Science of the Total Environment 912 (2024) 168738

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Studying mixture effects on uptake and tissue distribution of PFAS in zebrafish (*Danio rerio*) using physiologically based kinetic (PBK) modelling

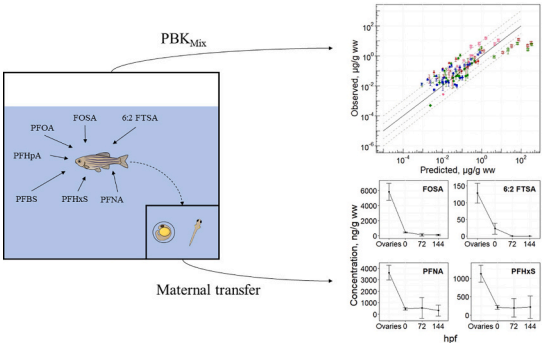
Elena Golosovskaia [a,*], Stefan Örn [b], Lutz Ahrens [c], Ioana Chelcea [a], Patrik L. Andersson [a,*]

[a] Department of Chemistry, Umeå University, Umeå, Sweden
[b] Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden
[c] Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

HIGHLIGHTS

- Novel data provided on kinetics of FOSA and 6:2 FTSA in adult female zebrafish.
- Maternal transfer into eggs were observed for all studied PFAS.
- Mixture PBK model predicted >60 % of external experimental data within 3-fold error.
- Competitive protein binding seemed not to be a critical factor for PFAS uptake.
- Gill surface pH influence the uptake of PFCAs, but not PFSAs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Henner Hollert

Keywords:
PFAS
Maternal transfer
Mixture
PBTK
ADME
Zebrafish

ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are ubiquitously distributed in the aquatic environment. They include persistent, mobile, bioaccumulative, and toxic chemicals and it is therefore critical to increase our understanding on their adsorption, distribution, metabolism, excretion (ADME). The current study focused on uptake of seven emerging PFAS in zebrafish (*Danio rerio*) and their potential maternal transfer. In addition, we aimed at increasing our understanding on mixture effects on ADME by developing a physiologically based kinetic (PBK) model capable of handling co-exposure scenarios of any number of chemicals. All studied chemicals were taken up in the fish to varying degrees, whereas only perfluorononanoate (PFNA) and perfluorooctanoate (PFOA) were quantified in all analysed tissues. Perfluorooctane sulfonamide (FOSA) was measured at concerningly high concentrations in the brain (C_{max} over 15 µg/g) but also in the liver and ovaries. All studied PFAS were maternally transferred to the eggs, with FOSA and 6:2 perfluorooctane sulfonate (6,2 FTSA) showing significant ($p < 0.02$) signs of elimination from the embryos during the first 6 days of development, while perfluorobutane sulfonate (PFBS), PFNA, and perfluorohexane sulfonate (PFHxS) were not eliminated in embryos during this time-frame. The mixture PBK model resulted in >85 % of predictions within a 10-fold error and 60 % of predictions within a 3-fold error. At studied levels of PFAS exposure, competitive binding was not a critical factor for PFAS kinetics. Gill surface pH influenced uptake for some carboxylates but not the sulfonates. The developed PBK

* Corresponding authors.
E-mail addresses: elena.dracheva@umu.se (E. Golosovskaia), patrik.andersson@umu.se (P.L. Andersson).

https://doi.org/10.1016/j.scitotenv.2023.168738
Received 18 September 2023; Received in revised form 17 November 2023; Accepted 19 November 2023
Available online 27 November 2023

model provides an important tool in understanding kinetics under complex mixture scenarios and this use of New Approach Methodologies (NAMs) is critical in future risk assessment of chemicals and early warning systems.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of diverse organic chemicals defined by the Organisation for Economic *Co*-operation and Development (OECD) as "with a few noted exceptions, any chemical with at least a perfluorinated methyl group ($-CF_3$) or a perfluorinated methylene group ($-CF_2-$)" (OECD, 2021), where "noted exceptions" being a carbon atom with a Cl/Br/I atom attached to it. This is a very wide definition including small molecules to more complex structures including polymers (Wang et al., 2021). In 2018, the OECD in cooperation with the United Nations Environment Programme (UNEP) published a database of >4700 manmade PFAS chemicals many of which are likely to be on the market (OECD, 2018). Due to environmental and health concerns, persistence, mobility, large production volumes and emissions of many PFAS, the European Chemicals Agency (ECHA) has recently published a restriction proposal covering around 10,000 PFAS (Registry of restriction intentions until outcome, n.d.). ECHA estimated total PFAS emissions in European economic area to be around 4.4 million metric tonnes over the next 30 years, in case no restrictions are placed soon (https://echa.europa.eu/-/echa-publishes-pfas-restriction-proposal, n.d.).

PFAS are characterized by the extremely strong and stable C—F bond (Smart, 1994) which gives them useful properties, such as thermal stability in addition to hydrophobicity and lipophobicity (Buck et al., 2011). Due to their chemical properties, PFAS are used extensively in applications such as textiles, plastic, paper, consumer articles, and in electronics (Gluge et al., 2020). PFAS include structures that are known to be persistent and bioaccumulative and are sometimes referred to as "forever chemicals". They are widespread in the environment and have been detected in, e.g., soils (Sörengård et al., 2022; Brusseau et al., 2020), drinking water (Wang et al., 2022a), humans, and wildlife (Ahrens and Bundschuh, 2014).

In surface water, PFAS are detected on a global scale in levels from ng/L to μg/L typically dominated by PFAS with eight or fewer carbons (Bai and Son, 2021). Perfluoroalkyl carboxylates (PFCAs), perfluoroalkyl sulfonates (PFSAs), and PFAS precursors have been measured in groundwater samples at concentrations up to 95, 26, and 20 ng/L (as sum of individual compounds), respectively, and in connection with this, levels in drinking water of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in the Netherlands were recently reported in ranges up to 4.3 and 8.6 ng/L, respectively (Sadia et al., 2023). However, much higher levels have been reported from contaminated sites where for example a municipality resident population in Sweden has been exposed to drinking water levels of PFOS and PFOA up to 8000 and 100 ng/L, respectively (Xu et al., 2021). In fish, PFAS have been detected in different species across the world (Kabore et al., 2022; Giari et al., 2023; Pickard et al., 2022; Mikolajczyk et al., 2023). Edible marine and freshwater fish and shellfish species are major sources of human exposure to PFAS (Torres and De-la-Torre, 2023) alongside with drinking water (Kabore et al., 2022; Giari et al., 2023; Pickard et al., 2022).

Several adverse health outcomes have been associated with PFAS exposure including developmental neurotoxicity (Foguth et al., 2020), liver and kidney cancers (Girardi and Merler, 2019; Stanifer et al., 2018), liver diseases (Bassler et al., 2019), various reproductive disorders (Fenton et al., 2021), and endocrine disruptive effects (National Toxicology Program, N., 2019; Weiss et al., 2009). However, their adsorption, distribution, metabolism, excretion (ADME) properties are not fully understood (Dickman and Aga, 2022) and even less is known on how their ADME properties are affected in mixture exposure scenarios (Fenton et al., 2021). These properties are however crucial for understanding the adverse effects caused by PFAS.

Physiologically based (toxico)kinetic (PB(*T*)K) models aim at estimating dose-at-target by simulating ADME using information about the organism's physiology and the chemical properties. This provides a translational linkage between external and internal chemical exposure of an organism. PBK models are routinely used in pharmacology and toxicology to estimate concentrations of chemicals in plasma and tissues without costly, time-consuming, and ethically questionable in vivo experiments in accordance with the 3Rs principle (replacement, reduction, and refinement) (Yuan et al., 2022). Several PBK models exist for studies of uptake and distribution of different PFAS in humans (Bernstein et al., 2021; Deepika et al., 2021; Worley et al., 2017) as well as in other mammals and fish species (Khazaee and Ng, 2018; Vidal et al., 2019; Mittal and Ng, 2018; Stadnicka et al., 2012; Brinkmann et al., 2016). Models are mainly developed for individual chemicals although exposure typically occurs in complex mixtures both in the environment but also in experimental laboratory settings. The ADME of a single chemical might be affected by other chemicals in a mixture as a result of modulation of physiological or physicochemical determinants of these processes, such as cardiac output, pH of gill surface, tissue:blood partition coefficients, distribution and transport processes, and metabolism (Haddad and Krishnan, 1998). PBK models tailored for chemical mixtures and inhalation exposure of rats have recently been reviewed (Desalegn et al., 2019). For fish, much less is known about effects of mixtures on ADME and published PBK mixture models are only trained on binary exposure scenarios (Tebby et al., 2019; Mit et al., 2021). This is a critical knowledge gap, as data indicates that long-chain PFAS have impact on kinetics of short-chain PFAS in fish (Wen et al., 2017).

Although data describing mixture effects on kinetics of PFAS is limited, various in vivo studies in adult fish have been done with complex mixtures (Wen et al., 2019; Chen et al., 2016; Huang et al., 2022). PFAS are known to bind to proteins in blood and tissues, and this binding affects their tissue distribution in mammalian species (Loccisano et al., 2013; Scinicariello et al., 2020). The present study took into account the plausible mixture effects on chemicals ADME with a focus on PFAS binding to transport proteins in blood. In addition, effects of gill surface pH on PFAS uptake were studied through PBK simulations. It is known that the pH of gills can decrease due to, e.g. carbon dioxide release from gills, and that process has been suggested to increase gill uptake of ionizable chemicals (Erickson et al., 2006).

The aim of the present study was to contribute to the development of in silico based tools for improved risk assessment of industrial chemicals by i) providing data on in vivo kinetics in adult female zebrafish of seven emerging PFAS including 6:2 perfluorooctanesulfonate (6:2 FTSA) and perfluorooctane sulfonamide (FOSA), which, to the best of our knowledge, have not been studied previously; ii) offering insights into maternal transfer of PFAS; and iii) developing a PBK mixture model capable of handling arbitrary number of chemicals and investigating changes in kinetics of individual PFAS in mixture scenarios.

2. Materials and methods

2.1. Compounds

Seven PFAS were selected for this study: perfluoroheptanoate (PFHpA) (CAS 375–85-9), PFOA (CAS 335–6-1), perfluorononanoate (PFNA) (CAS 375–95-1), perfluorobutane sulfonate (PFBS) (CAS 375–73-5), perfluorohexane sulfonate (PFHxS) (CAS 355–46-4), 6:2 FTSA (CAS 27619–97-2), and FOSA (CAS 754–91-6) (purity >97 %, Sigma Aldrich Sweden AB) (Table 1, Fig. S1). In addition, 7 isotopically mass-labelled standards (IS) were used for quantification including $^{13}C_4$-

PFHpA, $^{13}C_8$-PFOA, $^{13}C_9$-PFNA, $^{13}C_3$-PFBS, $^{13}C_3$-PFHxS, $^{13}C_2$–6:2 FTSA, $^{13}C_8$-FOSA (purities >99 %, Wellington Laboratories, Guelph, ON).

2.2. In vivo zebrafish experiment

Adult female zebrafish were purchased from a local supplier in Uppsala, Sweden, and kept under controlled and clean husbandry at the aquatic laboratory of the Department of Biomedical Sciences and Veterinary Public Health (BVF) for a month before the start of the experiment. The fish were kept in one 40-L glass aquarium with a flow-through system set at 3 daily water volume renewals (120*L*) for 32 days. The PFAS mixture stock solution of 1 mg/L per chemical was pumped into the tank with an ISMATEC 24 peristaltic pump at a flow rate of 1.2 L/day. The nominal exposure concentration of each compound was 10 μg/L, however, average measured water concentration was used as an input for the models (Table 2 and Table S1). Three individual fish (i.e., pseudoreplicates) were sampled at day 0 (control), 1, 3, 7, 21, and 32, for dissection of brain, liver, ovaries, and carcass. In addition, water was sampled at each sampling point. Prior to the sampling, fish were euthanized with immersion in a sodium bicarbonate-buffered tricaine methanesulfonate solution (MS222: 500 mg/L) and decapitated. The tissues and water samples were weighted and stored at −20 °C until chemical analysis was performed.

To study maternal transfer of the chemicals, reproduction studies with zebrafish were performed at two occasions: before start of exposure (controls) and after 32 days of exposure. Three females were selected at each occasion and placed individually in separate spawning tanks together with one unexposed male zebrafish. Spawning took place in clean water without chemical exposure. The day after, eggs were collected from the spawning pairs. Fertilized eggs were selected under stereomicroscope and placed in Petri dishes with clean water. For the control spawning fish, the embryos were sampled for chemical analyses immediately after spawning (day 0) and after six days (day 6). Embryos from the exposed females were sampled at days 0, 3 and 6. At sampling the embryos were transferred into 1.5 mL Eppendorf tubes and excess water was then pipetted off the tubes. Embryos were then immediately frozen and kept at −20 °C until chemical analysis.

Analysis of variance was performed with a Student's *t*-test with accepted level of significance of 0.05.

2.3. Chemical analysis

The zebrafish tissue, embryos and water samples were analysed as previously described (Menger et al., 2020; Sorengard et al., 2022). To all samples, 100 μL of the IS mixture were spiked (5 ng/mL for individual IS) before extraction. Briefly, the zebrafish tissue and embryo samples were extracted using solid-liquid extraction with methanol in 7 mL lysing tubes using a universal tissue homogeniser (ceramic beads, speed = 7500 rpm, cycle = 2 × 40 s, pause = 20 s, Precellys CK28, Precellys® Evolution, Bertin Technologies). The extracts were concentrated to 1 mL and cleaned-up using 25 mg ENVI-Carb™ plus 50 mL glacial acetic acid (for details see Menger et al., 2020 (Menger et al., 2020)). The water samples were extracted using weak anion exchange (WAX) cartridges (150 mg, 30 μm, 6 cc, Oasis, Waters, USA) for solid phase extraction (SPE). The cartridges were pre-conditioned with 4 mL 0.1 % ammonium hydroxide in methanol, 4 mL methanol and 4 mL Millipore water. After loading of the water samples, the cartridges were then washed with 4 mL 25 mM ammonium acetate buffer at pH 4, dried and then eluted using 4 mL methanol and 4 mL 0.1 % ammonium hydroxide in methanol. The extracts were evaporated with nitrogen gas to 1 mL. The instrumental analysis was performed using ultra-performance liquid chromatography (UPLC) coupled to a tandem mass spectrometry (MS/MS) (SCIEX Triple Quad 3500), with electrospray ionisation (ESI) in negative ionisation mode. For peak separation, a Phenomenex Gemini® 3 μm C18 HPLC column with a Phenomenex KJ0–4282 analytical guard column at 40 °C. For data analysis and quantification, SCIEX OS-MQ 1.7 was used (Table S2).

2.4. PBK model structure and parametrization

The PBK model structure was based on the previously developed models by Chelcea et al. (Chelcea et al., 2022), Grech et al. (Grech et al., 2019) and Wang et al. (Wang et al., 2022b) It consists of 10 compartments including blood, liver, brain, ovaries/eggs, kidney, gastrointestinal (GI)-tract, skin, richly perfused tissues, poorly perfused tissues, and adipose tissue. Physiological data on female zebrafish was obtained from the study of Grech et al. (Grech et al., 2019) Fish PBK models have been developed for few PFAS both based on blood flow limitation (perfusion rate-limited) (Vidal et al., 2019) and diffusion limitation (cell membrane permeability rate-limited) (Khazaee and Ng, 2018). Due to scarcity of experimental data on PFAS, it has been shown that complex diffusion-limited PBK models lead to less accurate predictions (Khazaee and Ng, 2018). Thus, we chose the use of perfusion-limited equations for the modelling.

In this study, we accounted for chemical absorption exclusively via the gills, since PFAS was administered via water exposure, however, the model can be easily parametrised to include oral and/or intravenous intake. In mammals, PFAS have been shown to be reabsorbed in the proximal tubules and eliminated via the urine (Louisse et al., 2023; Yang et al., 2010). PFAS reabsorption was previously studied in fish and no

Table 1
Studied compounds with their names, acronym, molecular structure, molecular weight (MW), acid dissociation constant (pK_a), octanol-water partitioning constant (K_{ow}), membrane-water distribution coefficient (D_{mw}), protein-water partition coefficient (K_{pw}) and human serum albumin (HSA) dissociation constant (K_d)

Test compound	Acronym	MW	pK_a[a]	Log K_{ow}[b]	Log D_{mw}[c]	Log K_{pw}[d]	K_d HSA[e], mM
Perfluoroheptanoate	PFHpA	363.05	0.47	3.94	0.87	2.94	0.44 ± 0.20
Perfluorooctanoate	PFOA	413.06	0.50	4.35	1.51	3.17	0.83 ± 0.38
Perfluorononanoate	PFNA	463.06	0.52	4.97	2.04	3.52	0.58 ± 0.21
Perfluorobutane sulfonate	PFBS	299.09	−3.57	3.90[f]	0.63	2.10	1.65 ± 0.69
Perfluorohexane sulfonate	PFHxS	399.10	−3.34	3.41	1.82	2.63	0.71 ± 0.47
6:2 perfluorooctanesulfonate	6:2 FTSA	427.16	1.31	2.66[f]	−0.50[g]	2.21	0.37 ± 0.34
Perfluorooctane sulfonamide	FOSA	499.15	7.01	5.87	0.30[g]	4.04	NA

[a] Estimated values, obtained from Scifinder[n] (2023).
[b] Measured values obtained from Chelcea et al. 2020 (Chelcea et al., 2022).
[c] log D_{mw} values were assumed to be 2 log units lower than log K_{mw}. log K_{mw} were measured previously (Droge, 2019).
[d] Values predicted as log K_{pw} = 0.57*log K_{ow} + 0.69
[e] Measured values from Jackson et al. (Jackson et al., 2021).
[f] Estimated value, obtained from EPI Suite V 4.1 (KOWWIN V1.68).
[g] D_{mw} (pH 7.4) values were determined following the approach outlined by Escher et al. for ionizable organic contaminants (Escher et al., 2009). As a first step, the liposome-water partition coefficient was determined as Log K_{lipw} = 0.90 × Log K_{ow} + 0.52. Subsequently, D_{mw} was determined as, $D_{mw} = f_N \times K_{lipw,\,N} + f_I \times K_{lipw,\,N} \times 0.1$ where f_N is the fraction of chemical in neutral form and f_I is the fraction of chemical in charged form at pH 7.4, as predicted by the Henderson-Hasselbalch equation.

Table 2
Bioconcentration factors (BCF) measured in this study and previously published studies on zebrafish[a].

Log BCF							
Compound	C_{water}, μg/L	Brain	Carcass	Liver	Ovaries	Whole body[a]	Reference
PFHpA	9.6 ± 1.9	ND	0.5 ± 0.7	ND	0.6 ± 0.1	0.5 ± 0.1	This study
	10.6 ± 0.1	0.2	NA	1.4	0.3	NA	Wen et al. (Wen et al., 2019)
PFOA	10 ± 1	NA	NA	NA	NA	1.7	Ulhaq et al.[b] (Ulhaq et al., 2015)
	8.9 ± 1.7	1.6 ± 0.5	1.4 ± 0.1	1.6 ± 0.2	1.6 ± 0.1	1.5 ± 0.1	This study
	0.9 ± 0.2	NA	NA	2.4	2.2	NA	Chen et al. (Chen et al., 2016)
	6.6 ± 1.2	NA	NA	2.4	2.0	NA	Chen et al. (Chen et al., 2016)
	10.6 ± 0.1	ND	NA	2.2	1.6	NA	Wen et al. (Wen et al., 2019)
PFNA	7.8 ± 2.3	2.0 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.7 ± 0.1	2.3 ± 0.1	This study
	0.7 ± 0.2	NA	NA	3.2	3.3	NA	Chen et al. (Chen et al., 2016)
	3.6 ± 0.8	NA	NA	3.5	3.4	NA	Chen et al. (Chen et al., 2016)
	12.1 ± 0.2	1.87	NA	2.6	2.3	NA	Wen et al. (Wen et al., 2019)
PFBS	8.7 ± 1.9	ND	0.0 ± 0.1	ND	0.6 ± 0.1	0.3 ± 0.1	This study
	2.2 ± 0.3	NA	NA	1.2	1.7	NA	Chen et al. (Chen et al., 2016)
	17.8 ± 1.3	NA	NA	1.3	1.8	NA	Chen et al. (Chen et al., 2016)
PFHxS	11.4 ± 2.6	ND	1.3 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	1.6 ± 0.1	This study
	0.7 ± 0.1	NA	NA	2.8 ± 0.0	2.5 ± 0.1	NA	Chen et al. (Chen et al., 2016)
	8.6 ± 1.5	NA	NA	2.7 ± 0.14	1.9 ± 0.1	NA	Chen et al. (Chen et al., 2016)
FOSA	9.6 ± 5.7	3.0 ± 0.1	ND	2.8 ± 0.1	2.7 ± 0.1	ND[c]	This study
6:2 FTSA	10.5 ± 2.0	ND	0.5 ± 0.0	1.0 ± 0.1	1.1 ± 0.1	0.7 ± 0.1	This study

PFHpA – perfluoroheptanoate, PFOA – perfluorooctanoate, PFNA – perfluorononanoate, PFBS – perfluorobutane sulfonate, PFHxS – perfluorohexane sulfonate, 6:2 FTSA – 6:2 perfluorooctanesulfonate, FOSA – perfluorooctane sulfonamide
ND – not determined due to high control levels (brain tissue) or unstable levels over the time of experiment (liver and carcass); NA – BCF not reported in the study.
[a] Calculated as weighted average of the tissues.
[b] Single chemical exposure scenario.
[c] Not determined due to missing carcass value.

significant difference have been found in model performance when PBK model was tested with various reabsorption parameters (Vidal et al., 2019). Thus we do not account for reabsorption process in our model. Contribution of branchial and renal clearances to the total clearance have been shown to differ between PFAS in fish (Consoer et al., 2016). For zebrafish, following the discussion by Khazaee et al. (Khazaee and Ng, 2018) and Wang et al. (Wang et al., 2022b), we considered urinary excretion for all studied PFAS negligible compared to the gill excretion. However, the model contains a kidney compartment and it can be parametrised to account for renal excretion when parameters for this elimination route become available. Excretion was modelled to occur primarily via gill elimination and to a smaller extent via spawning of eggs.

Since PFAS is present in the environment not only in neutral form, but also (and for some predominantly) in ionic form, we considered the ionisation potential of PFAS by using D_{ow} instead of K_{ow} in the gill uptake and excretion equations (Eqs. 2, 3). D_{ow} was estimated as follows:

$$Dow = fn_{fish} * 10^{logKow} + \left(1 - fn_{fish}\right) * 10^{logKow_{ion}} \quad (1)$$

where fn_{fish} represents the neutral form of chemical in fish body, and $log\ Kow_{ion}$ was assumed to be 3.1 log units lower than $log\ Kow$ (Armitage et al., 2013).

Developed models accounted for uptake and elimination of PFAS via the gills, where the gill uptake rate constant kx (mL/day) depends on gill ventilation, resistance of water ($\rho_{water} = 2.8*10^{-3}$ day·kg$^{0.75}$) and resistance of lipid layers ($\rho_{lip} = 68$ day·kg$^{0.75}$) (Erickson and Mckim, 1990; Hendriks et al., 2001) based on the study of Hendriks et al. (Hendriks et al., 2001) In addition, we added the blood perfusion parameter (Y_{blood}) as suggested in Wang et al. (Wang et al., 2022b)

$$kx = \frac{\left(\frac{BW}{1000}\right)^{0.75}}{\rho_{water} + \frac{\rho_{lip}}{Dow} + \frac{1}{Y_{water}} + \frac{1}{Y_{blood}}} * 1000 \quad (2)$$

where BW is the body weight of a fish (g), Y_{water} is gill ventilation coefficient under the given dissolved oxygen concentration in water, Y_{blood} is the mass-normalized blood perfusion coefficient, calculated as a product of cardiac output and blood:water partition coefficient.

The water elimination coefficient was determined as follows (Hendriks et al., 2001):

$$k_{out} = \left(\frac{1}{\rho_{lip} * (Dow - 1) + 1} + 1\right) * kx \quad (3)$$

Two approaches were tested in assessing the chemical fraction available for distribution in the blood. In the first approach (PBK_{Single} model), the free fraction of each chemical was calculated by taking the ratio of free concentration (C_{free}) to the total chemical concentration in the blood (C_{total}). This can be expressed as the inverse of the equilibrium association constant (K_a) times the total protein concentration in the blood (B_{total}), as proposed by Cheng et al. (Cheng and Ng, 2021) In this method, we assume immediate steady-state in the binding process and constant levels of bound fraction of the chemicals.

$$free\_fraction = \frac{C_{free}}{C_{total}} = \frac{1}{1 + K_a * B_{total}} \quad (4)$$

In the second approach (PBK_{Mix} model), a competitive binding algorithm (Blay et al., 2020) was implemented in the PBK model (Figure S2). We refer to the original publication for the detailed description of the algorithm (Blay et al., 2020). In brief, we assumed a 1:1 M binding between PFAS and protein and calculated the amount of chemical bound to protein iteratively depending on the chemicals dissociation constant (pK_d) and total amount of protein available, based on the algorithm proposed by Blay et al. (Blay et al., 2020) We assumed immediate steady-state of the binding of PFAS in the blood. As zebrafish lack the albumin gene (Noel et al., 2010; Nolte et al., 2018), it is unclear which protein is most relevant for distribution of small molecules, as a worse-case scenario we therefore used the total protein level in zebrafish serum, reported by Li et al. (Li et al., 2016) Studies have reported large variation in pK_d values measured with different methods, thus, we applied human serum albumin dissociation constant values from Jackson et al. (Jackson et al., 2021) who investigated all studied compounds, except FOSA. PBK_{Mix} model is capable of predicting dose-at-target levels for many chemicals by taking in the chemical-specific parameters in the vector format and assessing kinetic interactions between them with the above described algorithm.

Partition coefficients (PCs) between tissues and blood can be derived

from experimental data or predicted using Quantitative Structure-Property Relationship (QSPR) models. Several regression models exist for predicting PCs, however, they are less relevant for PFAS as most of them rely on K_{ow} and are guided by fat content of a tissue. K_{ow} is a challenging parameter to measure for PFAS as they are surface active chemicals (Rayne and Forest, 2009; Hidalgo and Mora-Diez, 2015), and the experimental measurements have large variation and are available for few PFAS (Chelcea et al., 2020). We therefore used PCs derived from experimental data by dividing the area under the concentration-time curve (AUC) of compound's tissue concentration by the AUC of the compound in the blood during the exposure period as suggested by Deepika et al. (Deepika and Kumar, 2023) (Table S3). Average values were used in the PBK model, where available. Muscle and gill PCs were used for poorly and richly perfused tissues, respectively.

Experimental data was however not available for the 6:2 FTSA and FOSA and therefore we applied a recently developed QSPR model that includes protein and phospholipid content of a tissue and chemical properties such as membrane-water partitioning (D_{mw}) and protein-water partitioning (K_{pw}) (Sun et al., 2022). This model has been shown to be predictive for PFAS yielding predictions closer to observed values for 5 out of 7 PFAS as compared to the previous model by Henriks et al. (Hendriks et al., 2001) (Table S4). We used the fish tissue composition from Wang et al. (Wang et al., 2022b) and zebrafish protein content from Wen et al. (Wen et al., 2019) for predicting PCs for these two compounds.

2.5. PBK model validation

Since the gill exchange parameters were obtained through mathematical calculations (i.e. kx and k_{out}) and tissue PCs were derived from the available data (i.e., PCs for PFHpA, PFOA, PFNA, PFBS, PFHxS) or predicted with a partitioning model (i.e., PCs for 6:2 FTSA and FOSA), no parameters were fitted to the data. All data sets with tissue levels presented in Table 2 were used as external validation sets.

Predictions obtained with the developed model were compared to the available *in vivo* data in terms of fold difference (FD). FD was calculated as follows:

$$FD = e^{\left|ln\left(\frac{P_{ij}}{O_{ij}}\right)\right|} \quad (5)$$

where P_{ij} is the predicted concentration of chemical i in organ j and O_{ij} is the measured concentration at a certain time point.

2.6. Sensitivity analysis

Global sensitivity analysis was performed by the variance-based Sobol method (Saltelli et al., 2010) for the PBK_{Single} model with PFOA and PFBS as representatives of studied PFAS. As for the PBKMix model, we performed a corresponding sensitivity analysis for a binary mixture of PFNA and PFHxS. For the analysis we used whole body AUC as output and both physiological and physicochemical parameters of the model were varied by 20 % of the mean values.

2.7. Software

All models were developed in R (v 4.2.1). Differential equations were solved with lsodes function of the "deSolve" package. The soboljansen function in R package "sensitivity" was used to perform the sensitivity analysis. PBK_{Mix} and PBK_{Single} model codes are available on GitHub (https://github.com/Feesterra/PBKMix_PFAS).

3. Results and discussion

3.1. In vivo zebrafish experiment

3.1.1. Adult female zebrafish

Seven PFAS including the less studied FOSA and 6:2 FTSA were analysed in the four zebrafish tissues brain, ovaries, liver, and carcass. Since tissue samples were analysed individually per fish, the data represents individual variation in organ levels (Table S5). All compounds studied showed significant signs of accumulation during the exposure period in at least carcass and ovaries compared to controls ($p < 0.05$). None of the PFAS were detected in the control water, however, some of the PFAS were detected in the brain (i.e., PFBS, PFHxS, 6:2 FTSA, FOSA, PFNA, and PFOA) and liver (i.e., 6:2 FTSA, PFHxS, PFOA, and PFBS) of control fish (Table S5). The levels of PFAS in the control fish samples may be explained with unintentional exposure via water and/or diet prior to this study. Different PFAS have been detected in various laboratory fish diets with PFOA being the most abundant (Cao et al., 2022). Water concentrations during the experiment are presented in Table 2 and Table S1. Fish food analysis of PFAS suggested no significant accumulation of studied compounds via the food, assuming fish eat 2 % of their body weight per day (Table S7).

PFNA and PFOA showed clear uptake curves in all analysed tissues, where the highest concentrations were detected for PFNA, almost one order of magnitude higher than PFOA (Fig. 1, Table S5). The uptake of PFHpA was approximately ten times lower than the longer chain PFOA and PFNA, while PFHpA was only detected in carcass and ovaries. The PFSA showed large differences in uptake, with the ovary concentrations peaking above 1000 ng/g wet weight (ww) for PFHxS as compared to 50 ng/g ww for PFBS. Among the two less studied PFAS, the levels in exposed zebrafish were very high for FOSA with brain maximal concentration of over 15,000 ng/g ww already at day 7. Our data suggests that brain accumulation of FOSA is at least at the same level as in the liver. The underlying reasons are unclear but could be related to active transport or variation in toxicokinetics. FOSA showed highest levels also at day 21 among the tested PFAS whereas 6:2 FTSA showed much lower uptake.

Bioconcentration factor (BCF) values (calculated as C_{tissue}/C_{water}) for the studied PFAS were compared with available literature data (Wen et al., 2019; Chen et al., 2016; Ulhaq et al., 2015) (Table 2). We calculated BCF values assuming that steady-state was reached after 32 days of exposure in each organ for all studied compounds. Thus, the calculated BCF might be underestimated for some PFAS. The variation in BCF was rather small between tissues with a notable highest accumulation in ovaries for most PFAS. This study showed lower BCF values compared to the study by Chen et al. (Chen et al., 2016), where adult female zebrafish were exposed to a mixture of 21 PFAS at two different concentrations for a 24 days period and a pool of 5 fish were analysed. Our data was more in line with the study by Wen et al. (Wen et al., 2019) who exposed adult zebrafish to a mixture of 11 PFAS at a nominal concentration of 10 μg/L each for 28 days. Since a larger variety of PFAS were used by Chen et al. (Chen et al., 2016) than in both current study and study by Wen et al. (Wen et al., 2019), it may indicate that certain PFAS, not considered in current study, may have a greater impact on the ADME of other PFAS.

3.1.2. Maternal transfer

The control embryo samples were measured at 0 dpf and 6 dpf whereas exposed embryos were measured at 0, 3, and 6 dpf. Low levels of PFNA and FOSA were measured in embryos of control fish, suggesting some background exposure either from mother or from surrounding water (Table S6). Low levels of some of the studied chemicals were found in ovaries in the control fish, e.g., PFOA, PFBS, and PFHxS (Fig. 2A). PFAS were detected in some control embryos but data showed in these cases overall large variation. Our measurements indicate that all studied PFAS, except PFHpA (quantified in one out of three samples), transferred from the exposed mother to embryos (Fig. 2B). PFBS, PFNA,

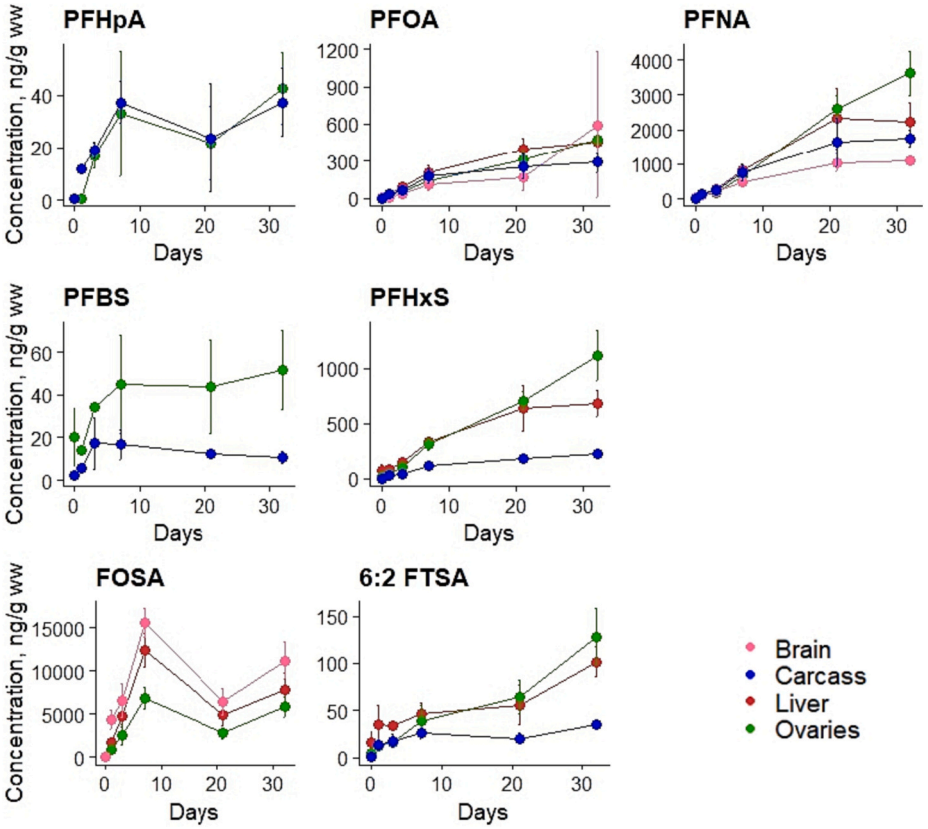


Fig. 1. Concentration over time profiles of studied PFAS in exposed adult female zebrafish. Day 0 indicates levels in control fish samples. Colours represent organs, dots average values of triplicate samples and error bars show the standard deviation. The full list of measured sample levels is provided in Table S5 in the Supporting Information.

and FOSA were found in embryos after 6 days in clean water, however, levels at day 6 were close to background levels. Significant decreases in concentrations of 6:2 FTSA and FOSA were observed in the embryo samples over the studied period ($p < 0.02$) (Fig. 2B, Figs. S3-S9). This is in contrast with the other studied PFAS for which the data suggest no decrease in embryo levels over 6 days of development. The underlying reasons behind the decrease in levels of 6:2 FTSA and FOSA in embryos is unclear, however, we hypothesize that several factors play a role in this process including 1) volume dilution and changes in tissue composition, as embryos grow fast and the yolk is absorbed (observed previously for some compounds (Billat et al., 2023; Chelcea et al., 2023)); 2) shedding of the chorion and as such the loss of any compounds on the chorion surface or within the perivitelline space (Vogs et al., 2019), and 3) metabolism of these chemicals.

Partition coefficients between eggs and ovaries were calculated using the concentrations in the embryos at 0 dpf and ovaries at the end of the exposure period (Table 3). These ranged between 0.08 (FOSA) and 0.34 (PFBS). The partitioning between eggs and ovaries correlated negatively with the size of a molecule and perfluorocarbon chain length (Fig. S10), indicating higher ratios of short chain PFAS in eggs compared to ovaries than that of long chain PFAS.

3.2. PBK modelling

3.2.1. Sensitivity analysis

According to the sensitivity analysis, the most influential parameter was log K_{ow} for both the PBK_{Single} and PBK_{Mix} model (Figs. S16, S17). Notably, parameters describing the spawning and egg laying process were among the ten most sensitive parameters. This means that it is critical to increase our understanding on maternal transfer of these chemicals and thus improve the parametrization of the process. Overall, maternal transfer of PFAS is an important elimination pathway with possible harmful consequences for the offspring, given that PFAS are known to affect embryo development (Menger et al., 2020; Truong et al., 2022; Ulhaq et al., 2013).

3.2.2. Model validation performance

We developed a PBK model for assessment of ADME of individual PFAS, the PBK_{Single} model, and the PBK_{Mix} model where the free fraction in the blood was adjusted for potential competitive binding to proteins in serum. Model outcome was compared with our data plus two additional *in vivo* studies (Table 4). Studied compounds included both PFAS with data available on tissue partitioning and less studied compounds for which QSPR models were used for predicting PCs (i.e., 6:2 FTSA and FOSA).

The PBK_{Mix} model showed reasonable accuracy in predicting the kinetics of our experimental data where 63 % of the predictions, for the 5 PFAS with experimental PCs (i.e., PFHpA, PFOA, PFNA, PFBS, PFHxS) fall within a 3-fold error and 85 % within a 10-fold error (Fig. 3A, Table 4, Fig. S15). The majority of datapoints predicted with >3-folds error is for PFHxS. The chemicals lacking experimental PCs (FOSA and 6:2 FTSA) showed larger errors with 73 % of the model predictions within a 10-fold error and 13 % within 3-fold (Fig. 4). All predictions for 6:2 FTSA were underestimated, whereas a large spread in data was observed for FOSA. Brain concentrations for FOSA were underestimated in the beginning of the exposure, but after 20 days (the last two data points) the predictions were accurate. The model overpredicted tissue levels of FOSA for ovaries and liver. Partition coefficients predicted with two tested QSPR models tended to be overestimated for the studied PFAS (Table S4). The molecular structures of FOSA and 6:2 FTSA differ from PFCAs and PFSAs which may result in different toxicokinetics, e.g., metabolism (Zhao et al., 2021). In zebrafish six different metabolites

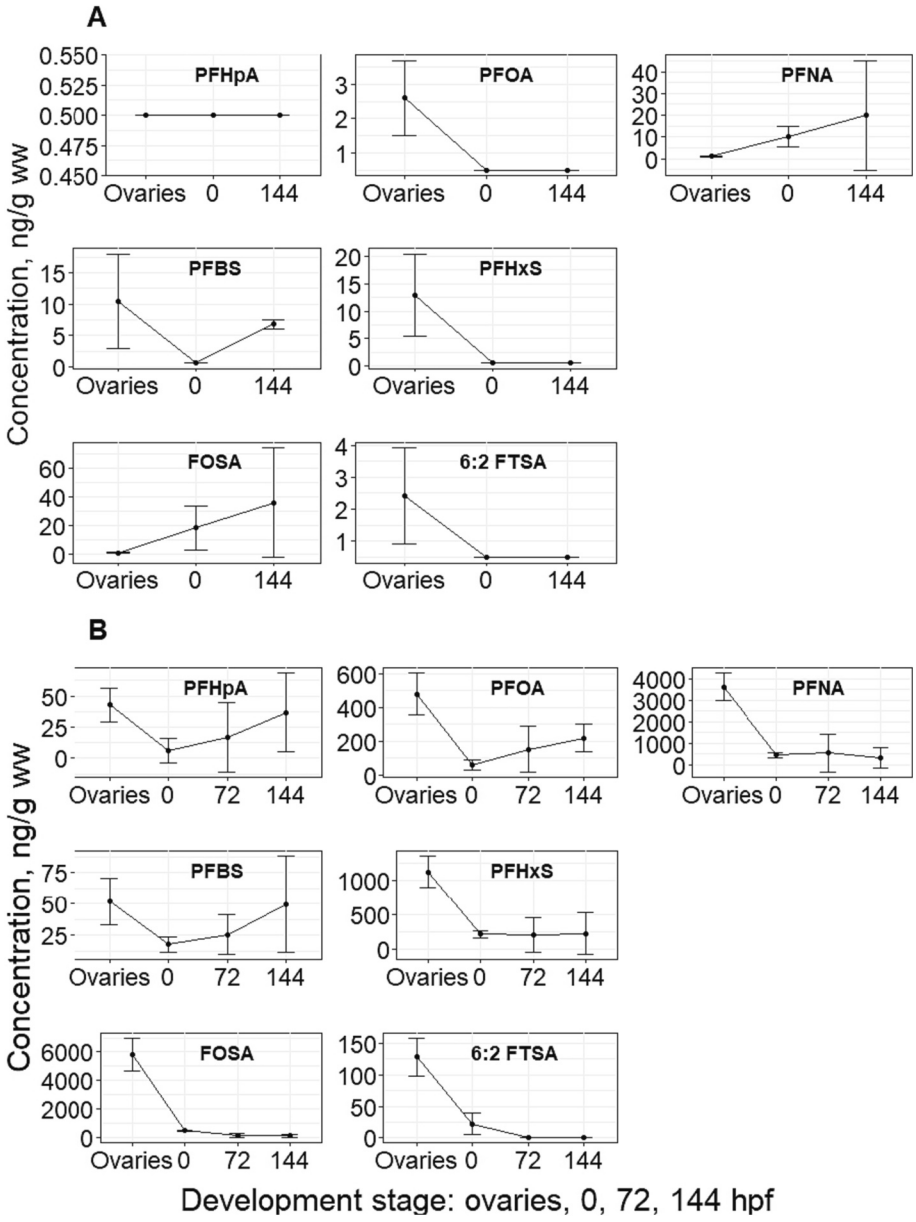


Fig. 2. Levels of studied PFAS in ovaries and embryos obtained from A) control females (unexposed), and B) females exposed for 32 days. Embryos were sampled at 0, 72, and 144 hpf.

Table 3
Partition coefficients between eggs and ovaries.

Compound	$PC_{egg:ovary}$
PFHpA	ND*
PFOA	0.13
PFNA	0.13
PFBS	0.34
PFHxS	0.19
FOSA	0.08
6:2 FTSA	0.18

PFHpA – perfluoroheptanoate, PFOA – perfluorooctanoate, PFNA – perfluorononanoate, PFBS – perfluorobutane sulfonate, PFHxS – perfluorohexane sulfonate, 6:2 FTSA – 6:2 perfluorooctanesulfonate, FOSA – perfluorooctane sulfonamide

* Not determined due to unstable data for the compound.

Table 4
Validation of PBK_{Mix} model reported as predictions within 3- and 10-fold error.

Validation data set	Predictions within 3-fold error, %	Predictions within 10-fold error, %
Our data (PFHpA, PFOA, PFNA, PFBS, PFHxS)[a]	63	85
Our data (6:2 FTSA and FOSA)[b]	13	73
Wen et al. (Wen et al., 2019),	67	98
Chen et al. (Chen et al., 2016), high dose[c]	22	47
Chen et al. (Chen et al., 2016), low dose[c]	22	45

[a] Compounds with experimentally derived PCs.
[b] Compounds with predicted PCs.
[c] Dosing presented in Table 2.

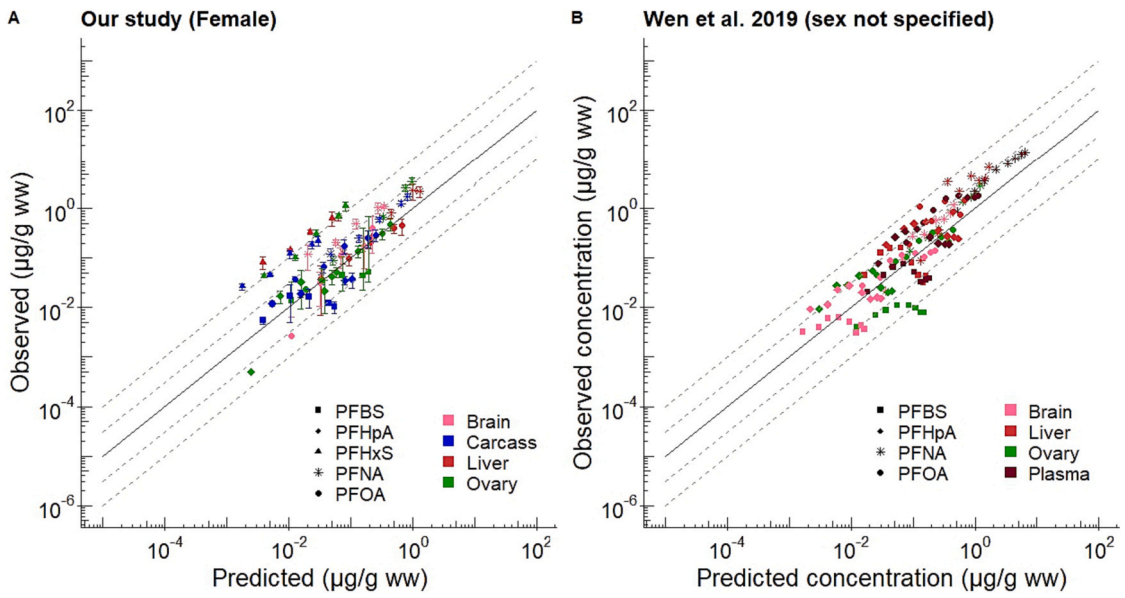


Fig. 3. Observed concentrations in exposed zebrafish versus predicted concentrations obtained with PBK_{Mix} model. Colours represent organs and shapes represent compounds in the mixture. Dashed lines represent 3-fold and 10-fold errors. Note that only experimental data which was not used for model calibration is presented in this figure.

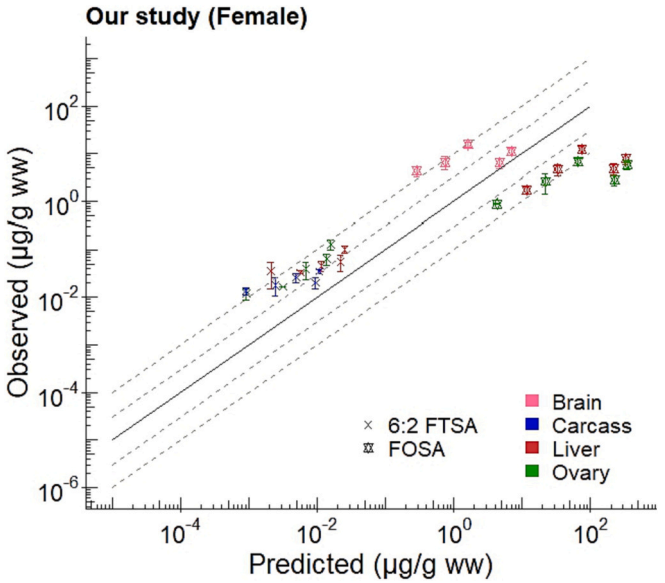


Fig. 4. PBK_{Mix} model performance of FOSA and 6:2 FTSA. Tissue:blood partition coefficients for these two PFAS were predicted with the model suggested by Sun et al. (Sun et al., 2022)

have been detected after FOSA exposure (Han et al., 2021) and fluorotelomer sulfonic acids have been reported to degrade in earthworms (Zhao et al., 2021). This may explain the larger model error of FOSA in the ovaries and liver being tissues with metabolic activity. Overall, more experimental data is required for the two less studied PFAS to better understand their kinetics (Table 2).

The experimental data presented by Wen et al. (Wen et al., 2019) includes four PFAS overlapping with our study, i.e., PFHpA, PFNA, PFOA, and PFBS, for which 67 % of predictions were within a 3-fold error and 98 % of predictions were within a 10-fold error (Fig. 3B). Generally similar model performance was expected based on our data and Wen et al. (Wen et al., 2019) as the study designs were similar. Differences were noted for some compounds (e.g., PFHpA and PFBS) that could be due to factors like sex variation and experimental variability.

Model predictions for PFCAs (PFHpA, PFOA, PFNA) showed better agreement with our experimental data, as compared with PFSAs (PFBS and PFHxS). These results indicate differences in gill uptake and depuration kinetics between PFCAs and PFSAs that the model was not capable to capture using the same gill uptake and elimination equations (Eqs. 2, 3). Higher BCFs for PFSAs as compared with PFCAs, of the same chain length, have been reported (e.g., BCF for PFOS is consistently higher than for PFOA) (Chen et al., 2016; Martin et al., 2003), which is also in line with our observations. Previous studies also showed concentration-dependent kinetics of PFAS in different organisms (Huang et al., 2022; Liu et al., 2011). This indicates that accounting for active transport and different gill exchange equations with concentration-dependent parameters might be required for the two groups when water exposure is considered. Among the PFSAs, PFHxS levels were overall underpredicted, which were not the case for PFBS levels. These differences might reflect kinetic differences due to variation in perfluorocarbon chain length.

The PBK_{Mix} model was also applied to predict the uptake of PFAS using data from Chen et al. (Chen et al., 2016) who studied a mixture of 21 PFAS where 4 were overlapping with current study (PFBS, PFHxS, PFNA, and PFOA). Our model underpredicted the tissue levels of PFNA and PFHxS (Fig. S11) by >10-fold for both high and low dose regimes (see Table 2 for water levels). This is in line with findings from the previously published PBK model for PFOA by Khazaee et al. (Khazaee and Ng, 2018), who were not able to predict data from Chen et al. (Chen et al., 2016), with the exception for the liver, using a permeability-limited PBK model. Most accurate predictions by our model were reached for PFOA and PFBS, with at least 85 % within a 10-fold error (Fig. S11). Different reasons may cause the imperfect predictions of the Chen et al. (Chen et al., 2016) data including potential PFAS precursors in the mixture that may transform to our chemicals or impact their ADME (Table S8). Notably higher BCFs were reported for all chemicals investigated in the current study in the two dosing regimens (Table 2) as compared with both current study and data of the Wen et al. (Wen et al., 2019) study.

As pH of the gill surface possibly has an effect on PFAS uptake, we investigated changes in tissue levels by setting gill pH to 7.4 (close to the pH of the fish body) (Kayim and Can, 2010), and two more acidic conditions (pH 5 and 6). The model predictions indicated that lower pH

increase gill uptake observed from the slightly higher concentrations in the tissues for PFNA, PFOA, FOSA and 6:2 FTSA, but not for PFSAs (Table S9). This is in agreement with previous studies where PFCAs uptake was reported to be dependent on the pH (Sun et al., 2022). Since the model tend to underestimate PFAS tissue levels, increased gill uptake led to decreased model error in most cases.

The PBK_{single} and PBK_{Mix} models reached similar free fractions of studied chemicals in blood serum, leading to comparable (1–3 % difference) model performance (Figs. S12-S14). This suggests no significant influence of competitive serum protein binding on the kinetics. It could be that this is not an important factor for the kinetics of the studied PFAS, the particular mixture, or the chosen exposure levels. Mixture effects on kinetics of individual PFAS have however been described previously where it was shown that long-chain PFAS lead to a decrease in internal concentrations of short-chain PFAS (Wen et al., 2017). Note however that that study included long-chain PFAS not present in the current study (PFOS, PFDA, PFUnA, and PFDoA).

3.2.3. Model limitations and further development

Modelling ADME of PFAS is a challenging task, considering their complex partitioning properties and acidity. PBK models are typically dependent on K_{ow}, which is known to be a less relevant physicochemical parameter for ionizable chemicals. To improve PBK models for PFAS, development of alternative equations that better describe water uptake and depuration processes or partitioning models to feed the PBK models with better estimates on e.g., membrane-water and protein-water partitioning using QSARs including polyparameter linear free energy relationships. Another known phenomena for PFAS is enterohepatic recirculation and it has for example been shown that PFOA is highly accumulating in gallbladder (Ulhaq et al., 2015). However, a previously developed PFOS model for rainbow trout showed no significant differences in PFOS kinetics when different reabsorption parameters were studied (Vidal et al., 2019). Thus, we decided to keep the model simple, however, as data become available, our model can be parametrized for higher accumulation in gallbladder and biliary clearance, which may improve the performance for some compounds.

The PBK_{Mix} model produced similar results as the PBK_{Single} model in estimating the unbound fraction in the blood serum under the current assumptions and rather low PFAS exposures. Specific data (when available) on protein levels and their function as transporters in zebrafish blood as well as data on PFAS affinity to fish-specific proteins, could improve the PBK_{Mix} model. The model may prove to be useful at higher exposure concentrations, where saturation of serum protein binding sites can lead to competitive binding effects. Future potential developments of the model include competitive binding to fatty acid binding proteins in the liver and inclusion co-exposed chemicals transforming to studied PFAS. It is also critical to generate more experimental data on PFAS tissue distribution and develop partitioning models covering a larger range of PFAS. This would increase the applicability domain and precision of the PBK_{Mix} model. The protein binding algorithm implemented in the current study can also be applied in mammalian PBK models, however, separate PBK model development would be needed for desired species.

4. Implications

This study provides new experimental data on uptake and distribution of PFAS chemicals in adult female zebrafish, maternal transfer, and novel data on kinetics of FOSA and 6:2 FTSA, which have not been studied in zebrafish previously. FOSA showed high bioaccumulation potential especially in the brain, while 6:2 FTSA showed low uptake potential. Special concerns should be taken when it comes to the brain as previous research suggests that some PFAS cause behavioural changes (Hawkey et al., 2023), and neurophysiological and neurochemical effects (Starnes et al., 2022). In fact, FOSA has been reported to induce hyperactive swimming behaviour in zebrafish (Truong et al., 2022). Research on neurotoxic effects induced by PFAS has mainly focused on PFOS and PFOA, but the current study highlights the need for more research on these effects for other PFAS such as FOSA. Most of the studied PFAS showed relatively high BCF in ovaries leading to maternal transfer. 6:2 FTSA and FOSA, however, showed signs of elimination from the embryo already within 72 h of development, which indicates a short half-life of these compounds in fish embryos.

To our knowledge, the PBK_{Mix} model is the first mixture model for zebrafish developed for and validated on several PFAS. Together with a permeability-limited PBK model for dioxin-like mixtures in humans (Liu et al., 2022), these are the first mixture PBK models, which are designed to be flexible in number of compounds. The PBK_{Mix} model showed good predictivity for the compounds investigated, being more precise for PFCAs than PFSAs. For 6:2 FTSA and FOSA, more data on tissue uptake and elimination is needed to calibrate the model for these types of PFAS. Development of models for tissue partitioning valid for a large PFAS domain is essential to achieve better predictive power for PFAS PBK models. The modelling results indicate no effects of competitive protein binding in blood on tissue levels under current modelling assumptions and low exposure concentrations. On the other hand, modelling indicated increased uptake of PFOA, PFNA, FOSA and 6:2 FTSA, but not for PFSAs, with decreasing pH of the gill surface. The development of PBK models including potential effects of co-exposed chemicals is a step towards improved New Approach Methodologies (NAMs) for assessing dose-at-target and thus next generation environmental risk assessment approaches (Nicola et al., 2023).

CRediT authorship contribution statement

Elena Golosovskaia: Conceptualization, Data curation, Formal analysis, Investigation, Software, Visualization, Writing – original draft, Writing – review & editing. **Stefan Örn:** Conceptualization, Formal analysis, Methodology, Resources, Writing – review & editing. **Lutz Ahrens:** Formal analysis, Methodology, Resources, Writing – review & editing. **Ioana Chelcea:** Formal analysis, Software, Writing – review & editing. **Patrik L. Andersson:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

In vivo data is available in the SI; PBK model code will be shared on GitHub

Acknowledgements

Thank you, Aleksandra Skrobonja, for helping with the PFAS analysis. The research was financially supported by the Swedish Research Council, grants no. 2019-01838, 2018-02264.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.168738.

References

Ahrens, L., Bundschuh, M., 2014. Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: a review. Environ. Toxicol. Chem. 33 (9), 1921–1929.
Armitage, J.M., Arnot, J.A., Wania, F., Mackay, D., 2013. Development and evaluation of a mechanistic bioconcentration model for ionogenic organic chemicals in fish. Environ. Toxicol. Chem. 32 (1), 115–128.

Bai, X., Son, Y., 2021. Perfluoroalkyl substances (PFAS) in surface water and sediments from two urban watersheds in Nevada, USA. Sci. Total Environ. 751, 141622.
Bassler, J., Ducatman, A., Elliott, M., Wen, S., Wahlang, B., Barnett, J., Cave, M.C., 2019. Environmental perfluoroalkyl acid exposures are associated with liver disease characterized by apoptosis and altered serum adipocytokines. Environ. Pollut. 247, 1055–1063.
Bernstein, A.S., Kapraun, D.F., Schlosser, P.M., 2021. A model template approach for rapid evaluation and application of physiologically based pharmacokinetic models for use in human health risk assessments: a case study on per- and Polyfluoroalkyl substances. Toxicol. Sci. 182 (2), 215–228.
Billat, P.A., Brochot, C., Brion, F., Beaudouin, R., 2023. A PBPK model to evaluate zebrafish eleutheroembryos' actual exposure: bisphenol a and analogs' (AF, F, and S) case studies. Environ. Sci. Pollut. Res. Int. 30 (3), 7640–7653.
Blay, V., Otero-Muras, I., Annis, D.A., 2020. Solving the competitive binding equilibria between many ligands: application to high-throughput screening and affinity optimization. Anal. Chem. 92 (18), 12630–12638.
Brinkmann, M., Schlechtriem, C., Reininghaus, M., Eichbaum, K., Buchinger, S., Reifferscheid, G., Hollert, H., Preuss, T.G., 2016. Cross-species extrapolation of uptake and disposition of neutral organic Chemicals in Fish Using a multispecies physiologically-based Toxicokinetic model framework. Environ. Sci. Technol. 50 (4), 1914–1923.
Brusseau, M.L., Anderson, R.H., Guo, B., 2020. PFAS concentrations in soils: background levels versus contaminated sites. Sci. Total Environ. 740, 140017.
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. Integr. Environ. Assess. Manag. 7 (4), 513–541.
Cao, D., Rericha, Y., Powley, C., Truong, L., Tanguay, R.L., Field, J.A., 2022. Background per- and polyfluoroalkyl substances (PFAS) in laboratory fish diet: implications for zebrafish toxicological studies. Sci. Total Environ. 842, 156831.
Chelcea, I., Orn, S., Hamers, T., Koekkoek, J., Legradi, J., Vogs, C., Andersson, P.L., 2022. Physiologically based Toxicokinetic modeling of bisphenols in zebrafish (Danio rerio) accounting for variations in metabolic rates, brain distribution, and liver accumulation. Environ. Sci. Technol. 56 (14), 10216–10228.
Chelcea, I., Vogs, C., Hamers, T., Koekkoek, J., Legradi, J., Sapounidou, M., Orn, S., Andersson, P.L., 2023. Physiology-informed toxicokinetic model for the zebrafish embryo test developed for bisphenols. Chemosphere 345, 140399.
Chelcea, I.C., Ahrens, L., Örn, S., Mucs, D., Andersson, P.L., 2020. Investigating the OECD database of per- and polyfluoroalkyl substances – chemical variation and applicability of current fate models. Environ. Chem. 17 (7), 498–508.
Chen, F.F., Gong, Z.Y., Kelly, B.C., 2016. Bioavailability and bioconcentration potential of perfluoroalkyl-phosphinic and -phosphonic acids in zebrafish (Danio rerio): comparison to perfluorocarboxylates and perfluorosulfonates. Sci. Total Environ. 568, 33–41.
Cheng, W., Ng, C.A., 2021. Bayesian refinement of the permeability-limited physiologically based pharmacokinetic model for Perfluorooctanoic acid in male rats. Chem. Res. Toxicol. 34 (11), 2298–2308.
Consoer, D.M., Hoffman, A.D., Fitzsimmons, P.N., Kosian, P.A., Nichols, J.W., 2016. Toxicokinetics of perfluorooctane sulfonate in rainbow trout (Oncorhynchus mykiss). Environ. Toxicol. Chem. 35 (3), 717–727.
Deepika, D., Kumar, V., 2023. The role of "physiologically based pharmacokinetic model (PBPK)" new approach methodology (NAM) in pharmaceuticals and environmental chemical risk assessment. Int. J. Environ. Res. Public Health *20* (4).
Deepika, D., Sharma, R.P., Schuhmacher, M., Kumar, V., 2021. Risk assessment of Perfluorooctane sulfonate (PFOS) using dynamic age dependent physiologically based pharmacokinetic model (PBPK) across human lifetime. Environ. Res. 199.
Desalegn, A., Bopp, S., Asturiol, D., Lamon, L., Worth, A., Paini, A., 2019. Role of physiologically based kinetic modelling in addressing environmental chemical mixtures - a review. Comput Toxicol 10, 158–168.
Dickman, R.A., Aga, D.S., 2022. A review of recent studies on toxicity, sequestration, and degradation of per- and polyfluoroalkyl substances (PFAS). J. Hazard. Mater. 436, 129120.
Droge, S.T.J., 2019. Membrane-water partition coefficients to aid risk assessment of Perfluoroalkyl anions and alkyl sulfates. Environ. Sci. Technol. 53 (2), 760–770.
Erickson, R.J., Mckim, J.M., 1990. A model for exchange of organic-Chemicals at Fish Gills - flow and diffusion limitations. Aquat. Toxicol. 18 (4), 175–197.
Erickson, R.J., McKim, J.M., Lien, G.J., Hoffman, A.D., Batterman, S.L., 2006. Uptake and elimination of ionizable organic chemicals at fish gills: I. Model formulation, parameterization, and behavior. Environ. Toxicol. Chem. 25 (6), 1512–1521.
Escher, B.I., Baumgartner, R., Lienert, J., Fenner, K., 2009. Predicting the ecotoxicological effects of transformation products. In: Boxall, A.B.A. (Ed.), Transformation Products of Synthetic Chemicals in the Environment. Springer, Berlin Heidelberg: Berlin, Heidelberg, pp. 205–244.
Fenton, S.E., Ducatman, A., Boobis, A., DeWitt, J.C., Lau, C., Ng, C., Smith, J.S., Roberts, S.M., 2021. Per- and Polyfluoroalkyl substance toxicity and human health review: current state of knowledge and strategies for informing future research. Environ. Toxicol. Chem. 40 (3), 606–630.
Foguth, R., Sepulveda, M.S., Cannon, J., 2020. Per- and polyfluoroalkyl substances (PFAS) neurotoxicity in sentinel and non-traditional laboratory model systems: potential utility in predicting adverse outcomes in human health. Toxics 8 (2).
Giari, L., Guerranti, C., Perra, G., Cincinelli, A., Gavioli, A., Lanzoni, M., Castaldelli, G., 2023. PFAS levels in fish species in the Po River (Italy): new generation PFAS, fish ecological traits and parasitism in the foreground. Sci. Total Environ. 876, 162828.
Girardi, P., Merler, E., 2019. A mortality study on male subjects exposed to polyfluoroalkyl acids with high internal dose of perfluorooctanoic acid. Environ. Res. 179 (Pt A), 108743.
Gluge, J., Scheringer, M., Cousins, I.T., DeWitt, J.C., Goldenman, G., Herzke, D., Lohmann, R., Ng, C.A., Trier, X., Wang, Z.Y., 2020. An overview of the uses of per- and polyfluoroalkyl substances (PFAS). Environ Sci-Proc Imp 22 (12), 2345–2373.
Grech, A., Tebby, C., Brochot, C., Bois, F.Y., Bado-Nilles, A., Dorne, J.L., Quignot, N., Beaudouin, R., 2019. Generic physiologically-based toxicokinetic modelling for fish: integration of environmental factors and species variability. Sci. Total Environ. 651 (Pt 1), 516–531.
Haddad, S., Krishnan, K., 1998. Physiological modeling of toxicokinetic interactions: implications for mixture risk assessment. Environ. Health Perspect. 106, 1377–1384.
Han, J., Gu, W., Barrett, H., Yang, D., Tang, S., Sun, J., Liu, J., Krause, H.M., Houck, K.A., Peng, H., 2021. A roadmap to the structure-related metabolism pathways of per- and polyfluoroalkyl substances in the early life stages of zebrafish (Danio rerio). Environ. Health Perspect. 129 (7), 77004.
Hawkey, A.B., Mead, M., Natarajan, S., Gondal, A., Jarrett, O., Levin, E.D., 2023. Embryonic exposure to PFAS causes long-term, compound-specific behavioral alterations in zebrafish. Neurotoxicol. Teratol. 97, 107165.
Hendriks, A.J., van der Linde, A., Cornelissen, G., Sijm, D.T.H.M., 2001. The power of size. 1. Rate constants and equilibrium ratios for accumulation of organic substances related to octanol-water partition ratio and species weight. Environ. Toxicol. Chem. 20 (7), 1399–1420.
Hidalgo, A., Mora-Diez, N., 2015. Novel approach for predicting partition coefficients of linear perfluorinated compounds. Theor. Chem. Accounts 135, 1.
https://echa.europa.eu/-/echa-publishes-pfas-restriction-proposal, 22.02.2023.
Huang, J., Liu, Y., Wang, Q., Yi, J., Lai, H., Sun, L., Mennigen, J.A., Tu, W., 2022. Concentration-dependent toxicokinetics of novel PFOS alternatives and their chronic combined toxicity in adult zebrafish. Sci. Total Environ. 839, 156388.
Jackson, T.W., Scheibly, C.M., Polera, M.E., Belcher, S.M., 2021. Rapid characterization of human serum albumin binding for per- and Polyfluoroalkyl substances using differential scanning Fluorimetry. Environ. Sci. Technol. 55 (18), 12291–12301.
Kabore, H.A., Goeury, K., Desrosiers, M., Vo Duy, S., Liu, J., Cabana, G., Munoz, G., Sauve, S., 2022. Novel and legacy per- and polyfluoroalkyl substances (PFAS) in freshwater sporting fish from background and firefighting foam impacted ecosystems in eastern Canada. Sci. Total Environ. 816, 151563.
Kayim, M., Can, E., 2010. The pH and Total fat values of fish meat in different iced storage period. Asian J. Anim. Vet. Adv. 5 (5), 346–348.
Khazaee, M., Ng, C.A., 2018. Evaluating parameter availability for physiologically based pharmacokinetic (PBPK) modeling of perfluorooctanoic acid (PFOA) in zebrafish. Environ Sci-Proc Imp 20 (1), 105–119.
Li, C., Tan, X.F., Lim, T.K., Lin, Q., Gong, Z., 2016. Comprehensive and quantitative proteomic analyses of zebrafish plasma reveals conserved protein profiles between genders and between zebrafish and human. Sci. Rep. 6, 24329.
Liu, C., Gin, K.Y., Chang, V.W., Goh, B.P., Reinhard, M., 2011. Novel perspectives on the bioaccumulation of PFCs–the concentration dependency. Environ. Sci. Technol. 45 (22), 9758–9764.
Liu, R., Zacharewski, T.R., Conolly, R.B., Zhang, Q., 2022. A physiologically based pharmacokinetic (PBPK) modeling framework for mixtures of dioxin-like compounds. Toxics 10, 11.
Loccisano, A.E., Longnecker, M.P., Campbell Jr., J.L., Andersen, M.E., Clewell 3rd, H.J., 2013. Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages. J. Toxicol. Environ. Health A 76 (1), 25–57.
Louisse, J., Dellafiora, L., van den Heuvel, J., Rijkers, D., Leenders, L., Dorne, J.C.M., Punt, A., Russel, F.G.M., Koenderink, J.B., 2023. Perfluoroalkyl substances (PFASs) are substrates of the renal human organic anion transporter 4 (OAT4). Arch. Toxicol. 97 (3), 685–696.
Martin, J.W., Mabury, S.A., Solomon, K.R., Muir, D.C.G., 2003. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (Oncorhynchus mykiss). Environ. Toxicol. Chem. 22 (1), 196–204.
Menger, F., Pohl, J., Ahrens, L., Carlsson, G., Orn, S., 2020. Behavioural effects and bioconcentration of per- and polyfluoroalkyl substances (PFASs) in zebrafish (Danio rerio) embryos. Chemosphere 245, 125573.
Mikolajczyk, S., Warenik-Bany, M., Pajurek, M., 2023. Perfluoroalkyl substances in Baltic fish - the risk to consumers. Environ. Sci. Pollut. Res. Int. 30 (21), 59596–59605.
Mit, C., Tebby, C., Gueganno, T., Bado-Nilles, A., Beaudouin, R., 2021. Modeling acetylcholine esterase inhibition resulting from exposure to a mixture of atrazine and chlorpyrifos using a physiologically-based kinetic model in fish. Sci. Total Environ. 773, 144734.
Mittal, V.K., Ng, C.A., 2018. Formation of PFAAs in fish through biotransformation: a PBPK approach. Chemosphere 202, 218–227.
National Toxicology Program, N, 2019. The toxicity studies of perfluoroalkyl sulfonates (perfluorobutane sulfonic acid, perfluorohexane sulfonate potassium salt, and perfluorooctane sulfonic acid) administered by gavage to Sprague Dawley (Hsd: Sprague Dawley SD) rats.
Nicola, M.R.D., Cattaneo, I., Nathanail, A.V., Carnesecchi, E., Astuto, M.C., Steinbach, M., Williams, A.J., Charles, S., Gestin, O., Lopes, C., Lamonica, D., Tarazona, J.V., Dorne, J., 2023. The use of new approach methodologies for the environmental risk assessment of food and feed chemicals. Curr Opin Environ Sci Health 31, 1–8.
Noel, E.S., Reis, M.D., Arain, Z., Ober, E.A., 2010. Analysis of the albumin/alpha-fetoprotein/Afamin/group specific component gene family in the context of zebrafish liver differentiation. Gene Expr. Patterns 10 (6), 237–243.
Nolte, T., Ryan, T., Gunnarsson, L., Verbruggen, B., Klüver, N., Owen, S., 2018. Binding of charged organic chemicals to fish plasma protein: current data availability, modelling aspects and uncertainties.
OECD, 2018. Toward a new comprehensive global database of per-and polyfluoroalkyl substances (PFASs): summary report on updating the OECD 2007 list of per-and

polyfluoroalkyl substances (PFASs). In: Organisation for Economic Cooperation and Development (OECD).
OECD, 2021. Reconciling terminology of the universe of per- and polyfluoroalkyl substances: recommendations and practical guidance. In: OECD Series on Risk Management, 61. OECD Publishing, Paris.
Pickard, H.M., Ruyle, B.J., Thackray, C.P., Chovancova, A., Dassuncao, C., Becanova, J., Vojta, S., Lohmann, R., Sunderland, E.M., 2022. PFAS and precursor bioaccumulation in freshwater recreational fish: implications for fish advisories. Environ. Sci. Technol. 56 (22), 15573–15583.
Rayne, S., Forest, K., 2009. Perfluoroalkyl sulfonic and carboxylic acids: a critical review of physicochemical properties, levels and patterns in waters and wastewaters, and treatment methods. J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng. 44 (12), 1145–1199.
Registry of restriction intentions until outcome. https://echa.europa.eu/registry-of-restriction-intentions/-/dislist/details/0b0236e18663449b, 10 July.
Sadia, M., Nollen, I., Helmus, R., Ter Laak, T.L., Been, F., Praetorius, A., van Wezel, A.P., 2023. Occurrence, fate, and related health risks of PFAS in raw and produced drinking water. Environ. Sci. Technol. 57 (8), 3062–3074.
Saltelli, A., Annoni, P., Azzini, I., Campolongo, F., Ratto, M., Tarantola, S., 2010. Variance based sensitivity analysis of model output. Design and estimator for the total sensitivity index. Comput. Phys. Commun. 181 (2), 259–270.
Scinicariello, F., Buser, M.C., Balluz, L., Gehle, K., Murray, H.E., Abadin, H.G., Attanasio, R., 2020. Perfluoroalkyl acids, hyperuricemia and gout in adults: analyses of NHANES 2009-2014. Chemosphere 259, 127446.
Smart, B., 1994. Characteristics of C-F systems. In: Tatlow, R.B.B.S.J. (Ed.), Organofluorine chemistry: Principles and commercial applications. New York (NY) Plenum, pp. 57–88.
Sörengård, M., Kikuchi, J., Wiberg, K., Ahrens, L., 2022. Spatial distribution and load of per- and polyfluoroalkyl substances (PFAS) in background soils in Sweden. Chemosphere 295, 133944.
Sorengard, M., Bergstrom, S., McCleaf, P., Wiberg, K., Ahrens, L., 2022. Long-distance transport of per- and polyfluoroalkyl substances (PFAS) in a Swedish drinking water aquifer. Environ. Pollut. 311, 119981.
Stadnicka, J., Schirmer, K., Ashauer, R., 2012. Predicting concentrations of organic chemicals in fish by using toxicokinetic models. Environ. Sci. Technol. 46 (6), 3273–3280.
Stanifer, J.W., Stapleton, H.M., Souma, T., Wittmer, A., Zhao, X., Boulware, L.E., 2018. Perfluorinated chemicals as emerging environmental threats to kidney health: a scoping review. Clin. J. Am. Soc. Nephrol. 13 (10), 1479–1492.
Starnes, H.M., Rock, K.D., Jackson, T.W., Belcher, S.M., 2022. A critical review and Meta-analysis of impacts of per- and Polyfluorinated substances on the brain and behavior. Front Toxicol 4, 881584.
Sun, J.M., Kelly, B.C., Gobas, F.A.P.C., Sunderland, E.M., 2022. A food web bioaccumulation model for the accumulation of per- and polyfluoroalkyl substances (PFAS) in fish: how important is renal elimination? Environ Sci Process Impacts 24 (8), 1152–1164.
Tebby, C., Brochot, C., Dorne, J.L., Beaudouin, R., 2019. Investigating the interaction between melamine and cyanuric acid using a physiologically-based Toxicokinetic model in rainbow trout. Toxicol. Appl. Pharmacol. 370, 184–195.
Torres, F.G., De-la-Torre, G.E., 2023. Per- and polyfluoroalkyl substances (PFASs) in consumable species and food products. J. Food Sci. Technol. 60, 2319–2336. https://doi.org/10.1007/s13197-022-05545-7.
Truong, L., Rericha, Y., Thunga, P., Marvel, S., Wallis, D., Simonich, M.T., Field, J.A., Cao, D., Reif, D.M., Tanguay, R.L., 2022. Systematic developmental toxicity assessment of a structurally diverse library of PFAS in zebrafish. J. Hazard. Mater. 431, 128615.
Ulhaq, M., Carlsson, G., Orn, S., Norrgren, L., 2013. Comparison of developmental toxicity of seven perfluoroalkyl acids to zebrafish embryos. Environ. Toxicol. Pharmacol. 36 (2), 423–426.
Ulhaq, M., Sundstrom, M., Larsson, P., Gabrielsson, J., Bergman, A., Norrgren, L., Orn, S., 2015. Tissue uptake, distribution and elimination of (14)C-PFOA in zebrafish (Danio rerio). Aquat. Toxicol. 163, 148–157.
Vidal, A., Babut, M., Garric, J., Beaudouin, R., 2019. Elucidating the fate of perfluorooctanoate sulfonate using a rainbow trout (Oncorhynchus mykiss) physiologically-based toxicokinetic model. Sci. Total Environ. 691, 1297–1309.
Vogs, C., Johanson, G., Naslund, M., Wulff, S., Sjodin, M., Hellstrandh, M., Lindberg, J., Wincent, E., 2019. Toxicokinetics of Perfluorinated alkyl acids influences their toxic potency in the zebrafish embryo (Danio rerio). Environ. Sci. Technol. 53 (7), 3898–3907.
Wang, J.Q., Nolte, T.M., Owen, S.F., Beaudouin, R., Hendriks, A.J., Ragas, A.M.J., 2022b. A generalized physiologically based kinetic model for fish for environmental risk assessment of pharmaceuticals. Environ. Sci. Technol. 56 (10), 6500–6510.
Wang, Y.Q., Hu, L.X., Liu, T., Zhao, J.H., Yang, Y.Y., Liu, Y.S., Ying, G.G., 2022a. Per- and polyfluoroalkyl substances (PFAS) in drinking water system: target and non-target screening and removal assessment. Environ. Int. 163, 107219.
Wang, Z., Buser, A.M., Cousins, I.T., Demattio, S., Drost, W., Johansson, O., Ohno, K., Patlewicz, G., Richard, A.M., Walker, G.W., White, G.S., Leinala, E., 2021. A new OECD definition for per- and Polyfluoroalkyl substances. Environ. Sci. Technol. 55 (23), 15575–15578.
Weiss, J.M., Andersson, P.L., Lamoree, M.H., Leonards, P.E., van Leeuwen, S.P., Hamers, T., 2009. Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin. Toxicol. Sci. 109 (2), 206–216.
Wen, W., Xia, X., Hu, D., Zhou, D., Wang, H., Zhai, Y., Lin, H., 2017. Long-chain Perfluoroalkyl acids (PFAAs) affect the bioconcentration and tissue distribution of short-chain PFAAs in zebrafish (Danio rerio). Environ. Sci. Technol. 51 (21), 12358–12368.
Wen, W., Xia, X.H., Zhou, D., Wang, H.T., Zhai, Y.W., Lin, H., Chen, J., Hu, D.X., 2019. Bioconcentration and tissue distribution of shorter and longer chain perfluoroalkyl acids (PFAAs) in zebrafish (Danio rerio): effects of perfluorinated carbon chain length and zebrafish protein content. Environ. Pollut. 249, 277–285.
Worley, R.R., Yang, X.X., Fisher, J., 2017. Physiologically based pharmacokinetic modeling of human exposure to perfluorooctanoic acid suggests historical non drinking-water exposures are important for predicting current serum concentrations. Toxicol. Appl. Pharmacol. 330, 9–21.
Xu, Y., Nielsen, C., Li, Y., Hammarstrand, S., Andersson, E.M., Li, H., Olsson, D.S., Engstrom, K., Pineda, D., Lindh, C.H., Fletcher, T., Jakobsson, K., 2021. Serum perfluoroalkyl substances in residents following long-term drinking water contamination from firefighting foam in Ronneby, Sweden. Environ. Int. 147, 106333.
Yang, C.H., Glover, K.P., Han, X., 2010. Characterization of cellular uptake of perfluorooctanoate via organic anion-transporting polypeptide 1A2, organic anion transporter 4, and urate transporter 1 for their potential roles in mediating human renal reabsorption of perfluorocarboxylates. Toxicol. Sci. 117 (2), 294–302.
Yuan, Y., He, Q., Zhang, S., Li, M., Tang, Z., Zhu, X., Jiao, Z., Cai, W., Xiang, X., 2022. Application of physiologically based pharmacokinetic modeling in preclinical studies: a feasible strategy to practice the principles of 3Rs. Front. Pharmacol. 13, 895556.
Zhao, S., Liu, T., Zhu, L., Yang, L., Zong, Y., Zhao, H., Hu, L., Zhan, J., 2021. Formation of perfluorocarboxylic acids (PFCAs) during the exposure of earthworms to 6:2 fluorotelomer sulfonic acid (6:2 FTSA). Sci. Total Environ. 760, 143356.