Per- and polyfluoroalkyl substances display structure-dependent inhibition towards UDP-glucuronosyltransferases

Yong-Zhe Liu a, c, d, 1, Zhi-Peng Zhang f, 1, Zhi-Wei Fu a, c, d, Kun Yang a, c, d, Ning Ding b, Li-Gang Hu e, Zhong-Ze Fang a, c, d, *, Xiaozhen Zhuo b, **

a Department of Toxicology and Sanitary Chemistry, School of Public Health, Tianjin Medical University, Tianjin 300070, China
b Department of Cardiology, First Affiliated Hospital of Xi’an Jiaotong University, 277 West Yanta Road, Shaanxi, Xi’an, 710061, China
c Tianjin Key Laboratory of Environment, Nutrition and Public Health, Tianjin 300070, China
d National Demonstration Center for Experimental Preventive Medicine Education, Tianjin Medical University, Tianjin 300070, China
e State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
f Department of Surgery, Peking University Third Hospital, Beijing, China

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Abstract
Per- and polyfluoroalkyl substances (PFASs) are a large group of chemicals and can be detected in environmental and human samples all over the world. Toxicity of existing and emerging PFASs will be a long-term source of concern. This study aimed to investigate structure-dependent inhibitory effects of 14 PFASs towards the activity of 11 UDP-glucuronosyltransferase (UGT) isoforms. In vitro UGTs-catalyzed glucuronidation of 4-methylumbelliferone (4-MU) was employed to determine the inhibition of PFASs towards different UGT isoforms. All the PFASs showed <75% of inhibition or stimulation effects on UGT1A3, UGT1A7, UGT1A9, UGT2B4, UGT2B7 and UGT2B17. However, PFASs showed broad inhibition on the activity of UGT1A1 and UGT1A8. The activity of UGT1A1 was inhibited by 98.8%, 98%, 79.9%, 77.1%, and 76.9% at 100 μmol/L of perfluorodecanoic acid (PFDA), perfluorooctanesulfonic acid potassium salt (PFOS), perfluorotetradecanoic acid (PFTA), perfluorooctanoic acid (PFOA) and perfluorododecanoic acid (PFDoA), respectively. UGT1A8 was inhibited by 97.6%, 94.8%, 86.3%, 83.4% and 77.1% by PFDA, PFTA, perfluorooctadecanoic acid (PFOcDA), PFDoA and PFOS, respectively. Additionally, PFDA significantly inhibited UGT1A6 and UGT1A10 by 96.8% and 91.6%, respectively. PFDoA inhibited the activity of UGT2B15 by 88.2%. PFDA and PFOS exhibited competitive inhibition towards UGT1A1, and PFDA and PFTA showed competitive inhibition towards UGT1A8. The inhibition kinetic parameter (K_i) were 3.15, 1.73, 13.15 and 20.21 μmol/L for PFDA-1A1, PFOS-1A1, PFDA-1A8 and PFTA-1A8, respectively. The values were calculated to be 0.3 μmol/L and 1.3 μmol/L for the in vivo inhibition of PFDA towards UGT1A1 and UGT1A8-catalyzed metabolism of substances, and 0.2 μmol/L and 2.0 μmol/L for the inhibition of PFOS towards UGT1A1 and the inhibition of PFTA towards UGT1A8, respectively. Molecular docking indicated that hydrogen bonds and hydrophobic interactions contributed to the interaction between PFASs and UGT isoforms. In conclusion, exposure to PFASs might inhibit the activity of UGTs to disturb metabolism of endogenous compounds and xenobiotics. The structure-related effects of PFASs on UGTs would be very important for risk assessment of PFASs.

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1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are a large group of man-made fluorinated chemicals. To date, over 4000 unique PFASs have been used in industrial and commercial products, including non-stick coatings, stain repellent and flame retardants (e.g., cooking utensils, food wrappers, electronics, clothes, upholstery, fire fighting foam, etc.). PFASs have been detected in the
environmental and human samples (Jian et al., 2018; Ritscher et al., 2018). Although the concentrations of PFOS, PFOA and other compounds in the population of the United States, Sweden, Germany and other countries have shown a downward trend (Glynn et al., 2012; Olsen et al., 2012; Shu et al., 2018; Toms et al., 2014; Tsai et al., 2018; Yeung et al., 2013), the body burden of PFASs in some developing countries is still high (Fu et al., 2016; Gao et al., 2015; Zhou et al., 2014b). Moreover, the concentration of some unidentified organic fluorides in human samples is increasing (Yeung and Mabury, 2016). Toxicity of PFASs will be a long-term source of concern (Liu et al., 2017).

Various toxicities of PFASs have been frequently reported, which contained hepatotoxicity (Frawley et al., 2018), endocrine disrupting effects (Kar et al., 2017), neurotoxicity (Shrestha et al., 2017), immunotoxicity (Peden-Adams et al., 2008), reproductive and developmental toxicity (Liu et al., 2015; Steves et al., 2018). The animal experiments have shown that PFASs can cause changes in the transcriptional level of UDP-glucuronosyltransferases (UGTs). For example, PFOS exposure in rats was found to cause a reduction in UGT1A1 and UGT2B4 activity in hepatic tissues (Krovel et al., 2008). However, the toxic effects of PFASs towards UGTs remain unclear. UGTs, one of the most important phase II drug-metabolizing enzymes (DMEs), have been reported to play a key role in the metabolism of various xenobiotics and endogenous substances. The inhibition of the activity of UGTs can disturb the metabolism elimination of endogenous substances, which might be a potential contributing factor for xenobiotics-induced toxicity. UGTs are vulnerable to xenogenous inhibitors and the inhibition of UGTs has been employed to evaluate the potential toxic effects of xenobiotics and provide new insights into risk assessment of xenobiotics (Du et al., 2018; Yang et al., 2018; Li et al., 2018a,b).

In this study, we aim to elucidate structure-dependent inhibitory effects of per- and polyfluoroalkyl substances towards the activity of UGTs in vitro. To collect comprehensive information, the inhibitory effect of 14 PFASs on 11 main human UGTs (UGT1A1, 1A3, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15 and 2B17) were determined. The abbreviations, molecular formula and structure of PFASs are shown in Supplemental Table 1. Half inhibition concentration (IC50), inhibition kinetic type and parameters (Ki) were determined. The threshold for the inhibition of PFASs towards UGTs in vivo was extrapolated. In silico docking method was employed to elucidate the inhibition mechanism.

2. Materials and methods

2.1. Chemicals and reagents

Perfluorooctanoic acid (PFOA, 96%), perfluorododecanoic acid (PFDoDA, 95%), and perfluorotetradecanoic acid (PFTA, 96%) were purchased from Sigma-Aldrich (St Louis, MO, USA). Perfluorooctanoic acid (PFOcDA, 97%) was obtained from Alfa Aesar (Bellingham, MA, USA). Perfluorohexanoic acid (PFHxA, 97%), perfluoroctanoic acid potassium salt (PFOS, 98%), perfluorooctyl iodide (POFOI, 98%), perfluorohexyl iodide (PFHxI, 98%), 1H,1H,2H,2H-Nonafluoro-1-hexanol (4:2 FTOH, 97%), 1H,1H-Undecafluoro-1-hexanol (5:1 FTOH, 98%), 1H,1H,2H,2H-Perfluorooctan-1-ol (6:2 FTOH, 98%), 1H,1H-Perfluoro-1-octanol (7:1 FTOH, 98%), and 3,3,4,4,5,5,6,7,8,8,8-Tridecafluorooctane-1-sulphonic Acid (6:2 FTSA, 98%) were purchased from J&K Scientific (Shanghai, China). Stock solutions of compounds were prepared in dimethyl sulfoxide (DMSO, Sigma-Aldrich) and some compounds were dissolved at 37 °C. Recombinant human UGT isoforms (UGT1A1, -1A3, -1A6, -1A7, -1A8, -1A9, -1A10, -2B4, -2B7, -2B15 and -2B17), expressed in baculovirus-infected insect cells, were commercially available from BD Gentest Corp. 4-methylumbelliferone (4-MU), uridine 5′-diphosphoglucuronic acid (UDPGA) (trisodium salt) and 7-hydroxycoumarin were bought from Sigma-Aldrich (St Louis, MO).

2.2. Inhibition of 14 PFASs towards 11 UGTs

In vitro determination of inhibition of 14 PFASs towards 11 UGTs was carried out as previously described (Du et al., 2018; Yang et al., 2018; Liu et al., 2016; Cao et al., 2017). All UGT isoforms except UGT1A4 catalyze the metabolism of 4-methylumbelliferone (4-MU). Therefore, UGT1A4 was not included in this study. The incubation system (200 μL) consists of 100 μm/L PFAS, 5 mm/M MgCl2, different concentrations of 4-MU and UGTs (110 μm/L 4-MU for 0.125 mg/ml UGT1A1, 1200 μm/L 4-MU for 0.05 mg/ml UGT1A3, 110 μm/L 4-MU for 0.025 mg/ml UGT1A6, 30 μm/L 4-MU for 0.05 mg/ml UGT1A7, 750 μm/L 4-MU for 0.025 mg/ml UGT1A8, 30 μm/L 4-MU for 0.05 mg/ml UGT1A9, 30 μm/L 4-MU for 0.05 mg/ml UGT1A10, 1000 μm/L 4-MU for 0.25 mg/ml UGT2B4, 350 μm/L 4-MU for 0.05 mg/ml UGT2B7, 250 μm/L 4-MU for 0.2 mg/ml UGT2B15 and 2000 μm/L 4-MU for 0.5 mg/ml UGT2B17), 50 mm/M Tris-HCl buffer and 2.5 mm/M UDPGA. Incubation system with DMSO was used as the control. After a 5 min pre-incubation at 37 °C, the reaction was begun by adding UDPGA. The reaction lasts for 30–120 min for different UGTs (120 min for UGT1A1, UGT1A3, UGT1A10, UGT2B4, UGT2B7, UGT2B15 and UGT2B17, and 30 min for UGT1A6, UGT1A7, UGT1A8 and UGT1A9, respectively). Ice-cold acetonitrile (volume = 200 μL, 100 μm/L of 7-hydroxycoumarin as the internal standard) was added to terminate the reaction. After the centrifugation at 10,625 × g for 10 min, 10 μL of supernatant was taken for ultra performance liquid chromatography (UPLC) analysis. Chromatographic separation was performed using a C18 column (2.1 × 100 mm, 2 μm, Kromasil) at an elution rate of 0.4 ml/min. All the substances were determined with photo-diode array (PDA) detector at 316 nm. The mobile phases contain H2O containing 0.5% (v/v) formic acid (phase A) and acetonitrile (phase B). Gradient condition was applied as followed: 0–3.5 min, 65% B; 3.5–5 min, 65% B; 5–5.5 min, 10% B; 5.5–7.5 min 10% B. 4-Methylutetrambeliferone β-D glucuronic (4-MUG) was the metabolite of 4-MU. The peak area ratio (4-MUG/internal standard) represents the activity of UGTs.

2.3. Half inhibition concentration (IC50) determination and inhibition kinetic analysis

The inhibitory effect of 0, 0.5, 1, 5, 10, 20, 40, 60, 80 and 100 μm/L of PFASs towards UGTs was determined. IC50 was calculated using probit procedure of SPSS. Different concentrations of 4-MU and PFASs were added in the reaction system to determine the glucuronidation reaction velocity of 4-MU. The concentrations of 4-MU depend on the isoforms of UGTs. The concentrations of PFASs were based on inhibitory rates of PFASs on UGTs in dose-response studies. Lineweaver-Burk plots were used to determine the inhibition type, which was evaluated through determining the intersection point in Lineweaver-Burk plots. The second plot was used to calculate the inhibition kinetic parameters (Ki), and was drawn using slopes from Lineweaver-Burk plot versus the concentrations of PFASs.

2.4. In vitro-in vivo extrapolation (IVIVE) for inhibition of PFASs towards UGTs catalyzed metabolism in vivo

In vitro-in vivo extrapolation (IVIVE) was utilized to predict the inhibition magnitude of UGTs-catalyzed metabolism in vivo. The
following equation was employed:

\[
\frac{AUC_i}{AUC} = 1 + \frac{[I]_{\text{in vivo}}}{K_i}
\]

\(AUC_i/AUC\) was the predicted rate of in vivo exposure of endogenous substances or xenobiotics with or without the exposure of PFASs. \([I]_{\text{in vivo}}\) was the exposure concentration of PFASs in vivo. The \(K_i\) value was inhibition kinetic parameters in vitro. The criteria for judging were as follows: \([I]_{\text{in vivo}}/K_i\) > 1, high possible; 0.1 < \([I]_{\text{in vivo}}/K_i\) < 1, medium possible; \([I]_{\text{in vivo}}/K_i\) < 0.1, low possible.

2.5. Explaining the molecular mechanism for the inhibitory effect of PFASs towards UGTs

In silico docking method was applied to explain the molecular mechanism for the inhibitory effect of PFASs towards UGTs. We constructed the homology model of UGT1A1 by homology modeling method with MODELLER9v14 program. Autodock Version 4.2 was utilized to dock the flexible structures of PFHxA, PFDA and PFOcDA into the cavity of UGT1A1, respectively. The protein domain was kept rigid. The polar hydrogen atoms were added to UGT1A1, and non-polar hydrogen atoms were merged. The grid box was generated with 60 \times 60 \times 60 in X, Y and Z coordinate to cover the entire ligand-binding site. Lamarckian Genetic Algorithm (LGA) method was employed to possess molecular docking study for the binding of PFASs towards UGT1A1. The LGA runs were set to 50 runs for each PFAS. The best conformation with the lowest docked energy was analyzed for the interactions between PFASs and UGT1A1, including hydrogen bonds and hydrophobic contacts.

3. Results

3.1. Inhibition potential of PFASs towards UGT isoforms

When the concentration of PFASs was 100 \(\mu\text{mol/L}\), the residual activity of UGT isoforms was shown in Fig. 1 and Supplemental Fig. 1. All the PFASs showed <75% of inhibition or stimulation effects on UGT1A3, UGT1A7, UGT1A9, UGT2B4, UGT2B7 and UGT2B17 (Supplemental Fig. 1). As shown in Fig. 1, PFASs showed broad inhibition on the activity of UGT1A1 and UGT1A8. The activity of UGT1A1 was inhibited by 98.8%, 98.0%, 79.9%, 77.1%, and 76.9% at 100 \(\mu\text{mol/L}\) of PFDA, PFOS, PFTA, PFOA and PFDoA, respectively. UGT1A8 was inhibited by 97.6%, 94.8%, 86.3%, 83.4% and 77.1% at 100 \(\mu\text{mol/L}\) of PFDA, PFTA, PFOcDA, PFDoA and PFOS, respectively. Additionally, 100 \(\mu\text{mol/L}\) of PFDA inhibited the activity of UGT1A6 and UGT1A10 by 96.8% and 91.6%, respectively. PFDoA (100 \(\mu\text{mol/L}\)) inhibited the activity of UGT2B15 by 88.2% (Fig. 1).

![Fig. 1. Inhibitory effects of 14 PFASs towards UGT1A1, UGT1A6, UGT1A8, UGT1A10 and UGT2B15. The residual activity of UGTs was given, and calculated using the following equations: Residual activity (% CTRL) = the activity at 100 \(\mu\text{mol/L}\) of PFASs / the activity at 0 \(\mu\text{mol/L}\) of PFASs. Data were presented as the mean value plus Standard Error. * The activity of UGTs was inhibited by more than 75%. The experiments were performed in triplicate.](image-url)
3.2. Half inhibition concentration (IC\textsubscript{50})

Given the broad inhibition of UGT1A1 and UGT1A8 by PFASs, the half inhibition concentration (IC\textsubscript{50}) was determined. For the inhibition of UGT1A1, PFDA and PFOS were selected as the representative PFASs due to their strongest inhibition towards UGT1A1. The IC\textsubscript{50} value was calculated to be 4.43 and 4.54 \( \mu \text{mol/L} \) for the inhibition of PFDA and PFOS towards UGT1A1. For the inhibition of UGT1A8, PFDA and PFTA were selected as the representative PFASs due to their strongest inhibition towards UGT1A8. The IC\textsubscript{50} value was calculated to be 13.39 and 38.81 \( \mu \text{mol/L} \) for the inhibition of PFDA and PFTA towards UGT1A8. The dose-response inhibition of PFDA and PFOS on UGT1A1 and PFDA and PFTA on UGT1A8 were shown in Fig. 2.

3.3. The inhibition kinetic type and parameter (K\textsubscript{i})

Furthermore, inhibition type and parameters (K\textsubscript{i}) were determined. As shown in Fig. 3A and Fig. 3B, the intersection point was located in the vertical axis in Lineweaver-Burk plot, indicating the competitive inhibition of PFDA and PFOS towards UGT1A1. Based on the second plot (Fig. 3C and D), the inhibition kinetic parameter (K\textsubscript{i}) was calculated to be 3.15 and 1.73 \( \mu \text{mol/L} \) for the inhibition of PFDA and PFOS towards UGT1A1, respectively. As shown in Fig. 4A and Fig. 4B, both PFDA and PFTA showed competitive inhibition towards UGT1A8. Based on the second plot (Fig. 4C and D), the inhibition kinetic parameter (K\textsubscript{i}) was calculated to be 13.15 and 20.21 \( \mu \text{mol/L} \) for the inhibition of PFDA and PFTA towards UGT1A8, respectively.

3.4. In vitro-in vivo extrapolation (IVIVE) for inhibition of PFASs towards UGTs

According to the evaluation standard (\(|I|_{\text{in vivo}}/K_{i}>1, \text{high possible}; 0.1 < |I|_{\text{in vivo}}/K_{i} < 1, \text{medium possible}; |I|_{\text{in vivo}}/K_{i} < 0.1, \text{low possible} \)), when the plasma concentration of compounds was more than one tenth of K\textsubscript{i}, the inhibition of the compounds towards UGTs-catalyzed metabolism \textit{in vivo} might occur. The value was calculated to be 0.3 \( \mu \text{mol/L} \) and 1.3 \( \mu \text{mol/L} \) for the \textit{in vivo} inhibition of PFDA towards UGT1A1 and UGT1A8-catalyzed metabolism of substances. The value was calculated to be 0.2 \( \mu \text{mol/L} \) and 2.0 \( \mu \text{mol/L} \) for the inhibition of PFOS towards UGT1A1 and the inhibition of PFTA towards UGT1A8, respectively.

3.5. Mechanism explanation of the stronger inhibition of PFDA than PFOA towards UGT1A1

We tried to explain the reason for the stronger inhibition of PFDA than other PFASs towards UGT1A1 using \textit{in silico} docking. PFHxA and PFOcDA were used as a control compound. The results showed that the lowest binding energy of PFDA was \(-3.40 \text{ kcal/mol} \), and the free energy scores of PFHxA and PFOcDA were \(-3.36 \text{ kcal/mol} \) and \(-1.91 \text{ kcal/mol} \), respectively. The lower the score is, the stronger the binding affinity of the PFASs with UGTs. The docking score orders were consistent with the \textit{ex vivo} UGT1A1 inhibition extent (PFDA > PFHxA > PFOcDA). The results suggested that the capabilities of PFASs for inhibiting UGTs were dependent on their structure-related binding affinities with UGTs. That is, the higher binding affinity of PFASs with UGTs caused more severe UGTs inhibition.

Amino acids residues in the binding cavity of UGT1A1 with PFDA contained Cys-152, Leu-182, Gln-183, Arg-184, Val-185, Phe-235, Val-236, Lys-237, Arg-378 and Lys-382. PFDA formed three hydrogen bonds with amino acids Arg-184, Val-236 and Arg-378. Hydrophobic contacts were formed between PFDA and 4 amino acids residues Cys-152, Ser-233, Asp-234 and Phe-235.10 amino acids residues (Pro-151, Cys-152, Leu-182, Gln-183, Arg-184, Val-185, Phe-235, Val-236, Lys-237, Arg-378) were included in the activity cavity of UGT1A1 binding with PFHxA. PFHxA generated six hydrogen bonds with Gln-183, Arg-184, Val-185, Ser-233, Asp-234 and Phe-235.10 amino acids residues (Pro-151, Cys-152, Leu-182, Gln-183, Arg-184, Val-185, Phe-235, Val-236, Lys-237, Arg-378) were included in the activity cavity of UGT1A1 binding with PFHxA.
and Arg-378.2 amino acids residues involved in the formation of hydrophobic interaction with PFHxA contained Cys-152 and Phe-235.14 amino acids residues in the binding cavity of UGT1A1 with PFOcDA contained Cys-152, Leu-182, Gln-183, Arg-184, Val-185, Ser-233, Asp-234, Phe-235, Val-236, Lys-237, Arg-378, Lys-382, Leu-418 and Leu-421. PFOcDA formed two hydrogen bonds with amino acids Arg-184 and Val-236. Hydrophobic contacts were formed between PFOcDA and 6 amino acids residues Cys-152, Gln-183, Ser-233, Asp-234, Arg-378 and Leu-421. The binding modes between PFDA and UGT1A1 were shown in Fig. 5 and Fig. 6. The results suggested that the longer the carbon chain, the fewer hydrogen bonds formed between PFASs and UGT1A1, but conversely, the more amino acids involved in the formation of hydrophobic interactions as the carbon chain increases. The factors that determine the ability to bind between the PFASs and UGT1A1 included both hydrogen bonds formation and hydrophobic interactions.

4. Discussion

The present study investigated the inhibition behaviour of 14 PFASs on the activity of UGTs, and the results showed that six PFASs (PFOA, PFDA, PFDoA, PFOcDA, PFTA and PFOS) exhibited strong inhibition on different isoform of UGTs. The activity of UGT1A1, 1A1, 1A6, 1A8, 1A10 and 2B15 was vulnerable to the inhibition of PFASs. IC50, the inhibition kinetic type and Kᵢ for the inhibition of PFDA and PFOS towards UGT1A1 and the inhibition of PFDA and PFTA towards UGT1A8 are shown in Supplemental Table 2.

UGT1A1 is essential for the efficient elimination of bilirubin (Sugatani, 2013; Gammal et al., 2016), and can catalyze the metabolism of estrogen (Piccinato et al., 2018) and thyroid hormone (Yamanaka et al., 2007). UGT1A1 can also catalyze the glucuronidation reaction of many clinical drugs (e.g. the toxic irinotecan metabolite SN38 (Toffoli et al., 2006), bakuchiol (Li et al., 2017), trovafloxacin (Fujihara et al., 2015)). UGT1A6 plays a key role in the metabolism of serotonin (Sakakibara et al., 2016; Krishnaswamy et al., 2003). UGT1A6 has also been implicated in the glucuronidation conjugation of many currently prescribed drugs including paracetamol (PCM) (Tankanitlert et al., 2007), antidepressants, neuroleptics, and β-adrenoreceptor blockers (Munisamy et al., 2013). UGTs especially UGT1A6 play an important role in bile acids-FXR axis in metabolic diseases (Zhou et al., 2014a; Sun et al., 2018). UGT1A8 and UGT1A10 are the only two UGT1A isoforms expressed in human intestinal tissue (small intestine, colon and rectum). They can catalyze the metabolic elimination of catechol estrogens and thyroid hormone (Yamanaka et al., 2007; Ran et al., 2016). UGT1A8 and UGT1A10 are enzymes for raloxifene glucuronidation (Mizuma, 2009), and play critical role in the metabolism of mycophenolic acid (MPA) (Wang et al., 2016). UGT2B15 is responsible for catalyzing the glucuronidation of androgen (Court et al., 2002; Chouinard et al., 2007) and sipoglitazar (Nishihara et al., 2013). Therefore, PFASs might exert its toxicity through disturbing the UGTs-catalyzed metabolism of endogenous substances and xenobiotics.
It is shown through the present study that PFDA, PFOS and PFTA had higher inhibition rate towards UGTs *in vitro*. PFDA, PFOS and PFTA are "long-chain" PFASs. Due to their persistence, bioaccumulation potential, toxicity, and long-range transport potential, PFDA and PFOS has been assigned as Persistent Organic Pollutants (POPs) since 2009 (Luo et al., 2017; Ritscher et al., 2018). We calculated concentrations of the compounds inhibiting UGTs-catalyzed metabolism, and the value was calculated to be 0.3 μmol/L and 1.3 μmol/L for the *in vivo* inhibition of PFDA towards UGT1A1-and UGT1A8-catalyzed metabolism of endogenous substances and xenobiotics. The value was calculated to be 2.0 μmol/L for the inhibition of PFTA towards UGT1A8. Jian et al. (2018) reported the level of PFAs in human matrices from 2010 to 2017, and the results showed that the highest concentration of PFDA in human blood is 20 ng/ml (0.04 μmol/L), the highest concentration of PFTA is 793.6 ng/ml (1.1 μmol/L), which was not
enough to inhibit the reaction mediated by UGTs in vivo. We also determined that the value was 0.2 μmol/L for the inhibition of PFOS towards UGT1A1. The experiments performed by Zhou et al. analyzed the serum samples from 39 Chinese fishery employees, and the highest median concentration of PFOS was determined to be 10,400 μg/L (20.8 μmol/L) (Zhou et al., 2014b). In the populations from Sweden, the concentration of PFOS was as high as 1,500 μg/L (3 μmol/L) (Li et al., 2018a,b). These values exceed the value of the inhibition of PFOS towards UGT1A1. These results indicate that the concentration of PFOS in some humans may be sufficient to inhibit the metabolism of endogenous and exogenous compounds catalyzed by UGT1A1.

In conclusion, we obtained the inhibition information of 14 PFASs on the activity of UGT isoforms. PFDA inhibited the most isoforms of UGTs, and UGT1A1 and UGT1A8 were most vulnerable UGT isoforms by PFASs. In silico docking study was used to elucidate the inhibition difference for the inhibition of PFDA, PFHxA and PFOcDA towards UGT1A1. Half inhibition concentration (IC50), inhibition kinetic type and parameters (K) were determined for PFDA-UGT1A1, PFOS-UGT1A1, PFDA-UGT1A8 and PFTA-UGT1A8, respectively. The threshold value was calculated, and high possibility for in vivo inhibition was obtained using PFOS-UGT1A1 as an example. All these results provide possible new insights for PFAS-induced toxicity.

Declaration of interests

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.113093.

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