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INTERACTION OF PFOS, PFOA AND 8:2 FTOH WITH HUMAN, RAT, AND MICROBIAL CYTOCHROME P450s: SIMILARITIES AND DIFFERENCES

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Introduction

Chemicals known as perfluoroalkyl and polyfluoroalkyl substances (PFASs) do not occur naturally, but because of wide use in chemical production since the 1950s, they can be found in the environment. Currently, the two most persistent and toxic PFASs are perfluorooctanoic acid (PFOA, $C_7F_{15}COOH$) and perfluorooctane sulfonic acid (PFOS, $C_8F_{17}SO_3H$). PFOS including the form of salts and its precursor, perfluorooctane sulfonyl fluoride, are classified as persistent organic pollutant (POPs) according to Stockholm Convention on POPs. A ubiquitous distribution of PFASs in wildlife and humans is confirmed [1-3] and thus it is important to determine mammalian and microbial metabolisms of PFOS and PFOA with cytochrome P450 (CYP) monooxygenases to understand distribution and impact of PFASs on human and wildlife.

P450 enzymes play important physiological roles in the detoxification of xenobiotics. For example, CYP1A1 proteins can metabolize some dioxins and PCBs by hydroxylation, but the activities of human and rat CYP1A1 proteins are very different. Recently it has been demonstrated [4] that rat CYP1A1 metabolized 3,3',4,4',5-pentachlorobiphenyl (PCB126) into 4-OH-3,3',4',5-tetrachlorobiphenyl and 4-OH-3,3',4',5,5'-pentachlorobiphenyl, but human CYP1A1 did not metabolize. Furthermore, indigenous microorganisms are a key for biotransformation and biodegradation of organic molecules such as xenobiotics, PCBs and pesticides as well as oil hydrocarbons [5-7]. Possible mechanisms of aerobic microbial biotransformation/biodegradation of fluorinated organic compounds in the polluted environment may include catalysis by fungal or bacterial CYP monooxygenases.

Material and methods

In order to determine behavior and metabolism of PFOS, PFOA and 8:2 FTOH, *in vitro* oxidation experiments of PFASs with rat CYP monooxygenases were conducted. Yeast with transformed rat CYP1A1 gene and human CYP1A1 gene were grown in SD media (N-base 6.7 g/L; Glucose 80 g/L and Histidine 160 mg/L) and YPAD media (yeast extract 10 g/L; BactoPeptone 20 g/L; Adenin sulphate 40 mg/L and Glucose 80 g/L). Cytochrome from *Bacillus megaterium* CYP102A1 (also referred to as P450 BM-3), were used for studying interaction of PFASs with microbial cytochromes.

Hydroxylation of PFOS and PFOA with rat CYP1A1 microsomal fraction was studied: Microsomal fraction (protein concentration 40pmol), PFOS and PFOA (100 ppb) and NADPH were used. Concentration of human CYP1A1 was to low to analyze interactions.

The extracts were loaded to Solid Phase Extraction cartridge (Presep PFC-II, Wako Pure Chemical Industries) and MeOH eluates were analyzed using LC/MS/MS.

Competition for CYP binding between chlorotoluron (CT) and PFAS were studied. Herbicide chlortoluron was used as positive control for CYP binding.

Results and discussion

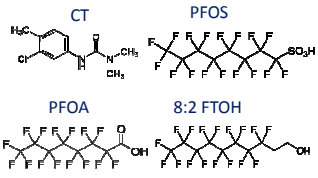
The concentration of microsomal fraction based on CO difference spectroscopy of rat CYP1A1 was 0.3pmol/µL (Fig 1).

1. Products of possible hydroxylation of PFAS after incubation with CYP1A1 were studied using 6 model systems (Table 1).

Table 1. Model systems and recovery rates

	MF	NADPH	PFOS	PFOA	ppb	%
1.	+	+	+	-	80.1	80
2.	+	+	-	+	81.8	82
3.	+	-	+	-	80.6	81
4.	+	-	-	+	96.2	96
5.	-	+	+	-	82.3	82
6.	-	+	-	+	99.5	99

MF-microsomal fraction



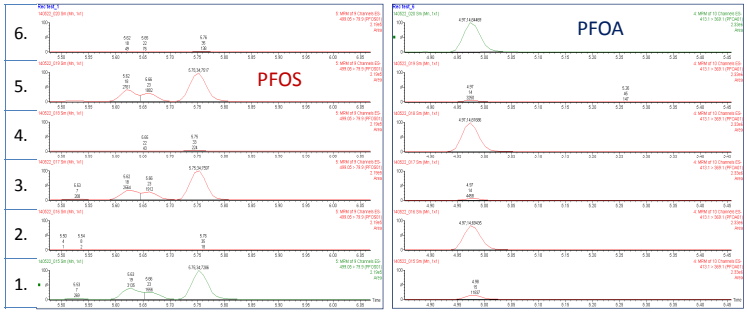


Fig 1. LC/MS chromatograms of 6 model systems extracts

However, recovery rate is suggesting that **PFOS and PFOA interact differently with rat CYP1A1 binding place.** Recovery rate of PFOS from solutions 3 and 5 indicate reduction because of abiotic factors.

High recovery rate in 4 and 6 of PFOA indicate that decrease in solution 2 is possible consequences of interaction of PFOA with MF and NADPH.

Oxidation of PFOA could lead to change of polarity and reduction of recovery rate. However, new peaks, **hydroxylation products were not detected using LC/MS/MS.**

2. Binding interaction of CT, PFOS, PFOA and 8:2 FTOH with rat CYP1A1 and microbial CYP102A1

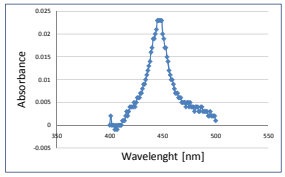


Fig 2. CO difference spectrum of rat CYP1A1

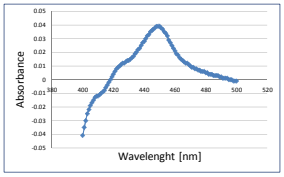
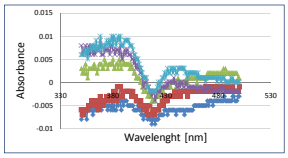


Fig 3. CO difference spectrum of microbial CYP102A1



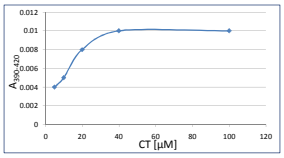
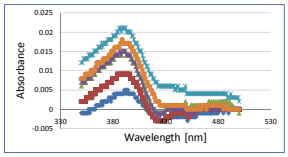


Fig 4a-b. Rat CYP1A1 spectrum of CT binding



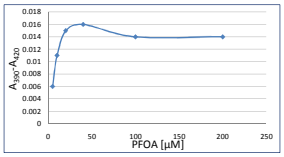


Fig 5a-b. Microbial CYP102A1 spectrum of PFOA binding

Table 2. Kd values of CT, PFOS, PFOA and 8:2 FTOH binding to rat CYP1A1 and microbial CYP102A1

Kd - rat CYP 1A1 [µM]						
CT	PFOS	PFOA	8:2 FTOH	CT & PFOS	CT & PFOA	CT & 8:2FTOH
10.2	44.2	3.94	-42.6	-1.47	0.164	-4.76
Kd - microbial CYP102A1 [µM]						
CT	PFOS	PFOA	8:2 FTOH	CT & PFOS	CT & PFOA	CT & 8:2FTOH
8.62	37.9	1.61	-0.00986	10.3	7.50	-2.32

Rat CYP1A1 Kd for CT is 10.2 µM, for PFOS is 44.2 µM and for PFOA is 3.94 µM. Microbial CYP102A1 Kd for CT is 8.62 µM, for PFOS is 37.9 µM and for PFOA is 1.61 µM. These results suggest that these three substrates are capable to enter binding pocket of P450 and to interact with this enzyme although CT is structurally not similar to PFOS and PFOA. Furthermore, Kd values suggest that **binding affinity increase in the following order: PFOS<CT<PFOA both in mammalian and microbial cytochroms tested**.

During incubation with 8:2 FTOH binding in the binding pocket was not determined in Rat CYP1A1 nor microbial CYP102A1. However, during incubation of Rat CYP1A1 and Microbial CYP102A1 cytochromes with CT & 8:2 FTOH it was observed that binding of CT was inhibited. In model systems with CT + PFOS and CT + PFOA it was observed that kinetics of the reaction did not followed the hyperbolic relationship between the rate of reaction and the concentration of substrate. This data suggest that there is a **competition between CT and PFOS as well as CT and PFOA for the binding pocket of P450**.

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References

1. Haug, L.S., et al., Environ. Int. 37, (2011) 687-693;
2. Croes K., et al., Chemosphere 89, (2012) 988-994;
3. Domingo J.L., Environ. Int. 40, (2012) 187-195;
4. Yamazaki K., et al., J. Biochem. 149, (2011) 487–494;
5. Winogradsky, S., C. R. Acad. Sci. Hebd. Seances Acad. Sci. D. 178 (1924) 1236-1239;
6. Gojgic-Cvijovic, G., et al., Biodegradation 23, (2012) 1-14;
7. Dionisi, D., ChemBioEng Rev 1, (2014) 67–82;