006. Determination of perfluorooctanoic acid and perfluorooctanesulfonate in human tissues by liquid chromatography/single quadrupole mass spectrometry. Rapid Communications in Mass Spectrometry 20, 2728-2734.

McKay, M.D., Beckman, R.J., Conover, W.J., 1979. Comparison of three methods for selecting values of input variables in tha analysis of outpur from a computer code. Technometrics 21, 239-245.

McNally, K., Cotton, R., Loizou, G.D., 2011. A Workflow for Global Sensitivity Analysis of PBPK Models. Frontiers in pharmacology 2, 31.

Mörk, A.K., Jonsson, F., Johanson, G., 2009. Bayesian population analysis of a washin-washout physiologically based pharmacokinetic model for acetone. Toxicology & Applied Pharmacology 240, 423-432.

Mumtaz, M., Fisher, J., Blount, B., Ruiz, P., 2012. Application of physiologically based pharmacokinetic models in chemical risk assessment. Journal of Toxicology 2012.

Nestorov, I., 2007. Whole-body physiologically based pharmacokinetic models. Expert Opinion on Drug Metabolism & Toxicology 3, 235-249.

Pérez, F., Nadal, M., Navarro-Ortega, A., Fàbrega, F., Domingo, J.L., Barceló, D.. Farré, M., 2013. Accumulation of perfluoroalkyl substances in human tissues. Environment International 59, 354-362.

Peyret, T., Poulin, P., Krishnan, K., 2010. A unified algorithm for predicting partition coefficients for PBPK modeling of drugs and environmental chemicals. Toxicology & Applied Pharmacology 249, 197-207.

Rowland, M., Peck, C., Tucker, G., 2011. Physiologically-based pharmacokinetics in drug development and regulatory science. Annual Review of Pharmacology & Toxicology 51, 45-73.

Snedecor, G.W.. Cochran, W.G., 1989. Statistical Methods. Iowa State University Press.

Sweeney, L.M., Tyler, T.R., Kirman, C.R., Corley, R.A., Reitz, R.H., Paustenbach, D.J., Holson, J.F., Whorton, M.D., Thompson, K.M., Gargas, M.L., 2001. Proposed occupational exposure limits for select ethylene glycol ethers using PBPK models and Monte Carlo simulations. Toxicological Sciences 62, 124-139.

Tan, Y.M., Clewell III, H.J., Andersen, M.E., 2008. Time dependencies in perfluorooctylacids disposition in rat and monkeys: a kinetic analysis. Toxicology Letters 177, 38-47.

Teorell, T., 1937. Kinetics of distribution of substances administered to the body. Archives Internationales de Pharmacodynamie et de Therapie 57, 205-240.

Thompson, M.D., Beard, D.A., 2011. Development of appropriate equations for physiologically based pharmacokinetic modeling of permeability-limited and flow-limited transport. Journal of Pharmacokinetics & Pharmacodynamics 38, 405-421.

Thompson, M.D., Beard, D.A., 2012. Physiologically based pharmacokinetic tissue compartment model selection in drug development and risk assessment. Journal of Pharmacological Sciences 101, 424-435.

US EPA, 2011. Exposure factors handbook. In: EPA/600/R-09/052F. US Environmental Protection Agency, Washington, DC.

WHO., 2010. International Programme on Chemical Safety Harmonization Project. Characterization and Application of Physiologically Based Pharmacokinetic Models in Risk Assessment. Harmonization Project Document No. 9. World Health Organization, Geneva, Switzerland; 2010.

Woodruff, T.J., Bois, F.Y., Auslander, D., Spear, R.C., 1992. Structure and parameterization of pharmacokinetic models: their impact on model predictions. Risk Analysis 12, 189-201.

Discussion of Chapter VI

The uncertainty associated to the PBPK model was studied to the case-study of PFOS and PFOA. The visual validation of the model suggests that the PBPK model was able to simulate very precisely PFOS and PFOA in plasma, liver, kidney, brain and lungs. However, a deep statistical treatment, based on the Student's t-test, revealed that models may not be fully validated. In general terms, there was a good fitting for plasma, while the statistical validation for the rest of the tissues was not so successful. Assuming a level of significance of the 1% (α=1%), significant differences were noted for PFOA in brain and lungs, and PFOS in lung and kidney. Assuming α=5%, significant differences were observed for PFOA in liver, brain and lung, and for PFOS in liver, lung and kidney. This means that the PBPK model can perfectly predict the concentration in plasma, but there is more uncertainty regarding its suitability for other body compartments. In liver, which is a target organ for both chemicals, the PBPK model may be considered as valid according to the visual validation, but not validated according to the statistical analysis. This uncertainties could be linked to the Pks, whose values were obtained by using experimental data coming from only 20 autopsy individuals. To adjust the simulations results, more accurete Pks should be found by using better experimental data.

In conclusion, it is here confirmed that the visual study of the PBPK model outcomes must be complemented with some statistical treatment for a correct model validation. Although the statistic validation of PBPK models has been poorly used in the past, our results indicate that this should be an extended practice to avoid visual biases.

GENERAL DISCUSSION

PCDD/Fs and PFASs are widely spread environmental contaminants. They are highly persistent in the environment and bioaccumulative in living organisms. Moreover, these pollutants are very toxic for human health, while they own a long-range transport capacity. PCDD/Fs were originally included in the Stockholm Convention as POPs, while PFOS was also added in 2009. In addition PFOA is a serious candidate to enter that list. Consequently, the human health risk associated to these two families of pollutants need to be carefully assessed.

In silico techniques are receiving an important attention as alternative methods to classical toxicological methods. PBPK models may improve the assessment of human health risk, and therefore there is a need of reliable PBPK models. The use of PBPK models, as techniques to estimate the internal dose to environmental pollutants, has been incipient in the past. Even more, approaches on PCDD/Fs and PFASs are particularly scarce. Previous studies have been mainly focused on specific PCDD/F congeners, on one hand, and only on PFOS and PFOA, on the other one. PBPK models may improve the assessment of health risks by predicting the time-course concentration of pollutants in human tissues. The use of PBPK in human health risk assessment allows extrapolating the cross-species, cross-route, the dose and the time of the predictions, without the need of experimental analysis.

The main objective of this thesis was to demonstrate the capability of PBPK models to estimate the body burdens of mixtures of environmental pollutants. The present work was focused on the development of a PBPK model to assess the time course concentration of PCDD/Fs and PFASs in human tissues, as a tool of potential use in health risk assessment.

In the process of PBPK model development, PCDD/Fs were chosen as classic POPs, while there are also extensive PK data available in the scientific literature. Moreover, PCDD/Fs have a relatively simply PK for its high absorption, since they are not metabolized in humans and whose elimination mechanism takes place mainly through bile. On the other hand, PFASs were also selected as representative of “new” POPs. In order to prioritize chemicals, a SOM-based hazard index was here developed. PFASs are toxic chemicals whose hazard was compared to other pollutants, including pharmaceuticals, pesticides, and endocrine disruptors among others. In addition to PCDD/Fs, the PBPK model was applied to PFOS and PFOA, being the most well known

PFASs in the research literature. After that, the model was also extended to PFASs other than PFOS and PFOA, for the very first time.

Extrapolation of the PBPK model to other groups of environmental pollutants is subject to PK/PD knowledge. For chemicals with similar PK/PD characteristics to PCDD/Fs and PFASs, the PBPK model may be extended by changing the parametric data (basically the Pks and elimination constants) and slightly modifying the mechanism of elimination and/or metabolism. Thus, POPs like polychlorinated biphenyls (PCBs) or polybrominated diphenyl ethers (PBDEs) are good candidates to be modelled by applying the present PBPK model. However, the robustness and accuracy of PBPK models should be improved in the future. For instance, temporal dynamics of exposure concentration and physiological parameters should be included to consider the long-term exposure to POPs. Furthermore, taking into account toxicity of POPs and related substances in the neonate development, PBPK models could incorporate the mammary gland in the model structure in order to assess the transfer of the chemicals. On the other hand, since humans are really exposed to chemical mixtures, future PBPK modeling studies should incorporate possible interactions among the chemical compounds.

The simulations of both PCDD/Fs and PFASs were, in general terms, reasonably coincident with experimental results. Moreover, the uncertainty in the PBPK model was incorporated to improve the process of model validation. The PBPK model developed in the present thesis has been demonstrated to be a reliable tool to simulate the levels of PCDD/Fs and PFASs in human tissues. PBPK models are not only a promising tool, but also a reality to simulate the concentrations of environmental pollutants in human tissues, for their subsequent use in human health risk assessment.

CONCLUSIONS

General conclusion

The PBPK model here developed has proven to be a reliable tool to simulate 17 PCDD/F congeners and 11 PFASs in human tissues. The model was successfully validated for PCDD/Fs in adipose tissue for the first time, being this the target tissue where PCDD/Fs accumulate. Moreover, the model was simulated for PFOS and PFOA as well as other PFASs, elucidating its mechanism of elimination. Finally, the statistical analysis carried out in the PBPK model for PFOS and PFOA demonstrates that statistical analysis of the final outcomes is a necessary practice to be integrated in PBPK modeling to ensure the reliability of the simulations.

Specific conclusions

1) A SOM-based hazard index (HI) have been able to create a ranking of environmental chemicals based on their PBT properties. The pollutants studied using HI were 205 pollutants, including pharmaceutic compounds, pesticides, illicit drugs, PFASs, endocrine disruptors and UV filters. According to the HI outcomes, PFASs are among the most hazardous environmental pollutants for human health.

2) IRICAP may assess the human health risk associated to the exposure of chemical mixtures and its aggregate effect. Although chemical interactions have not been considered in the IRICAP, the ranking should be able to prioritize chemical mixtures in river water, being extensible to other environmental compounds.

3) PBPK models have proven to be a reliable tool to assess the concentration of PCDD/Fs in plasma and adipose tissue. Values from PBPK model simulations and experimental results of PCDD/Fs in plasma and adipose tissue were very similar.

4) PBPK models are a feasible tool to reduce the cost of biological surveillance studies by minimizing the number of biological samples (e.g., blood and adipose tissue) where PCDD/Fs must be analyzed. They also allow estimating the long-term accumulation of PCDD/Fs in human tissues, highlighting adipose tissue as the target tissue where PCDD/Fs mainly accumulate.

5) For the first time, a generic PBPK model was developed for PFASs. The structure of the PBPK model simulated a mechanism of urinary elimination with a mechanism of resorption of PFASs from urine to plasma, and a strong protein binding for all the compounds. According to data from the population in Tarragona County (Spain), the model was able to successfully simulate the concentration of PFASs in plasma. Although it is not the definitive proof of the pharmacokinetics of the PFASs under study, it demonstrates that this mechanism is able to simulate PFASs in plasma.

6) The PBPK model for PFASs was validated in a case study in Norway, where experimental data of 5 PFASs in blood were available for the PFAS exposure for general population. The model was validated for PFHxS, PFOS, PFHpA, PFOA and PFNA.

7) The visual and the statistical validation of the PBPK model differed for some tissues depending on the level of significance (α). The visual validation seemed to be sufficient for validation purposes, being the most largely extended method of PBPK validation. However, the statistical tests showed that the visual validation may not be enough to assure the reliability of the model.

REFERENCES

Alcock, R.E., Sweetman, A.J., Jones, K.C., 2001. A congener-specific PCDD/F emissions inventory for the UK: do current estimates account for the measured atmospheric burden? Chemosphere 43, 183-194.

Andersen, M.E., Birnbaum, L.S., Barton, H.A., Eklund, C.R., 1997. Regional hepatic CYP1A1 and CYP1A2 induction with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin evaluated with a multicompartment geometric model of hepatic zonation. Toxicology & Applied Pharmacology 144, 145-155.

Andersen, M.E., Clewell III, H.J., Tan, Y.M., Butenhoff, J.L., Olsen, G.W., 2006. Pharmacokinetic modeling of saturable, renal resorption of perfluoroalkylacids in monkeys--probing the determinants of long plasma half-lives. Toxicology 227, 156-164.

Andersen, M.E., Mills, J.J., Gargas, M.L., Kedderis, L., Birnbaum, L.S., Neubert, D., Greenlee, W.F., 1993. Modeling receptor-mediated processes with dioxin: Implications for pharmacokinetics and risk assessment. Risk Analysis 13, 25-36.

Apelberg, B.J., Witter, F.R., Herbstman, J.B., Calafat, A.M., Halden, R.U., Needham, L.L., Goldman, L.R., 2007. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environmental Health Perspectives 115, 1670-1676.

ATSDR, 1998. Agency of Toxic Substances and Desease Registry (1998) Toxicological profile for chlorinated dibenzo-*p*-dioxins. U.S. Department of health and human services. Public health service.

Austin, M.E., Kasturi, B.S., Barber, M., Kannan, K., MohanKumar, P.S., MohanKumar, S.M., 2003. Neuroendocrine effects of perfluorooctane sulfonate in rats. Environmental Health Perspectives 111, 1485-1489.

Baccarelli, A., Giacomini, S.M., Corbetta, C., Landi, M.T., Bonzini, M., Consonni, D., Grillo, P., Patterson Jr, D.G., Pesatori, A.C., Bertazzi, P.A., 2008. Neonatal thyroid function in Seveso 25 years after maternal exposure to dioxin. PLoS Medicine 5, e161.

Bakhtyari, N., Baderna, D., Boriani, E., Schuhmacher, M., Heise, S., Benfenati, E., 2013. Toxicological and ecotoxicological studies for additives. In: Bilitewski, B., Darbra, R.M., Barceló, D. (Eds.) Global Risk-Based Management of Chemical Additives II, vol. 23. Springer Berlin Heidelberg, pp. 73-89.

Barry, V., Winquist, A., Steenland, K., 2013. Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environmental Health Perspectives 121, 1313-1318.

Beaudouin, R., Micallef, S., Brochot, C., 2010. A stochastic whole-body physiologically based pharmacokinetic model to assess the impact of inter-individual variability on tissue dosimetry over the human lifespan. Regulatory Toxicology and Pharmacology 57, 103-116.

Bischoff, K.B., Dedrick, R.L., Zaharko, D.S., Longstreth, J.A., 1971. Methotrexate pharmacokinetics. Journal of Pharmaceutical Sciences 60, 1128-1133.

Boethling, R., Fenner, K., Howard, P., Klečka, G., Madsen, T., Snape, J.R., Whelan, M.J., 2009. Environmental persistence of organic pollutants: Guidance for development and review of POP risk profiles. Integrated Environmental Assessment & Management 5, 539-556.

Boffetta, P., Mundt, K.A., Adami, H.O., Cole, P., Mandel, J.S., 2011. TCDD and cancer: A critical review of epidemiologic studies. Critical Reviews in Toxicology 41, 622-636.

Bouvier, D.Y.M., Prieto, P., Blaauboer, B.J., Bois, F.Y., Boobis, A., Brochot, C., Coecke, S., Freidig, A., Gundert-Remy, U., Hartung, T., Jacobs, M.N., Lave, T., Leahy, D.E., Lennernas, H., Loizou, G.D., Meek, B., Pease, C., Rowland, M., Spendiff,

M., Yang, J.. Zeilmaker, M., 2007. Physiologically-based pharmacokinetic modelling (PBPK modelling): meeting the 3Rs agenda. The report and recommendations of ECVAM Workshop 63. Alternatives to Laboratory Animals 35, 661-671.

Brown, R.P., Delp, M.D., Lindstedt, S.L., Rhomberg, L.R., Beliles, R.P., 1997. Physiological parameter values for physiologically based pharmacokinetic models. Toxicology & Industrial Health 13, 407-484.

Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P., 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integrated Environmental Assessment & Management 7, 513-541.

Butenhoff, J.L., Kennedy Jr, G.L., Hinderliter, P.M., Lieder, P.H., Jung, R., Hansen, K.J., Gorman, G.S., Noker, P.E., Thomford, P.J., 2004. Pharmacokinetics of perfluorooctanoate in cynomolgus monkeys. Toxicological Sciences 82, 394-406.

Cahill, T.M., Cousins, I., Mackay, D., 2003. Development and application of a generalized physiologically based pharmacokinetic model for multiple environmental contaminants. Environmental Toxicology & Chemistry 22, 26-34.

Calafat, A.M., Wong, L.Y., Kuklenyik, Z., Reidy, J.A., Needham, L.L., 2007. Polyfluoroalkyl chemicals in the U.S. population: Data from the national health and nutrition examination survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. Environmental Health Perspectives 115, 1596-1602.

Carrier, G., Brunet, R.C., Brodeur, J., 1995a. Modeling of the toxicokinetics of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in mammalians, including humans. I. Nonlinear distribution of PCDD/PCDF body burden between liver and adipose tissues. Toxicology & Applied Pharmacology 131, 253-266.

Carrier, G., Brunet, R.C., Brodeur, J., 1995b. Modeling of the toxicokinetics of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in mammalians, including humans. II. Kinetics of absorption and disposition of PCDDs/PCDFs. Toxicology & Applied Pharmacology 131, 267-276.

Chan, J.K.Y., Wong, M.H., 2013. A review of environmental fate, body burdens, and human health risk assessment of PCDD/Fs at two typical electronic waste recycling sites in China. Science of The Total Environment 463-464, 1111-1123.

Chang, S.C., Noker, P.E., Gorman, G.S., Gibson, S.J., Hart, J.A., Ehresman, D.J., Butenhoff, J.L., 2012. Comparative pharmacokinetics of perfluorooctanesulfonate (PFOS) in rats, mice, and monkeys. Reproductive Toxicology 33, 428-440.

Clark, L.H., Setzer, R.W., Barton, H.A., 2004. Framework for evaluation of physiologically-based pharmacokinetic models for use in safety or risk assessment. Risk Analysis 24, 1697-1717.

Clewell III, H.J., Andersen, M.E., Barton, H.A., 2002. A consistent approach for the application of pharmacokinetic modeling in cancer and noncancer risk assessment. Environmental Health Perspectives 110, 85-93.

Cole, P., Trichopoulos, D., Pastides, H., Starr, T., Mandel, J.S., 2003. Dioxin and cancer: a critical review. Regulatory Toxicology & Pharmacology 38, 378-388.

Cui, L., Zhou, Q.F., Liao, C.Y., Fu, J.J., Jiang, G.B., 2009. Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis. Archives of Environmental Contamination & Toxicology 56, 338-349.

D'eon, J.C., Mabury, S.A., 2011. Is indirect exposure a significant contributor to the burden of perfluorinated acids observed in humans? Environmental Science & Technology 45, 7974-7984.

Di Muria, M., Lamberti, G., Titomanlio, G., 2010. Physiologically based pharmacokinetics: A simple, all purpose model. Industrial & Engineering Chemistry Research 49, 2969-2978.

Domingo, J.L., 2012. Health risks of dietary exposure to perfluorinated compounds. Environment International 40, 187-195.

Edginton, A.N., Theil, F.P., Schmitt, W., Willmann, S., 2008. Whole body physiologically-based pharmacokinetic models: their use in clinical drug development. Expert Opinion in Drug Metabolism on Toxicology 4, 1143-1152.

EFSA, 2008. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. Scientific opinion of the panel on contaminants in the food chain. EFSA J 653, 1-131.

Ericson, I., Martí-Cid, R., Nadal, M., van Bavel, B., Lindström, G., Domingo, J.L., 2008. Human exposure to perfluorinated chemicals through the diet: Intake of perfluorinated compounds in foods from the Catalan (Spain) market. Journal of Agricultural & Food Chemistry 56, 1787-1794.

Ericson, J.I., Nadal, M., van Bavel, B., Lindstrom, G.. Domingo, J.L., 2012. Per- and polyfluorinated compounds (PFCs) in house dust and indoor air in Catalonia, Spain: implications for human exposure. Environment International 39, 172-180.

Falk, S., Brunn, H., Schröter-Kermani, C., Failing, K., Georgii, S., Tarricone, K., Stahl, T., 2012. Temporal and spatial trends of perfluoroalkyl substances in liver of roe deer (Capreolus capreolus). Environmental Pollution 171, 1-8.

Fiedler, H., 1996. Sources of PCDD/PCDF and impact on the environment. Chemosphere 32, 55-64.

Flesch-Janys, D., Becher, H., Gurn, P., Jung, D., Konietzko, J., Manz, A., Papke, O., 1996. Elimination of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in occupationally exposed persons. Journal of Toxicology & Environmental Health 47, 363-378.

Fuentes, S., Colomina, M.T., Rodriguez, J., Vicens, P., Domingo, J.L., 2006. Interactions in developmental toxicology: concurrent exposure to perfluorooctane sulfonate (PFOS) and stress in pregnant mice. Toxicology Letters 164, 81-89.

Fuentes, S., Colomina, M.T., Vicens, P., Domingo, J.L., 2007a. Influence of maternal restraint stress on the long-lasting effects induced by prenatal exposure to perfluorooctane sulfonate (PFOS) in mice. Toxicology Letters 171, 162-170.

Fuentes, S., Vicens, P., Colomina, M.T., Domingo, J.L., 2007b. Behavioral effects in adult mice exposed to perfluorooctane sulfonate (PFOS). Toxicology 242, 123-129.

Gentry, P.R., Covington, T.R., Clewell III, H.J., 2003. Evaluation of the potential impact of pharmacokinetic differences on tissue dosimetry in offspring during pregnancy and lactation. Regulatory Toxicology & Pharmacology 38, 1-16.

Giesy, J.P., Kannan, K., 2001. Global distribution of perfluorooctane sulfonate in wildlife. Environmental Science & Technology 35, 1339-1342.

Han, X., Nabb, D.L., Russell, M.H., Kennedy, G.L., Rickard, R.W., 2011. Renal elimination of perfluorocarboxylates (PFCAs). Chemical Research in Toxicology 25, 35-46.

Han, X., Snow, T.A., Kemper, R.A., Jepson, G.W., 2003. Binding of perfluorooctanoic acid to rat and human plasma proteins. Chemical Research in Toxicology 16, 775-781.

Harris, L.A., Barton, H.A., 2008. Comparing single and repeated dosimetry data for perfluorooctane sulfonate in rats. Toxicology Letters 181, 148-156.

Hassanin, A., Lee, R.G., Johnston, A.E., Jones, K.C., 2006. Reductions and changing patterns of ambient PCDD/Fs in the UK: Evidence and implications. Chemosphere 65, 530-539.

Hu, K.K., Bunce, N.J., 1999. Metabolism of polychlorinated dibenzo-*p*-dioxins and related dioxin-like compounds. Journal of Toxicology & Environmental Health-Part B-Critical Reviews 2, 183-210.

Huang, S.M., Abernethy, D.R., Wang, Y., Zhao, P., Zineh, I., 2013. The utility of modeling and simulation in drug development and regulatory review. Journal of Pharmacological Sciences 102, 2912-2923.

Hundley, S.G., Sarrif, A.M., Kennedy, G.L., 2006. Absorption, distribution, and excretion of ammonium perfluorooctanoate (APFO) after oral administration to various species. Drug Chemical Toxicology 29, 137-145.

IARC, 1997. International Agency for Research no Cancer (1997). Polychlorinated dibenzo-para-doxins and polychlorinated dibenzofurans. Monographs on the Evaluation of Carcinogenic Risk to Humans, volume 69.

IARC, 2012. International Agency for Research no Cancer (2012). A review of human carcinogenics: Chemical agents and related occupations. Monographs on the Evaluation of Carcinogenic Risk to Humans, volume 100F.

Iida, T., Todaka, T., Hirakawa, H., Hori, T., Tobiishi, K., Matsueda, T., Watanabe, S., Yamada, T., 2007. Concentration and distribution of dioxins and related compounds in human tissues. Chemosphere 67, S263-271.

Ji, K., Kim, Y., Oh, S., Ahn, B., Jo, H., Choi, K., 2008. Toxicity of perfluorooctane sulfonic acid and perfluorooctanoic acid on freshwater macroinvertebrates (Daphnia magna and Moina macrocopa) and fish (Oryzias latipes). Environmental Toxicology & Chemistry 27, 2159-2168.

Johansson, J.H., Berger, U., Vestergren, R., Cousins, I.T., Bignert, A., Glynn, A., Darnerud, P.O., 2014. Temporal trends (1999-2010) of perfluoroalkyl acids in commonly consumed food items. Environmental Pollution 188, 102-108.

Jones, H.M., Rowland-Yeo, K., 2013. Basic concepts in physiologically based pharmacokinetic modeling in drug discovery and development. CPT: Pharmacometrics and Systems Pharmacology 2, e63.

Karrman, A., Domingo, J.L., Llebaria, X., Nadal, M., Bigas, E., van Bavel, B., Lindstrom, G., 2010. Biomonitoring perfluorinated compounds in Catalonia, Spain: concentrations and trends in human liver and milk samples. Environmental Science & Pollution Research 17, 750-758.

Karrman, A., Ericson, I., van Bavel, B., Darnerud, P.O., Aune, M., Glynn, A., Lignell, S., Lindstrom, G., 2007. Exposure of perfluorinated chemicals through lactation: Levels of matched human milk and serum and a temporal trend, 1996-2004, in Sweden. Environmental Health Perspectives 115, 226-230.

Kemper, R.A., 2003. Perfluorooctanoic acid: Toxicokinetics in the rat. DuPont Haskell Laboratories, Project No. DuPont–7473. US EPA Administrative Record, AR-226-1499.

King, F.G., Dedrick, R.L., Collins, J.M., Matthews, H.B., Birnbaum, L.S., 1983. Physiological model for the pharmacokinetics of 2,3,7,8-tetrachlorodibenzofuran in several species. Toxicology & Applied Pharmacology 67, 390-400.

Kohn, M.C., Sewall, C.H., Lucier, G.W., Portier, C.J., 1996. A mechanistic model of effects of dioxin on thyroid hormones in the rat. Toxicology & Applied Pharmacology 136, 29-48.

Kulkarni, P.S., Crespo, J.G., Afonso, C.A.M., 2008. Dioxins sources and current remediation technologies — A review. Environment International 34, 139-153.

Kutz, F.W., Barnes, D.G., Bottimore, D.P., Greim, H., Bretthauer, E.W., 1990. The international toxicity equivalency factor (I-TEF) method of risk assessment for complex mixtures of dioxins and related compounds. Chemosphere 20, 751-757.

Lau, C., 2012. Perfluoroalkyl acids: Recent research highlights. Reproductive Toxicology 33, 405-409.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: A review of monitoring and toxicological findings. Toxicological Sciences 99, 366-394.

Lau, C., Butenhoff, J.L., Rogers, J.M., 2004. The developmental toxicity of perfluoroalkyl acids and their derivatives. Toxicology & Applied Pharmacology 198, 231-241.

Lawrence, G.S., Gobas, F.A., 1997. A pharmacokinetic analysis of interspecies extrapolation in dioxin risk assessment. Chemosphere 35, 427-452.

Leahy, D.E., 2006. Integrating in vitro ADMET data through generic physiologically based pharmacokinetic models. Expert Opinion on Drug Metabolism & Toxicology 2, 619-628.

Leung, H.W., Ku, R.H., Paustenbach, D.J., Andersen, M.E., 1988. A physiologically based pharmacokinetic model for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in C57BL/6J and DBA/2J mice. Toxicology Letters 42, 15-28.

Li, M.H., 2009. Toxicity of perfluorooctane sulfonate and perfluorooctanoic acid to plants and aquatic invertebrates. Environmental Toxicology 24, 95-101.

Linares, V., Perello, G., Nadal, M., Gomez-Catalan, J., Llobet, J.M., Domingo, J.L., 2010. Environmental versus dietary exposure to POPs and metals: A probabilistic assessment of human health risks. Journal of Environmental Monitoring 12, 681-688.

Linden, J., Lensu, S., Tuomisto, J., Pohjanvirta, R., 2010. Dioxins, the aryl hydrocarbon receptor and the central regulation of energy balance. Frontiers in Neuroendocrinology 31, 452-478.

Llorca, M., Farre, M., Pico, Y., Teijon, M.L., Alvarez, J.G., Barcelo, D., 2010. Infant exposure of perfluorinated compounds: Levels in breast milk and commercial baby food. Environment International 36, 584-592.

Loccisano, A.E., Campbell, J.L., Andersen, M.E., Clewell, H.J., 2011. Evaluation and prediction of pharmacokinetics of PFOA and PFOS in the monkey and human using a PBPK model. Regulatory Toxicology & Pharmacology 59, 157-175.

Loccisano, A.E., Campbell Jr, J.L., Butenhoff, J.L., Andersen, M.E., Clewell III, H.J., 2012a. Comparison and evaluation of pharmacokinetics of PFOA and PFOS in the adult rat using a physiologically based pharmacokinetic model. Reproductive Toxicology 33, 452-467.

Loccisano, A.E., Campbell Jr, J.L., Butenhoff, J.L., Andersen, M.E., Clewell III, H.J., 2012b. Evaluation of placental and lactational pharmacokinetics of PFOA and PFOS in the pregnant, lactating, fetal and neonatal rat using a physiologically based pharmacokinetic model. Reproductive Toxicology 33, 468-490.

Loccisano, A.E., Longnecker, M.P., Campbell, J.L., Andersen, M.E., Clewell, H.J., 2013. Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages. Journal of Toxicology and Environmental Health - Part A: Current Issues 76, 25-57.

Lou, I., Wambaugh, J.F., Lau, C., Hanson, R.G., Lindstrom, A.B., Strynar, M.J., Zehr, R.D., Setzer, R.W., Barton, H.A., 2009. Modeling single and repeated dose pharmacokinetics of PFOA in mice. Toxicological Sciences 107, 331-341.

Luebker, D.J., Hansen, K.J., Bass, N.M., Butenhoff, J.L., Seacat, A.M., 2002. Interactions of fluorochemicals with rat liver fatty acid-binding protein. Toxicology 176, 175-185.

Martí-Cid, R., Bocio, A., Domingo, J.L., 2008. Dietary exposure to PCDD/PCDFs by individuals living near a hazardous waste incinerator in Catalonia, Spain: Temporal trend. Chemosphere 70, 1588-1595.

Maruyama, W., Yoshida, K., Tanaka, T., Nakanishi, J., 2002. Determination of tissue-blood partition coefficients for a physiological model for humans, and estimation of dioxin concentration in tissues. Chemosphere 46, 975-985.

Maruyama, W., Yoshida, K., Tanaka, T., Nakanishi, J., 2003. Simulation of dioxin accumulation in human tissues and analysis of reproductive risk. Chemosphere 53, 301-313.

McLachlan, M.S., 1993. Digestive tract absorption of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in a nursing Infant. Toxicology & Applied Pharmacology 123, 68-72.

Michalek, J.E., Pirkle, J.L., Caudill, S.P., Tripathi, R.C., Patterson Jr, D.G., Needham, L.L., 1996. Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 10-year follow-up. Journal of Toxicological & Environmental Health 47, 209-220.

Mocarelli, P., Gerthoux, P.M., Patterson Jr, D.G., Milani, S., Limonta, G., Bertona, M., Signorini, S., Tramacere, P., Colombo, L., Crespi, C., Brambilla, P., Sarto, C., Carreri, V., Sampson, E.J., Turner, W.E., Needham, L.L., 2008. Dioxin exposure, from infancy through puberty, produces endocrine disruption and affects human semen quality. Environmental Health Perspectives 116, 70-77.

Mocarelli, P., Needham, L.L., Marocchi, A., Patterson Jr, D.G., Brambilla, P., Gerthoux, P.M., Meazza, L., Carreri, V., 1991. Serum concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and test results from selected residents of Seveso, Italy. Journal of Toxicological & Environmental Health 32, 357-366.

Mumtaz, M., 2010. Principes and practices of mixtures toxicology. Wiley Vch Verlag Gmbh, Weinheim (Germany).

Nadal, M., Fabrega, F., Schuhmacher, M., Domingo, J.L., 2013. PCDD/Fs in plasma of individuals living near a hazardous waste incinerator. A comparison of measured levels and estimated concentrations by PBPK modeling. Environmental Science & Technology 47, 5971-5978.

Nadal, M., Schuhmacher, M., Domingo, J.L., 2011. Long-term environmental monitoring of persistent organic pollutants and metals in a chemical/petrochemical area: Human health risks. Environmental Pollution 159, 1769-1777.

Noker, P., Gorman, G., 2003. A pharmacokinetic study of potassium perfluorooctanesulfonate in the cynomolgus monkey. Southern Research Institute, Study ID: 9921.6, USEPA Public Docket, Administrative Record AR-226-1356.

Olsen, G.W., Chang, S.C., Noker, P.E., Gorman, G.S., Ehresman, D.J., Lieder, P.H., Butenhoff, J.L., 2009. A comparison of the pharmacokinetics of perfluorobutanesulfonate (PFBS) in rats, monkeys, and humans. Toxicology 256, 65-74.

Olsen, G.W., Mair, D.C., Church, T.R., Ellefson, M.E., Reagen, W.K., Boyd, T.M., Herron, R.M., Medhdizadehkashi, Z., Nobiletti, J.B., Rios, J.A., Butenhoff, J.L., Zobel, L.R., 2008. Decline in perfluorooctanesulfonate and other polyfluoroalkyl chemicals in American red cross adult blood donors, 2000-2006. Environmental Science & Technology 42, 4989-4995.

Olson, J.R., 1986. Metabolism and disposition of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in guinea pigs. Toxicology & Applied Pharmacology 85, 263-273.

Paul, A.G., Jones, K.C., Sweetman, A.J., 2009. A first global production, emission, and environmental inventory for perfluorooctane sulfonate. Environmental Science & Technology 43, 386-392.

Peyret, T., Poulin, P., Krishnan, K., 2010. A unified algorithm for predicting partition coefficients for PBPK modeling of drugs and environmental chemicals. Toxicology & Applied Pharmacology 249, 197-207.

Pirkle, J.L., Wolfe, W.H., Patterson, D.G., Needham, L.L., Michalek, J.E., Miner, J.C., Peterson, M.R., Phillips, D.L., 1989. Estimates of the half-life of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Vietnam Veterans of Operation Ranch Hand. Journal of Toxicology & Environmental Health 27, 165-171.

Poulin, P., Theil, F.P., 2009. Development of a novel method for predicting human volume of distribution at steady-state of basic drugs and comparative assessment with existing methods. Journal of Pharmacological Sciences 98, 4941-4961.

Prevedouros, K., Cousins, I.T., Buck, R.C., Korzeniowski, S.H., 2006. Sources, fate and transport of perfluorocarboxylates. Environmental Sciences & Technology 40, 32-44.

Price, K., Haddad, S., Krishnan, K., 2003. Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. Journal of Toxicology & Environmental Health, Part A 66, 417-433.

Ramsey, J.C., Andersen, M.E., 1984. A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. Toxicology & Applied Pharmacology 73, 159-175.

Reddy, M.B., Yang, R.S.H., Clewell III, H.J., Andersen, M.E., 2005. Physiologically based on pharmacokinetic modelling: Science and Applications. Wiley and Sons, Inc., Publication.

Robson, M.G., Toscano, W., 2007. Risk assessment for environmental Health John Willey and sons, Inc., San Francisco (USA).

Roncaglioni, A., Toropov, A.A., Toropova, A.P., Benfenati, E., 2013. In silico methods to predict drug toxicity. Current Opinion in Pharmacology 13, 802-806.

Rotander, A., Kärrman, A., Bavel, B.V., Polder, A., Rigét, F., Audunsson, G.A., Víkingsson, G., Gabrielsen, G.W., Bloch, D., Dam, M., 2012. Increasing levels of long-chain perfluorocarboxylic acids (PFCAs) in Arctic and North Atlantic marine mammals, 1984-2009. Chemosphere 86, 278-285.

Santostefano, M.J., Wang, X., Richardson, V.M., Ross, D.G., DeVito, M.J., Birnbaum, L.S., 1998. A pharmacodynamic analysis of TCDD-induced cytochrome P450 gene expression in multiple tissues: dose- and time-dependent effects. Toxicology & Applied Pharmacology 151, 294-310.

Schecter, A., Papke, O., Ball, M., 1990. Evidence for transplacental transfer of dioxins from mother to fetus: Chlorinated dioxin and dibenzofuran levels inthe liver of stillborn infants. Chemosphere 21, 1017-1022.

Schlummer, M., Gruber, L., Fiedler, D., Kizlauskas, M., Muller, J., 2013. Detection of fluorotelomer alcohols in indoor environments and their relevance for human exposure. Environment International 57-58, 42-49.

Schlummer, M., Moser, G.A., McLachlan, M.S., 1998. Digestive tract absorption of PCDD/Fs, PCBs, and HCB in humans: Mass balances and mechanistic considerations. Toxicology & Applied Pharmacology 152, 128-137.

Schmitt, W., Willmann, S., 2004. Physiology-based pharmacokinetic modeling: Ready to be used. Drug Discovery Today: Technologies 1, 449-456.

Schuhmacher, M., Kiviranta, H., Ruokojärvi, P., Nadal, M., Domingo, J.L., 2009. Concentrations of PCDD/Fs, PCBs and PBDEs in breast milk of women from Catalonia, Spain: A follow-up study. Environment International 35, 607-613.

Schuhmacher, M., Kiviranta, H., Ruokojärvi, P., Nadal, M., Domingo, J.L., 2013. Levels of PCDD/Fs, PCBs and PBDEs in breast milk of women living in the vicinity of a hazardous waste incinerator: Assessment of the temporal trend. Chemosphere 93, 1533-1540.

Seacat, A.M., Thomford, P.J., Hansen, K.J., Clemen, L.A., Eldridge, S.R., Elcombe, C.R., Butenhoff, J.L., 2003. Sub-chronic dietary toxicity of potassium perfluorooctanesulfonate in rats. Toxicology 183, 117-131.

Sonne, C., Gustavson, K., Rigét, F.F., Dietz, R., Birkved, M., Letcher, R.J., Bossi, R., Vorkamp, K., Born, E.W., Petersen, G., 2009. Reproductive performance in East Greenland polar bears (Ursus maritimus) may be affected by organohalogen contaminants as shown by physiologically-based pharmacokinetic (PBPK) modelling. Chemosphere 77, 1558-1568.

Stahl, T., Mattern, D., Brunn, H., 2011. Toxicology of perfluorinated compounds. Environmental Sciences Europe 23, 38.

Sweeney, M.H., Mocarelli, P., 2000. Human health effects after exposure to 2,3,7,8-TCDD. Food Additives & Contaminants 17, 303-316.

Takekuma, M., Saito, K., Falandysz, J., Nakazawa, H., 2011. Ratio variation of congener profiles of PCDD/Fs and dioxin-like PCBs in human milk during lactation. Science of The Total Environment 409, 1368-1377.

Tan, Y.M., Clewell III, H.J., Andersen, M.E., 2008. Time dependencies in perfluorooctylacids disposition in rat and monkeys: A kinetic analysis. Toxicology Letters 177, 38-47.

Teodorell, T., 1937. Kinetics of distribution of substances administred to the body II. The intravascular modes of administration. Archives Internationales De Pharmacodynamie Et De Therapie 57, 226-240.

Thompson, M.D., Beard, D.A., 2011. Development of appropriate equations for physiologically based pharmacokinetic modeling of permeability-limited and flow-limited transport. Journal of Pharmacokinetics & Pharmacodynamics 38, 405-421.

Thompson, M.D., Beard, D.A., Wu, F., 2012. Use of partition coefficients in flow-limited physiologically-based pharmacokinetic modeling. Journal of Pharmacokinetics & Pharmacodynamics 39, 313-327.

U.S.EPA, 2000. (2000) Draft exposure and human health reassessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. EPA/600/P-00/001. US Environmental Protection Agency. Washington, DC.

U.S.EPA, 2012. U.S. Environmental Protection Agency (2012). EPA's Reanalysis of key issues related to dioxin toxicity and response to NAS comments, Volume 1.

Van Asselt, E.D., Kowalczyk, J., van Eijkeren, J.C.H., Zeilmaker, M.J., Ehlers, S., Fürst, P., Lahrssen-Wiederholt, M., van der Fels-Klerx, H.J., 2013. Transfer of perfluorooctane sulfonic acid (PFOS) from contaminated feed to dairy milk. Food Chemistry 141, 1489-1495.

Van den Berg, M., Birnbaum L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2005. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicological Sciences 93, 223-241

Van Leeuwen, F.X., Feeley, M., Schrenk, D., Larsen, J.C., Farland, W., Younes, M., 2000. Dioxins: WHO's tolerable daily intake (TDI) revisited. Chemosphere 40, 1095-1101.

Vasseur, P., Cossu-Leguille, C., 2006. Linking molecular interactions to consequent effects of persistent organic pollutants (POPs) upon populations. Chemosphere 62, 1033-1042.

Vestergren, R., Cousins, I.T., 2009. Tracking the pathways of human exposure to perfluorocarboxylates. Environmental Science & Technology 43, 5565-5575.

Von, K.M., Huisinga, W., 2007. Physiologically based pharmacokinetic modelling: a sub-compartmentalized model of tissue distribution. Journal of Pharmacokinetics and Pharmacodynamics 34, 789-806.

Wang, S.L., Chang, Y.C., Chao, H.R., Li, C.M., Li, L.A., Lin, L.Y., Päpke, O., 2006. Body burdens of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls and their relations to estrogen metabolism in pregnant women. Environmental Health Perspectives 114, 740-745.

Wang, T., Wang, Y., Liao, C., Cai, Y., Jiang, G., 2009. Perspectives on the inclusion of perfluorooctane sulfonate into the Stockholm Convention on Persistent Organic Pollutants. Environmental Science & Technology 43, 5171-5175.

Warner, M., Mocarelli, P., Brambilla, P., Wesselink, A., Patterson Jr, D.G., Turner, W.E., Eskenazi, B., 2013. Serum TCDD and TEQ concentrations among Seveso women, 20 years after the explosion. Journal of Exposure Science and Environmental Epidemiology.

Weber, L.W.D., Ernst, S.W., Stahl, B.U., Rozman, K., 1993. Tissue distribution and toxicokinetics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats after intravenous injection. Fundamental & Applied Toxicology 21, 523-534.

Wendling, J.M., Orth, R.G., Poiger, H., 1990. Determination of [3H]-2,3,7,8-tetrachlorodibenzo-*p*-dioxin in human feces to ascertain its relative metabolism in man. Analytical Chemistry 62, 796-800.

Wolfe, W.H., Michalek, J.E., Miner, J.C., Pirkle, J.L., Caudill, S.P., Patterson Jr, D.G., Needham, L.L., 1994. Determinants of TCDD half-life in veterans of operation ranch hand. Journal of Toxicology & Environmental Health 41, 481-488.

Yang, C.H., Glover, K.P., Han, X., 2009. Organic anion transporting polypeptide (Oatp) 1a1-mediated perfluorooctanoate transport and evidence for a renal reabsorption mechanism of Oatp1a1 in renal elimination of perfluorocarboxylates in rats. Toxicology Letters 190, 163-171.

Yeung, L.W., Guruge, K.S., Taniyasu, S., Yamashita, N., Angus, P.W., Herath, C.B., 2013. Profiles of perfluoroalkyl substances in the liver and serum of patients with liver cancer and cirrhosis in Australia. Ecotoxicology & Environmental Safety 96, 139-146.

Zafeiraki, E., Costopoulou, D., Vassiliadou, I., Bakeas, E., Leondiadis, L., 2014. Determination of perfluorinated compounds (PFCs) in various foodstuff packaging materials used in the Greek market. Chemosphere 94, 169-176.

ANNEX 1

Supporting Information in Chapter 1

Integrated risk index of chemical aquatic pollution (IRICAP): Case studies in Iberian rivers

Table S1

Geographical distribution of 77 sampling points where IRICAP was applied.

Code	River	River Basin	Coordinates UTM		
			Zone	X	Y
LLO1	Llobregat	Llobregat	31	416304	4679359
LLO2	Llobregat	Llobregat	31	407020	4659392
LLO3	Llobregat	Llobregat	31	405907	4617415
CAR1	Cardener	Llobregat	31	381484	4656936
CAR2	Cardener	Llobregat	31	387429	4643298
CAR3	Cardener	Llobregat	31	397127	4629284
CAR4	Cardener	Llobregat	31	403881	4616871
LLO4	Llobregat	Llobregat	31	403792	4607459
LLO5	Llobregat	Llobregat	31	410078	4594291
ANO1	Anoia	Llobregat	31	378856	4606045
ANO2	Anoia	Llobregat	31	388339	4602206
ANO3	Anoia	Llobregat	31	401051	4586728
LLO6	Llobregat	Llobregat	31	411036	4592524
LLO7	Llobregat	Llobregat	31	420247	4577928
EBR1	Ebro	Ebro	30	405193	4761644
OCA	Oca	Ebro	30	466118	4731520
EBR2	Ebro	Ebro	30	503672	4726140
ZAD	Zadorra	Ebro	30	517732	4742302
EBR3	Ebro	Ebro	30	513141	4715725
NAJ	Nájerilla	Ebro	30	523620	4703281
ARG	Arga	Ebro	30	602161	4740847
EBR4	Ebro	Ebro	30	565335	4696194
EBR5	Ebro	Ebro	30	619147	4653811
GAL1	Gállego	Ebro	30	714638	4705571
GAL2	Gállego	Ebro	30	681725	4622524
HUE	Huerva	Ebro	30	673724	4609044
EBR6	Ebro	Ebro	30	692418	4604252
MAR	Martín	Ebro	30	693300	4535853
ESE	Ésera	Ebro	30	280915	4676203
CIN1	Cinca	Ebro	31	271142	4667380
CIN2	Cinca	Ebro	31	264776	4642241

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Code	River	River Basin	Zone	X	Y
RS	Ribera Salada	Ebro	31	370389	4658269
SEG	Segre	Ebro	31	292482	4601301
MAT	Matarranya	Ebro	31	262933	4564305
ALG	Algars	Ebro	31	265824	4554895
EBR7	Ebro	Ebro	31	299521	4559714
EBR8	Ebro	Ebro	31	294619	4513636
EBR9	Ebro	Ebro	31	306788	4509432
JUC1	Júcar	Júcar	30	598583	4453975
JUC2	Júcar	Júcar	30	573092	4436231
JUC3	Júcar	Júcar	30	562110	4414506
JUC4	Júcar	Júcar	30	601713	4336027
JUC5	Júcar	Júcar	30	665927	4340496
CAB1	Cabriel	Júcar	30	627162	4439354
CAB2	Cabriel	Júcar	30	612534	4422400
CAB3	Cabriel	Júcar	30	614272	4410987
CAB4	Cabriel	Júcar	30	628595	4376486
CAB5	Cabriel	Júcar	30	642129	4355758
JUC6	Júcar	Júcar	30	707741	4328283
JUC7	Júcar	Júcar	30	720584	4336933
MAG1	Magro	Júcar	30	667953	4362542
MAG2	Magro	Júcar	30	711245	4348964
JUC8	Júcar	Júcar	30	729360	4343192
BOR	Borosa	Guadalquivir	30	512435	4207084
GUA1	Guadalquivir	Guadalquivir	30	497027	4214205
GUAM	Guadiana	Guadalquivir	30	481267	4192450
GUA2	Guadalquivir	Guadalquivir	30	452771	4200519
MAG	Magaña	Guadalquivir	30	456338	4242400
GUAN	Guadabullón	Guadalquivir	30	431850	4199348
GUA3	Guadalquivir	Guadalquivir	30	395434	4207864
YEG	Yeguas	Guadalquivir	30	384664	4246754
GUAL	Guadalmoral	Guadalquivir	30	375522	4166800
GUA4	Guadalquivir	Guadalquivir	30	334794	4189933
PIC	Picachos	Guadalquivir	30	315008	4180807
BEM	Bembézar	Guadalquivir	30	279446	4224770
CAC	Cacín	Guadalquivir	30	423215	4086558
GEN1	Genil	Guadalquivir	30	396109	4116460
GEN2	Genil	Guadalquivir	30	314734	4161417
GUA5	Guadalquivir	Guadalquivir	30	294339	4174415
COR	Corbones	Guadalquivir	30	272990	4153778
HER	Herreros	Guadalquivir	30	235090	4156650
GUAA	Guadaira	Guadalquivir	30	267816	4123619
GUA6	Guadalquivir	Guadalquivir	29	761545	4129986
GUA7	Guadalquivir	Guadalquivir	29	759226	4107247
GUA8	Guadalquivir	Guadalquivir	29	751405	4094029
GUAR	Guadiamar	Guadalquivir	29	742717	4130496
GUA9	Guadalquivir	Guadalquivir	29	736448	4084204

Table S2.

SOM input PBT values after normalization.

	Half-life	Bioaccumulationn	Toxicity
Acetaminophen	0.066	0.117	0.268
Acridone	0.089	0.045	0.346
Albendazol	0.067	0.397	0.535
Alprazolam	0.120	0.250	0.547
Amlodipine	0.077	0.386	0.536
Amoxicillin	0.033	0.117	0.238
Atenolol	0.049	0.117	0.297
Atorvastatin	0.050	0.410	0.684
Azaperol	0.155	0.334	0.493
Azaperone	0.179	0.295	0.506
Azithromycin	0.274	0.544	0.466
Bezafibrate	0.072	0.117	0.520
Carazolol	0.066	0.417	0.472
Carbamazepine	0.082	0.301	0.435
Cefalexin	0.022	0.117	0.202
Cimetidine	0.092	0.117	0.355
Ciprofloxacin	0.115	0.117	0.177
Citalopram	0.162	0.501	0.540
Clarithromycin	0.218	0.411	0.462
Clopidogrel	0.131	0.513	0.546
Codeine	0.118	0.106	0.379
Desloratadine	0.127	0.638	0.602
Dexamethasone	0.161	0.221	0.307
Diazepam	0.084	0.358	0.506
Diclofenac	0.105	0.117	0.442
Diltiazem	0.085	0.339	0.465
Dimetridazole	0.086	0.117	0.411
Enalapril	0.029	0.117	0.335
Enalaprilat	0.014	0.117	0.112
Erithromycin	0.212	0.395	0.416
Famotidine	0.098	0.117	0.244
Fluoxetine	0.111	0.513	0.603
Fluvastatin	0.044	0.117	0.443
Furosemide	0.129	0.117	0.354
Gemfibrozil	0.067	0.117	0.524
Glibenclamide	0.119	0.663	0.664
Hydrochlrothiazide	0.124	0.117	0.258
Hydrocodone	0.154	0.133	0.385
Ibuprofen	0.054	0.117	0.438
Indomethacine	0.083	0.117	0.602
Iopromide	0.088	0.117	0.000
Irbesartan	0.077	0.742	0.716
Ketoprofen	0.055	0.117	0.350

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	Half-life	Bioaccumulationn	Toxicity
Levamisol	0.077	0.207	0.459
Loratadine	0.121	0.726	0.724
Lorazepam	0.095	0.292	0.475
Losartan	0.123	0.407	0.507
Meloxicam	0.072	0.452	0.530
Metformin	0.066	0.117	0.157
Metoprolol	0.135	0.203	0.317
Metronidazole	0.077	0.117	0.332
Metronidazole-OH	0.060	0.117	0.203
Nadolol	0.070	0.117	0.370
Naproxen	0.044	0.117	0.365
Norfluoxetine	0.108	0.569	0.574
Ofloxacin	0.156	0.117	0.160
Olanzapine	0.154	0.386	0.468
Oxycodone	0.164	0.117	0.335
Paroxetine	0.073	0.656	0.607
Phenazone	0.074	0.117	0.557
Piroxicam	0.077	0.117	0.326
Pravastatin	0.019	0.117	0.381
Propranolol	0.061	0.400	0.474
Propyphenazone	0.080	0.222	0.619
Ranitidine	0.118	0.117	0.311
Ronidazole	0.068	0.117	0.308
Salbutamol	0.064	0.117	0.347
Sertraline	0.137	0.740	0.646
Sotalol	0.070	0.117	0.323
Sulfamethoxazole	0.104	0.117	0.357
Tamsulosin	0.086	0.304	0.444
Tenoxicam	0.100	0.000	0.272
Tetracyclin	0.161	0.117	0.262
Thiabendazole	0.077	0.304	0.467
Torasemide	0.123	0.443	0.431
Trazodone	0.215	0.419	0.498
Trimethoprim	0.096	0.117	0.307
Valsartan	0.030	0.117	0.458
Venlafaxine	0.142	0.429	0.517
Warfarin	0.065	0.339	0.554
Xylazine	0.107	0.620	0.617
9-Tetrahydrocannabinol (THC)	0.084	1.000	0.886
2-oxo-3-hydroxy-LSD	0.104	0.117	0.311
1S,2R(+)Ephedrine	0.055	0.037	0.368
11-hydri-9-THC	0.073	0.686	0.768
11-nor-9-carboxy-9-THC	0.051	0.410	0.673
6-acetylmorphine	0.116	0.162	0.404
Amphetamine	0.069	0.194	0.449

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	Half-life	Bioaccumulationn	Toxicity
Benzoilecgonine	0.057	0.117	0.095
Cannabidiol	0.072	0.850	0.847
Cannabinol	0.083	0.940	0.859
Cocaethylene	0.076	0.333	0.469
Cocaine	0.074	0.278	0.438
EDDP perchlorate	0.141	0.686	0.628
Heroin	0.100	0.166	0.404
Lysergic acid diethylamide (LSD)	0.113	0.378	0.441
MDMA	0.063	0.255	0.467
Metaamphetamine	0.071	0.242	0.476
Methadonehydrochloride	0.151	0.530	0.571
Morphine	0.120	0.117	0.344
1H-Benzotriazole (BT)	0.065	0.145	0.457
Benzil paraben	0.054	0.473	0.561
Bisphenol a (BPA)	0.089	0.436	0.578
Caffeine	0.076	0.117	0.263
Etilparaben	0.047	0.304	0.499
Diethylstilbestrol (DES)	0.078	0.706	0.704
Estradiol (E2)	0.101	0.542	0.598
Estradiol 17-glucuronide (E2-17G)	0.062	0.117	0.448
Estriol (E3)	0.089	0.301	0.434
Estriol 16-glucuronide (E3-16G)	0.038	0.117	0.082
Estriol 3-sulfate (E3-3S)	0.107	0.117	0.182
Estrone (E1)	0.119	0.406	0.557
Estrone 3-glucuronide (E1-3G)	0.072	0.117	0.318
Estrone 3-sulfate (E1-3S)	0.140	0.117	0.245
Ethinyl estradiol (EE2)	0.141	0.489	0.556
Metilparaben	0.045	0.225	0.472
Nonylphenol (NP)	0.056	0.491	0.776
Nonylphenol diethoxylate (NP2EO)	0.054	0.360	0.678
Nonylphenol monocarboxylate	0.028	0.234	0.621
Nonylphenol monoethoxylate	0.047	0.402	0.712
Octylphenol (OP)	0.054	0.450	0.738
Octylphenol diethoxylate (OP2EO)	0.054	0.360	0.678
Octylphenol monocarboxylate	0.070	0.234	0.553
Octylphenol monoethoxylate	0.045	0.326	0.667
Propilparaben	0.049	0.392	0.527
Tolytriazon (TT)	0.106	0.117	0.393
Triclocaraban	0.159	0.680	0.636
Triclosan	0.137	0.658	0.654
Tris (2-cloroetil) phosphate (TCEP)	0.072	0.000	0.419
Tris (butoxietil) phosphate (TBEP)	0.000	0.308	0.489
Tris (cloroisopropil) phosphate	0.081	0.251	0.521
3-hydroxycarbofuran	0.058	0.117	0.327
Acethochlor	0.092	0.391	0.627

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	Half-life	Bioaccumulationn	Toxicity
Alachlor	0.092	0.466	0.627
Atrazine	0.130	0.204	0.511
Azynphos ethyl	0.052	0.448	0.569
Azinphos methyl	0.049	0.347	0.486
Buprofezin	0.110	0.587	0.644
Carbofuran	0.075	0.281	0.494
Chlorfenvinphos	0.098	0.318	0.567
Chlorpyriphos	0.106	0.689	0.666
Deisopropylatrazine	0.122	0.000	0.426
Desethylatrazine	0.125	0.034	0.447
Diazinon	0.059	0.511	0.591
Dichlofenthion	0.080	0.726	0.677
Dimethoate	0.022	0.117	0.342
Diuron	0.119	0.336	0.470
Ethion	0.020	0.706	0.787
Fenitrothion	0.059	0.432	0.558
Fenoxon	0.048	0.086	0.466
Fenoxon sulfone	0.052	0.117	0.331
Fenoxon sulfoxide	0.050	0.117	0.326
Fenthion	0.050	0.555	0.609
Fenthion sulfone	0.054	0.239	0.471
Fenthion sulfoxide	0.052	0.218	0.465
Hexythiazox	0.120	0.784	0.706
Imazalil	0.133	0.513	0.742
Imdacloprid	0.105	0.117	0.688
Isoproturon	0.085	0.366	0.490
Malathion	0.015	0.287	0.453
Methiocarb	0.074	0.374	0.536
Metolachlor	0.094	0.370	0.621
Molinate	0.056	0.418	0.514
Omethoate	0.021	0.117	0.279
Parathion ethyl	0.055	0.514	0.584
Parathion methyl	0.051	0.364	0.525
Prochloraz	0.161	0.556	0.589
Propanil	0.093	0.397	0.535
Propazine	0.133	0.254	0.564
Pyriproxyphen	0.072	0.780	0.700
Simazine	0.128	0.138	0.457
Terbutryn	0.134	0.379	0.634
Tolclofos methyl	0.085	0.627	0.651
i,p-PFNA	0.329	0.234	0.583
I,p PFNS	0.458	0.234	0.525
L-PFBS	0.165	0.117	0.226
L-PFDS	0.570	0.234	0.586
L-PFHpS	0.305	0.117	0.404

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	Half-life	Bioaccumulationn	Toxicity
L-PFHxS	0.249	0.117	0.344
L-PFOS	0.373	0.117	0.464
PFBA	0.066	0.117	0.289
PFDA	0.276	0.234	0.567
PFDoA	0.550	0.410	0.748
PFHpA	0.197	0.117	0.445
PFHxA	0.160	0.117	0.387
PFHxDA	1.000	0.117	1.000
PFNA	0.295	0.234	0.565
PFOA	0.242	0.117	0.505
PFODA	1.000	0.117	0.891
PFOSA	0.438	0.819	0.689
PFPeA	0.128	0.117	0.590
PFTeDA	0.897	0.234	0.875
PFTrDA	0.694	0.234	0.811
PFUdA	0.550	0.410	0.748
2,2'-dihydroxy-4-	0.069	0.376	0.586
4,4'-dihydroxybenzophenone (4DHB)	0.073	0.123	0.486
4-hydroxybenzophenone (4HB)	0.074	0.259	0.510
4-methylbenzylidene camphor	0.132	0.838	0.674
Benzophenone-1 (BP1)	0.072	0.243	0.536
Benzophenone-2 (BP2)	0.070	0.215	0.518
Benzophenone-3 (BP3)	0.071	0.371	0.573
Ethyl 4-aminobenzoate (Et-PABA)	0.060	0.210	0.454
Ethylexyl dimethyl PABA (OD-PABA)	0.067	0.814	0.683
Ethylexyl methoxycinnamate (EHMC)	0.036	0.819	0.653
Octocrylene (OC)	0.056	0.986	0.672

Table S3.

PBT properties of the studied compounds after SOM application.

	Half-life	Bioaccumulation	Toxicity
PFHxDA	2.48	0.77	2.33
PFODA	2.48	0.77	2.33
PFTeDA	2.48	0.77	2.33
PFTrDA	2.48	0.77	2.33
PFDoA	2.11	0.93	2.53
PFUdA	2.11	0.93	2.53
THC	0.35	2.70	2.40
Cannabidiol	0.35	2.70	2.40
Cannabinol	0.35	2.70	2.40
4MBC	0.35	2.70	2.40
OD-PABA	0.35	2.70	2.40
EHMC	0.35	2.70	2.40
OC	0.35	2.70	2.40
Hexythiazox	0.42	2.57	2.33
PFOSA	0.42	2.57	2.33
Irbesartan	0.30	2.57	2.40
Pyriproxyphen	0.30	2.57	2.40
Loratadine	0.36	2.47	2.30
Sertraline	0.36	2.47	2.30
Dichlofenthion	0.36	2.47	2.30
L-PFDS	1.93	0.80	2.33
Glibenclamide	0.41	2.30	2.20
EDDPperchlorate	0.41	2.30	2.20
Triclocaraban	0.41	2.30	2.20
Triclosan	0.41	2.30	2.20
Chlorpyriphos	0.41	2.30	2.20
DES	0.29	2.27	2.30
11-hydri-9-THC	0.25	2.20	2.40
Ethion	0.25	2.20	2.40
Paroxetine	0.34	2.13	2.17
Tolclofos methyl	0.34	2.13	2.17
Desloratadine	0.44	2.07	2.07
Xylazine	0.44	2.07	2.07
Buprofezin	0.37	1.97	2.03
NP	0.24	1.70	2.40
Inazalil	0.24	1.70	2.40
Fenthion	0.26	1.87	2.13
I,p PFNS	1.46	0.73	2.00
L-PFOS	1.46	0.73	2.00
Norfluoxetine	0.43	1.80	1.90
Methadonehydrochloride	0.43	1.80	1.90
Prochloraz	0.43	1.80	1.90
Fluoxetine	0.29	1.77	2.00

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	Half-life	Bioaccumulation	Toxicity
E2	0.29	1.77	2.00
Diazinon	0.29	1.77	2.00
Parathion ethyl	0.29	1.77	2.00
i,p-PFNA	1.12	0.87	2.00
Atorvastatin	0.24	1.47	2.27
11-nor-9-carboxy-9-THC	0.24	1.47	2.27
NP1EO	0.24	1.47	2.27
OP	0.24	1.47	2.27
Terbutryn	0.44	1.27	2.23
Azithromycin	0.50	1.63	1.77
Citalopram	0.50	1.63	1.77
Clopidogrel	0.50	1.63	1.77
EE2	0.50	1.63	1.77
Alachlor	0.23	1.50	2.17
NP2EO	0.27	1.30	2.17
OP2EO	0.27	1.30	2.17
OP1EO	0.27	1.30	2.17
Acethochlor	0.27	1.30	2.17
Meloxicam	0.26	1.50	1.87
Benzilparaben	0.26	1.50	1.87
BPA	0.26	1.50	1.87
Azynphos ethyl	0.26	1.50	1.87
Fenitrothion	0.26	1.50	1.87
DHMB	0.24	1.33	2.03
Venlafaxine	0.48	1.43	1.67
PFDA	0.86	0.80	1.87
PFNA	0.86	0.80	1.87
Metolachlor	0.33	1.13	2.00
E1	0.29	1.40	1.77
Molinate	0.29	1.40	1.77
Propanil	0.29	1.40	1.77
Azaperone	0.58	0.93	1.93
Clarithromycin	0.53	1.33	1.53
Erithromycin	0.53	1.33	1.53
Olanzapine	0.53	1.33	1.53
Torasemide	0.53	1.33	1.53
Trazodone	0.53	1.33	1.53
Carazolol	0.35	1.30	1.67
Losartan	0.35	1.30	1.67
Warfarin	0.25	1.17	1.87
BP3	0.25	1.17	1.87
Albendazol	0.24	1.30	1.73
Amlodipine	0.24	1.30	1.73
Propilparaben	0.24	1.30	1.73
Methiocarb	0.24	1.30	1.73

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	Half-life	Bioaccumulation	Toxicity
Azaperol	0.43	1.23	1.57
LSD	0.43	1.23	1.57
Diazepam	0.27	1.23	1.67
Propranolol	0.27	1.23	1.67
Isoproturon	0.27	1.23	1.67
Chlorfenvinphos	0.33	0.97	1.87
Alprazolam	0.46	0.83	1.87
Propazine	0.46	0.83	1.87
Parathion_methyl	0.21	1.13	1.73
Carbamazepine	0.32	1.13	1.57
Diltiazem	0.32	1.13	1.57
Lorazepam	0.32	1.13	1.57
Tamsulosin	0.32	1.13	1.57
Thiabendazole	0.32	1.13	1.57
Cocaethylene	0.32	1.13	1.57
Diuron	0.32	1.13	1.57
Azinphos methyl	0.22	1.10	1.63
NP1EC	0.29	0.80	1.83
OP1EC	0.29	0.80	1.83
BP1	0.29	0.80	1.83
Propyphenazone	0.34	0.67	1.90
Etilparaben	0.20	0.93	1.67
TBEP	0.20	0.93	1.67
Carbofuran	0.20	0.93	1.67
Metaamphetamine	0.22	0.80	1.70
Metilparaben	0.22	0.80	1.70
TCCP	0.22	0.80	1.70
Fenthion sulfone	0.22	0.80	1.70
Fenthion sulfoxide	0.22	0.80	1.70
4HB	0.22	0.80	1.70
BP2	0.22	0.80	1.70
E3	0.37	0.97	1.37
MDMA	0.21	0.90	1.57
Malathion	0.21	0.90	1.57
Indomethacine	0.29	0.50	1.87
Phenazone	0.29	0.50	1.87
Imdacloprid	0.29	0.50	1.87
PFPeA	0.29	0.50	1.87
Atrazine	0.26	0.63	1.70
Cocaine	0.28	0.87	1.40
Bezafibrate	0.25	0.47	1.70
Gemfibrozil	0.25	0.47	1.70
Levamisol	0.22	0.67	1.47
Amphetamine	0.22	0.67	1.47
Et-PABA	0.22	0.67	1.47

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	Half-life	Bioaccumulation	Toxicity
L-PFHpS	0.60	0.33	1.37
L-PFHxS	0.60	0.33	1.37
PFHpA	0.60	0.33	1.37
PFOA	0.60	0.33	1.37
Fenoxon	0.26	0.47	1.57
4DHB	0.26	0.47	1.57
Fluvastatin	0.24	0.43	1.53
Ibuprofen	0.24	0.43	1.53
Valsartan	0.24	0.43	1.53
BT	0.24	0.43	1.53
E2-17G	0.24	0.43	1.53
Deisopropylatrazine	0.48	0.29	1.43
Desethylatrazine	0.48	0.29	1.43
Hydrocodone	0.49	0.37	1.30
Oxycodone	0.49	0.37	1.30
PFHxA	0.49	0.37	1.30
Dimetridazole	0.30	0.43	1.40
Diclofenac	0.32	0.33	1.47
TCEP	0.32	0.33	1.47
Simazine	0.32	0.33	1.47
6-acetylmorphine	0.26	0.50	1.33
Heroin	0.26	0.50	1.33
Codeine	0.36	0.40	1.30
TT	0.36	0.40	1.30
Dexamethasone	0.42	0.67	0.93
Pravastatin	0.22	0.50	1.27
Cimetidine	0.34	0.37	1.20
Furosemide	0.34	0.37	1.20
Sulfamethoxazole	0.34	0.37	1.20
Morphine	0.34	0.37	1.20
Nadolol	0.26	0.40	1.20
Naproxen	0.26	0.40	1.20
Enalapril	0.23	0.40	1.13
Ketoprofen	0.23	0.40	1.13
Metronidazole	0.23	0.40	1.13
Piroxicam	0.23	0.40	1.13
Salbutamol	0.23	0.40	1.13
3-hydrosycarbofuran	0.23	0.40	1.13
Dimethoate	0.23	0.40	1.13
Fenoxon sulfone	0.23	0.40	1.13
Fenoxon sulfoxide	0.23	0.40	1.13
Acridone	0.25	0.37	1.13
1S,2R(+)Ephedrine	0.25	0.37	1.13
Ranitidine	0.26	0.40	1.03
Trimethoprim	0.26	0.40	1.03

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	Half-life	Bioaccumulation	Toxicity
2-oxo-3-hydroxy-LSD	0.26	0.40	1.03
Metoprolol	0.04	0.60	1.00
Atenolol	0.24	0.37	1.03
Ronidazole	0.24	0.37	1.03
Sotalol	0.24	0.37	1.03
E1-3G	0.24	0.37	1.03
Famotidine	0.31	0.40	0.87
Hydrochlrothiazide	0.31	0.40	0.87
Tetracyclin	0.31	0.40	0.87
E1-3S	0.31	0.40	0.87
Acetaminophen	0.26	0.37	0.90
Tenoxicam	0.26	0.37	0.90
Caffeine	0.26	0.37	0.90
Omethoate	0.26	0.37	0.90
PFBA	0.26	0.37	0.90
L-PFBS	0.32	0.43	0.70
Amoxicillin	0.27	0.40	0.73
Cefalexin	0.27	0.40	0.73
Metronidazole-OH	0.27	0.40	0.73
Ciprofloxacin	0.28	0.40	0.57
Enalaprilat	0.28	0.40	0.57
Iopromide	0.28	0.40	0.57
Metformin	0.28	0.40	0.57
Ofloxacin	0.28	0.40	0.57
Benzoilecgonine	0.28	0.40	0.57
E3-16G	0.28	0.40	0.57
E3-3S	0.28	0.40	0.57

ANNEX 2

Supporting Information in Chapter 2

PCDD/Fs in plasma of individuals living near a hazardous waste incinerator. A comparison of measured levels and estimated concentrations by PBPK modeling

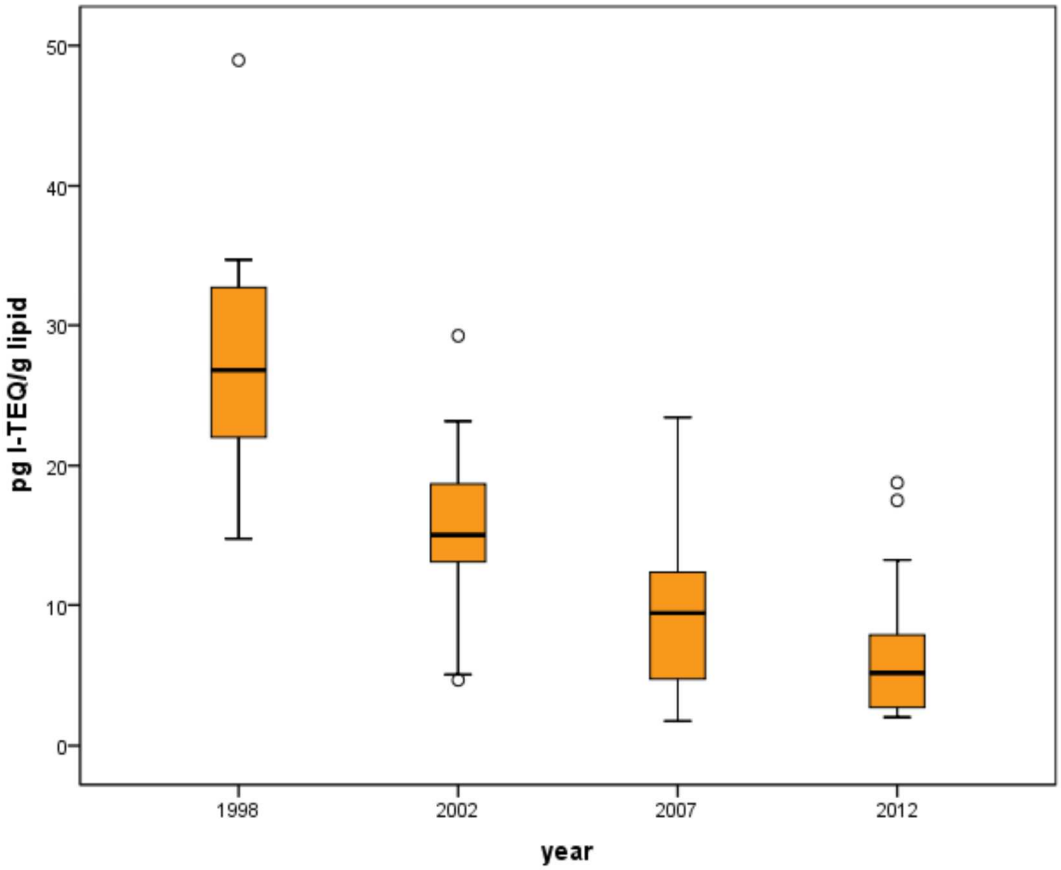

Figure S1. Temporal trends of PCDD/Fs in plasma of residents near the HWI of Constantí, Tarragona County (Catalonia, Spain).

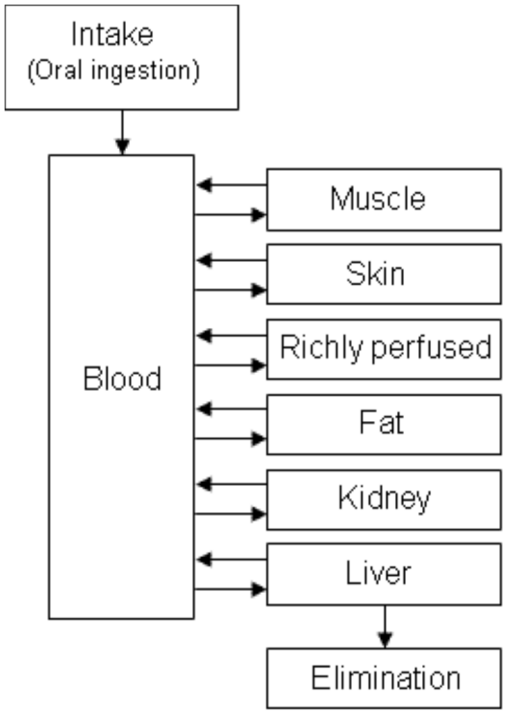

Figure S2. Conceptual representation of the PBPK model for PCDD/Fs in humans.

Table S1.

PCDD/F concentrations (pg/g lipid) in plasma of 40 individuals living in the vicinity of the HWI.

Sample	Age	Gender	I-TEQ	WHO-TEQ
1	49	Male	7.47	5.91
2	50	Male	13.3	12.7
3	61	Male	18.8	25.4
4	56	Female	7.67	6.73
5	40	Male	8.89	7.54
6	50	Female	7.67	7.14
7	65	Female	17.5	16.1
8	47	Male	8.12	7.73
9	48	Female	5.26	5.25
10	28	Female	6.54	5.5
11	33	Female	9.21	7.8
12	53	Male	4.4	4.6
13	55	Male	4.86	4.53
14	22	Female	3.83	3.88
15	56	Female	7.68	7.99
16	51	Male	11	10.7
17	52	Female	6.89	6.81
18	23	Male	3.82	4.05
19	51	Female	4	4.15
20	26	Male	12.1	11.9
21	36	Female	3	3.29
22	32	Female	2.04	2.16
23	44	Male	6.02	5.33
24	41	Male	8.05	7.5
25	67	Female	5.72	6.63
26	48	Male	2.67	2.87
27	43	Female	4.14	4.46
28	52	Male	5.08	5.62
29	45	Male	2.7	3.03
30	51	Female	2.35	2.51
31	44	Male	2.75	2.95
32	50	Female	2.03	2.59
33	64	Female	5.93	7.38
34	43	Female	2.5	2.77
35	48	Male	2.21	2.52
36	50	Male	2.56	2.87
37	49	Male	2.67	2.7
38	49	Female	3.8	4.04
39	60	Female	9.75	10.5
40	47	Male	2.11	2.17

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	I-TEQ	WHO-TEQ
Mean	6.18	6.26
Standard deviation	5.37	4.06
Minimum	2.03	2.16
Maximum	18.8	25.4

ANNEX 3

Supporting Information in Chapter 3

A PBPK model to estimate PCDD/F levels in adipose tissue: Comparison with experimental values of residents near a Hazardous Waste Incinerator.

Table S1.

Absorption and intake used in the PBPK model.

		Intake of PCCD/F (pg day-1)			
	Absorption	**1998**	**2002**	**2007**	**2012**
2,3,7,8-TCDD	97	28.6	6.1	3.9	5.6
1,2,3,7,8-PeCDD	99	14.9	9.6	8.5	7.6
1,2,3,4,7,8-HxCDD	98	43.4	11.6	5.3	3.6
1,2,3,6,7,8-HxCDD	97	114	21.5	7.1	10.9
1,2,3,7,8,9-HxCDD	96	41.6	11.5	4.8	6.9
1,2,3,4,6,7,8-HpCDD	86	1292	92.4	28.3	69.6
OCDD	76	9623	525	141	297
2,3,7,8-TCDF	97	192	48.9	25.2	40.6
1,2,3,7,8-PeCDF	99	125	28.2	11.4	9.2
2,3,4,7,8-PeCDF	98	109	46.8	12.4	25.7
1,2,3,4,7,8-HxCDF	97	231	53.3	20.9	33.4
1,2,3,6,7,8-HxCDF	97	107	27.6	12.3	13.9
1,2,3,7,8,9-HxCDF	95	10.2	10.4	3.1	6.6
2,3,4,6,7,8-HxCDF	96	42	32.7	5.8	9.3
1,2,3,4,6,7,8-HpCDF	87	708	69.8	72.2	127
1,2,3,4,7,8,9-HpCDF	99	89.7	15.8	13	23.9
OCDF	95	4420	93.4	476	201

Table S2.

Physiological parameters used in the PBPK model.

Tissue	**Tissue volume**	**Cardiac output (L/h)**
Blood	4.5	-
Liver	1.5	182.2
Fat	10.3	11.1
Kidney	0.3	3.5
Muscle	24	43.2
Richly perfused	1.8	51.1
Skin	2.2	3.2

Table S3.

Partition coefficients (Pk) used in the PBPK model (unitless).

	Liver	Kidney	Fat	Muscle	Richly perfused	Skin
2,3,7,8-TCDD	9.8	3.1	247	17	4.1	2.5
1,2,3,7,8-PeCDD	17	2.9	432	19	4.5	2
1,2,3,4,7,8-HxCDD	30	1.2	117	5.1	2.4	2.6
1,2,3,6,7,8-HxCDD	55	3	219	13	2.7	1.7
1,2,3,7,8,9-HxCDD	297	11	1466	134	30	2.6
1,2,3,4,6,7,8-HpCDD	34	2.3	143	39	14	14
OCDD	56	2.8	55	26	16	14
2,3,7,8-TCDF	18	0.97	55	4.7	2.3	9.4
1,2,3,7,8-PeCDF	19	1.4	130	10	6.3	9.2
2,3,4,7,8-PeCDF	45	2.1	336	38	3.5	2.6
1,2,3,4,7,8-HxCDF	25	1.6	75	11	6	2.1
1,2,3,6,7,8-HxCDF	45	1.7	130	25	2.2	2.4
1,2,3,7,8,9-HxCDF	1.8	0.4	172	2.9	3.7	11
2,3,4,6,7,8-HxCDF	3.8	0.9	48	3.1	3.5	6.2
1,2,3,4,6,7,8-HpCDF	22	0.9	139	7.3	2.3	11
1,2,3,4,7,8,9-HpCDF	8.7	0.3	113	3.1	1.9	11
OCDF	15	1.7	144	5.5	4.5	14