

In vitro toxicological characterisation of PFOA and homologous substances with variant carbon chain length

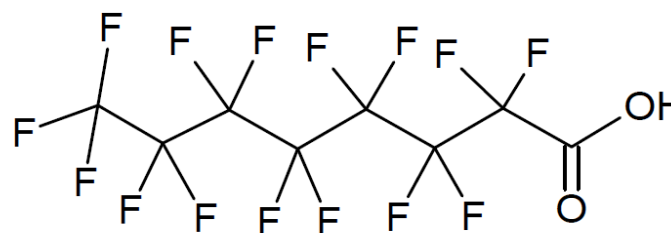
Dr. Thorsten Buhrke

Various PFCAs have been detected in human blood samples

PFCA	Name	Mean Serum Conc. [nM]	Detection Frequency (N = 2094 = 100 %)
PFHpA	Perfluoroheptanoic acid	0.8	6.2 %
PFOA	Perfluorooctanoic acid	9.4	99.7 %
PFNA	Perfluorononanoic acid	2.2	98.8 %
PFDCa	Perfluorodecanoic acid	0.6	31.3 %

Calafat et al. (2007) Environ Health Perspect 115: 1596-1602

PFOA: C8 compound



Correlation between carbon chain length and renal excretion in rats

PFCA	Name	Half-Life $t_{1/2}$ [days]	
		Male Rats	Female Rats
PFHpA	Perfluoroheptanoic acid	0.1	0.05
PFOA	Perfluorooctanoic acid	5.6	0.08
PFNA	Perfluorononanoic acid	29.6	2.4
PFDCa	Perfluorodecanoic acid	39.9	58.6

Ohmori et al. (2007) Toxicology 184: 135-140

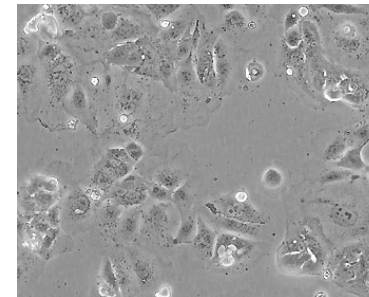
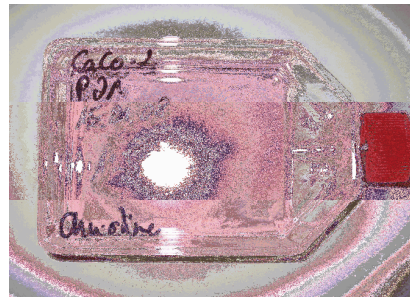
Human: very slow excretion; $t_{1/2}$ for PFOA ~ 3.8 years

Olsen et al. (2007) Environ Health Perspect 115: 1298-1305

PFCAs used in this study

PFCA	Name	Number of Carbon Atoms
PFBA	Perfluorobutyric acid	4
PFHxA	Perfluorohexanoic acid	6
PFHpA	Perfluoroheptanoic acid	7
PFOA	Perfluorooctanoic acid	8
PFNA	Perfluorononanoic acid	9
PFDCa	Perfluorodecanoic acid	10
PFDoA	Perfluorododecanoic acid	12

Model system:
Human liver cell line HepG2



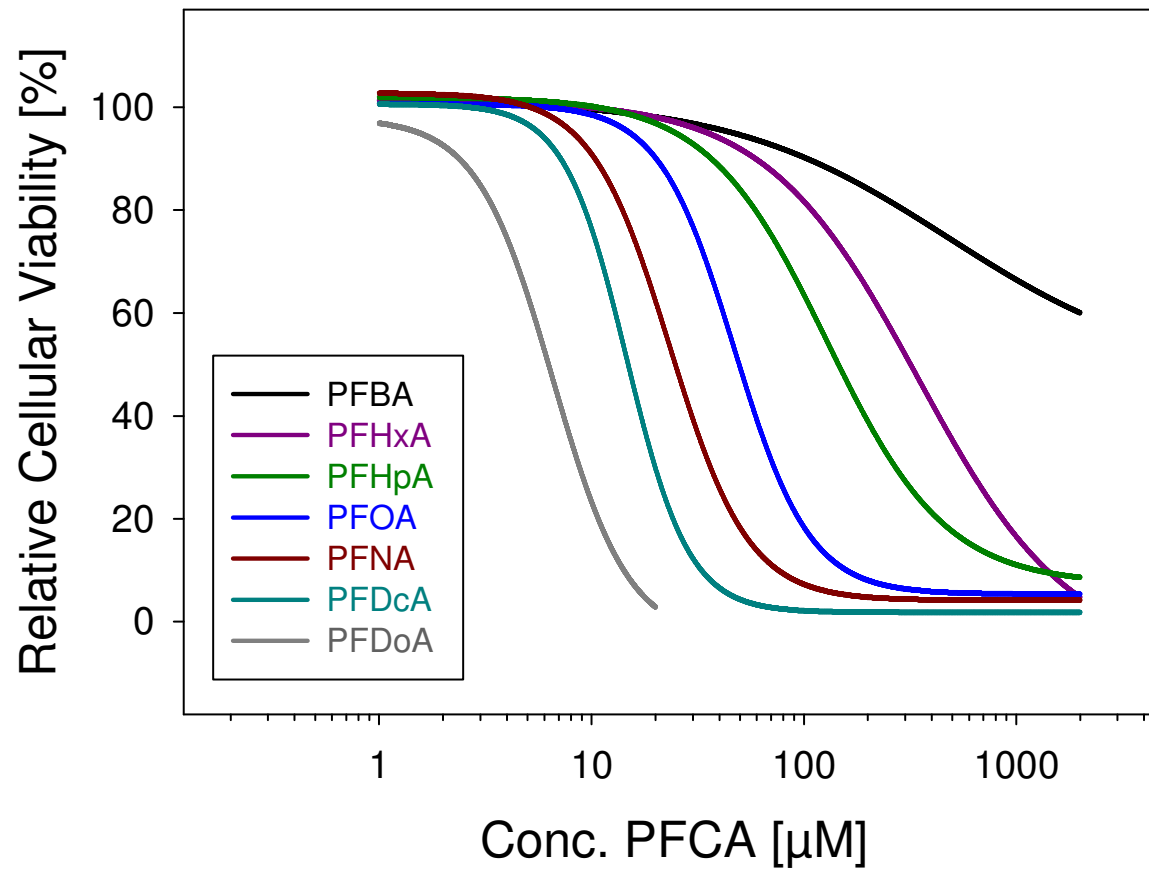
Genotoxicity

PFCAs were tested in two independent in vitro assays for genotoxicity

- Ames Test
- V79 Mikronucleus Assay

→ No incidence of genotoxicity for PFCAs

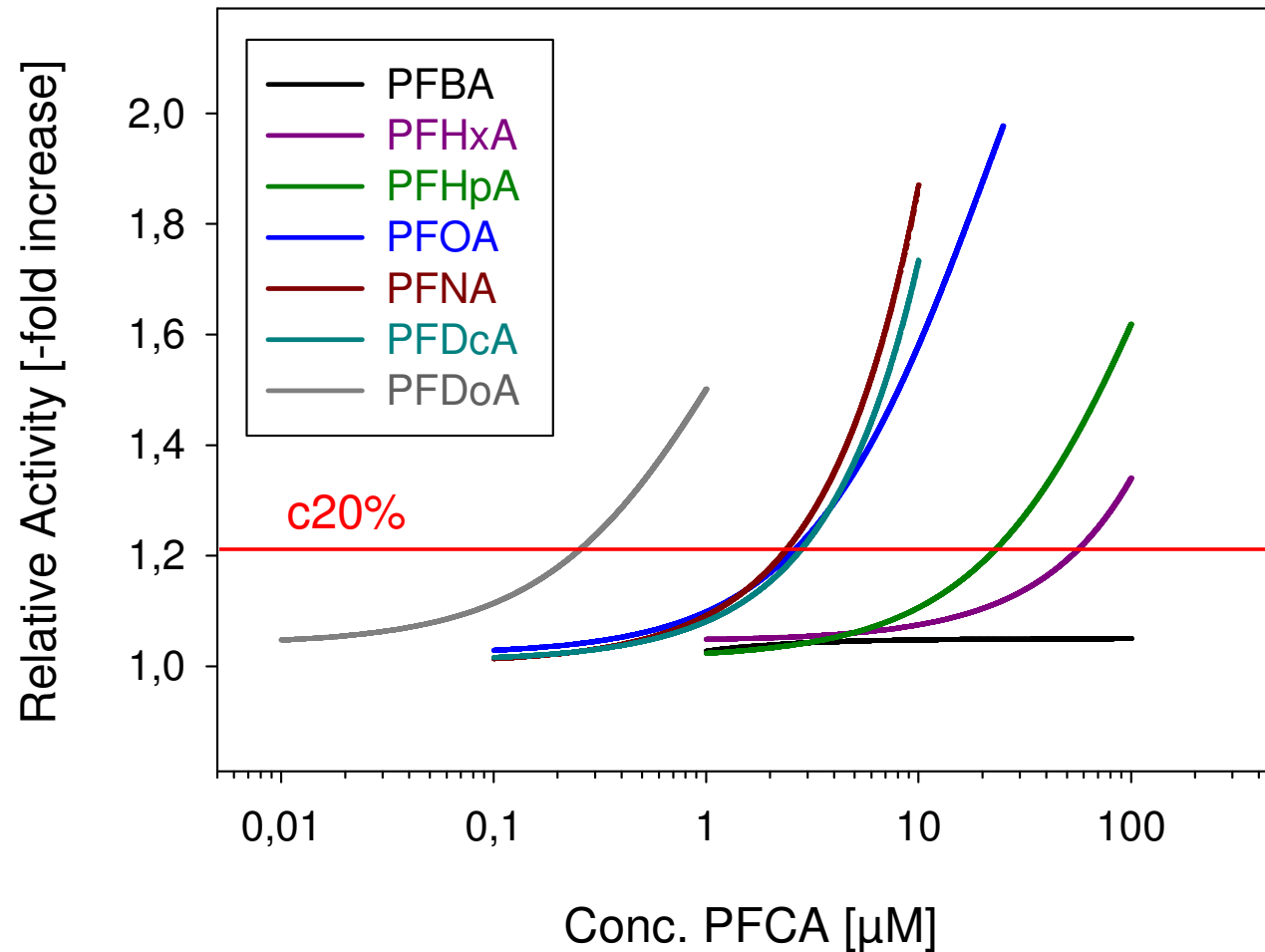
Cytotoxicity of PFCAs (Neutral Red Assay)



PFCA	EC50
PFBA	> 1000 μM
PFHxA	344 μM
PFHpA	128 μM
PFOA	47 μM
PFNA	23 μM
PFDoA	15 μM
PFDcA	7 μM

→ Cytotoxicity of PFCAs increased with increasing carbon chain length

Cellular Proliferation (MTT Assay)



PFCA	c20%
PFBA	> 100 μM
PFHxA	74.3 μM
PFHpA	23.6 μM
PFOA	3.0 μM
PFNA	2.3 μM
PFDCa	3.1 μM
PFDoA	0.3 μM

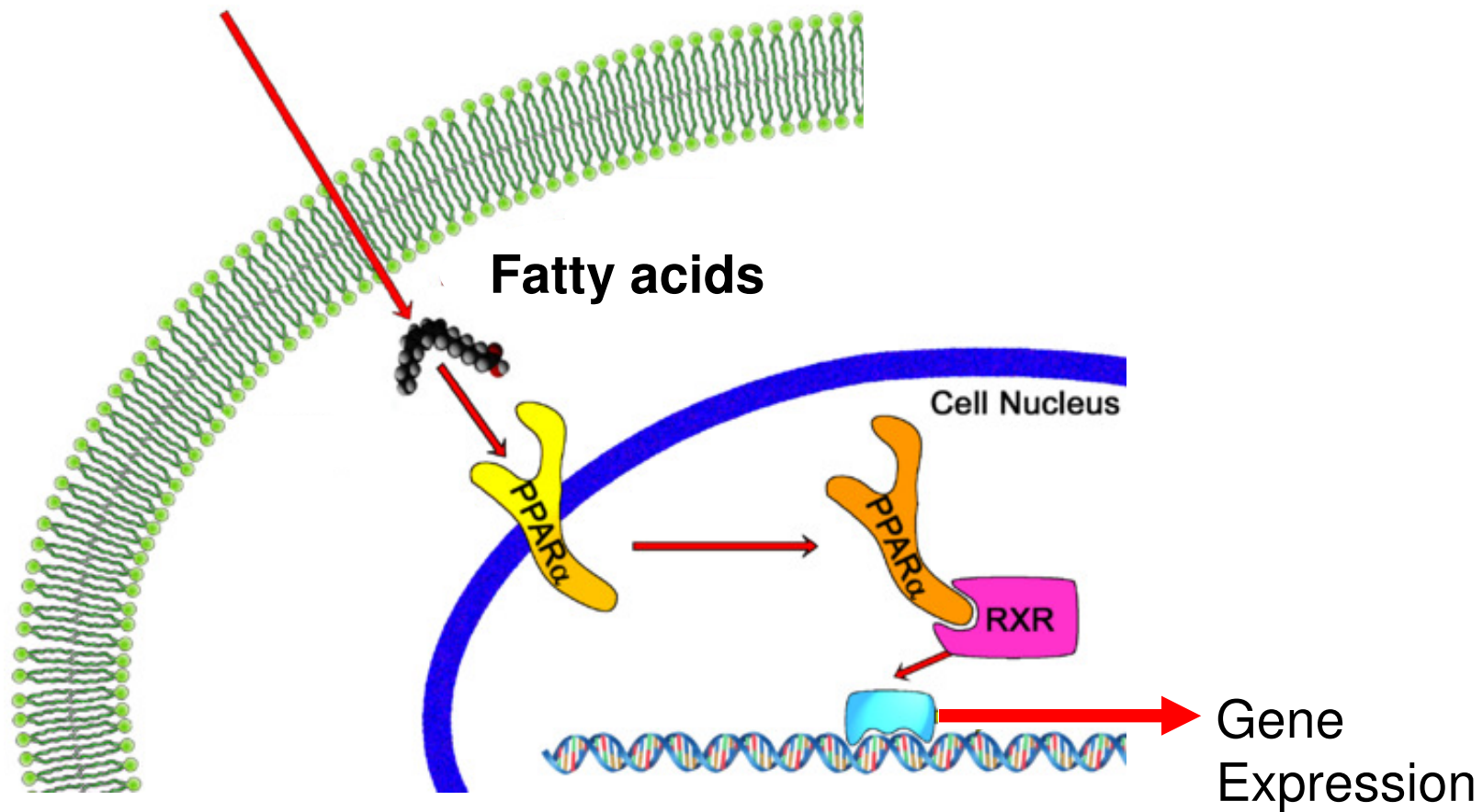
→ Stimulation of proliferation of HepG2 cells by PFCAs increased with increasing carbon chain length

Activation of PPAR α by fatty acids

PPAR α :

Peroxisome Proliferator-activated Receptor alpha

Fatty acids



PPAR isoforms

PPAR Isoform	Organ specificity	Function
PPAR α	Liver	Fatty acid metabolism
PPAR γ	Adipose tissue	Lipid storage
PPAR δ	ubiquitous	Energy homoeostasis

→ PFOA activates PPAR α and PPAR γ (but not PPAR δ)

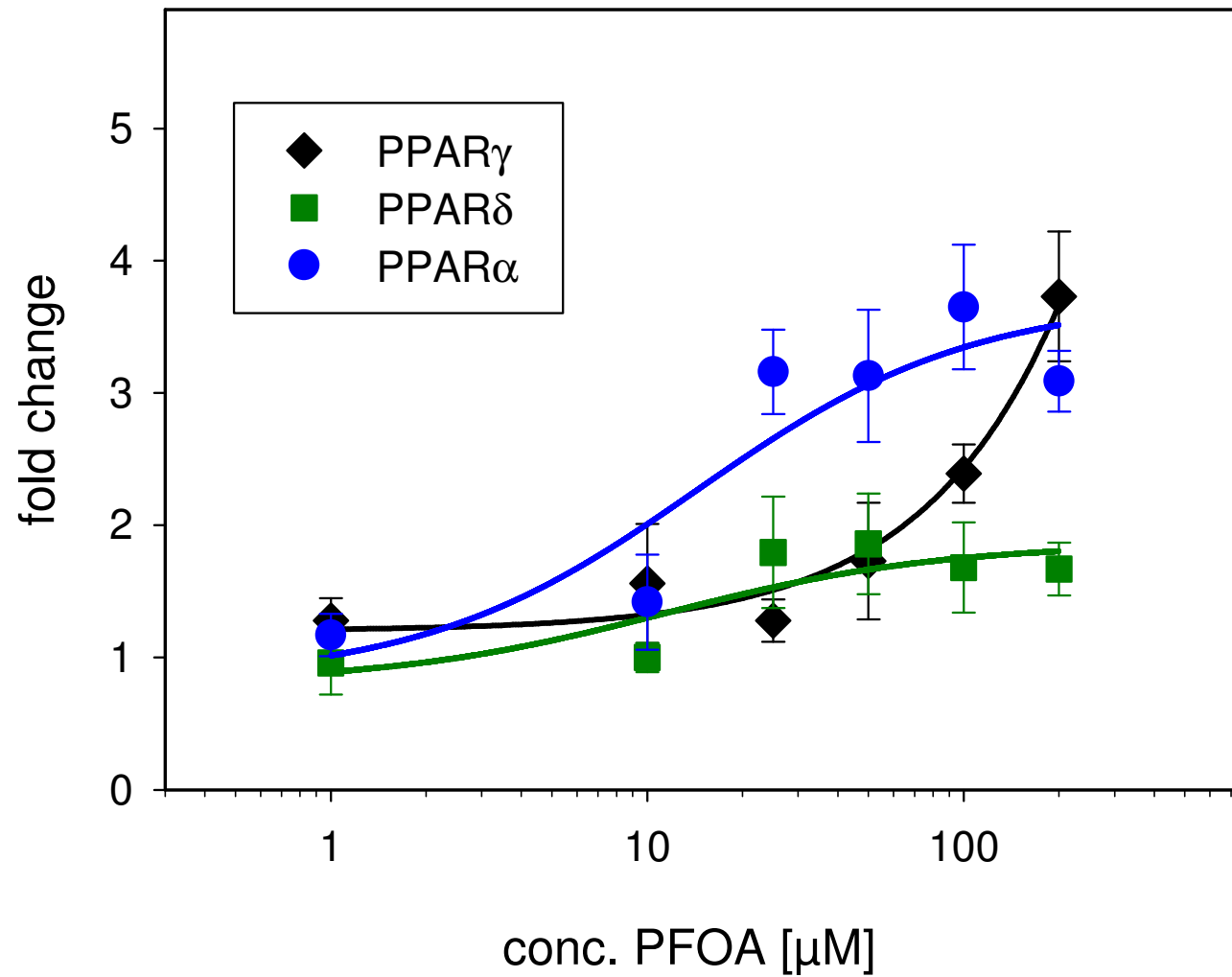
Vanden Heuvel et al. (2006) Toxicol Sci 92: 476-489

→ PFCAs activate PPAR α

positive correlation between carbon chain length and the level of PPAR α activation

Wolf et al. (2008) Toxicol Sci 106: 162-171

PPAR activation by PFOA



PPAR activation by PFCAs

PFCA	Activation of PPAR isoform; c20% values		
	PPAR α	PPAR γ	PPAR δ
PFHxA	15.4 μ M	43.2 μ M	> 200.0 μ M
PFHpA	6.4 μ M	23.2 μ M	51.0 μ M
PFOA	0.9 μ M	19.7 μ M	2.1 μ M

- PFCAs activate PPAR α , PPAR γ , and PPAR δ
- Positive correlation between PFCA carbon chain length and PPAR activation

Summary

	PFOA	PFHxA
Cytotoxicity	47 µM (EC50)	344 µM (EC50)
Stimulation of cellular proliferation	3 µM (c20% value)	74 µM (c20% value)
Activation of PPAR α	0.9 µM (c20% value)	15.4 µM (c20% value)
Activation of PPAR γ	19.7 µM (c20% value)	43.2 µM (c20% value)
Activation of PPAR δ	2.1 µM (c20% value)	> 200 µM (c20% value)

For comparison: PFOA blood serum concentrations in the population

„normal“ burden:	9.4 nM (Calafat et al. (2007) Environ Health Perspect 115: 1596-1602)
	13.8 nM (Fromme et al. (2007) Int Arch Occup Environ Health 80: 313-319)
high burden:	0.2 µM (Steenland et al. (2009) Environ Health Perspect 117: 1083-1088)
	1.7 µM (Olsen et al. (2007) Environ Health Perspect 115: 1298-1305)
Peak values:	12.3 µM (Olsen et al. (2007) Environ Health Perspect 115: 1298-1305)
	17.7 µM (Ehresman et al. (2007) Environ Res 103: 176-184)

Thanks to

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PPAR activation

Cytotoxicity

Cellular Proliferation

Ames Test

V79 Mikronucleus assay

Prof. Dr. Dr. Alfonso Lampen

Thank you for your attention

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