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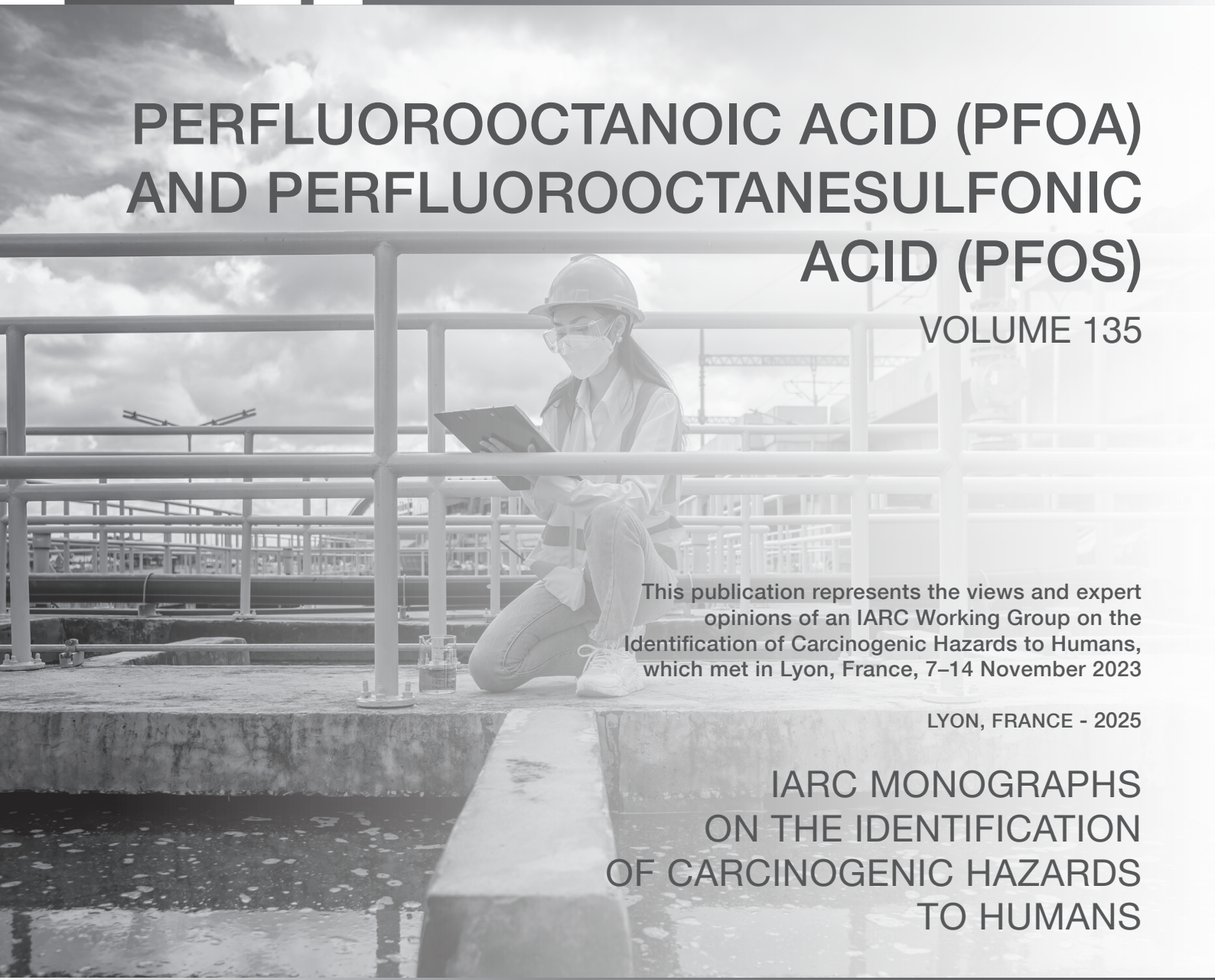

PERFLUOROOCTANOIC ACID (PFOA) AND PERFLUOROOCTANESULFONIC ACID (PFOS)

VOLUME 135

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Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
Peroxisome proliferator-activated receptor α (PPARα) receptor – primary cells							
PPARα-mediated gene transcription	mRNA expression (RT-qPCR)	Primary human hepatocytes	PFOA – form not stated 5–200 μM, 24 h	*ACOX* – no effect *ACOT* – no effect ↑ *CYP4A11*	LEC – 20	No antagonist used	Bjork and Wallace (2009)
			PFOS – form not stated 25 μM, 24 h	*ACOX* – no effect *ACOT* – no effect *CYP4A11* – no effect			
PPARα-mediated gene transcription	mRNA expression (RT-qPCR)	Primary human hepatocytes	PFOA – form not stated 25 μM, 24 h	↑ *ACOX1* *EHHADH* – no effect ↑ *CYP4A11*	N/A	Single concentration No antagonist used	Bjork et al. (2011)
			PFOS – form not stated 25 μM, 24 h	*ACOX1* – no effect *EHHADH* – no effect *CYP4A11* – no effect	N/A		
PPARα-mediated gene transcription	mRNA expression (RT-qPCR)	Primary human hepatocytes	PFOA – form not stated 5–100 μM, 48 h	↑ *PDK4* ↑ *FABP1* *MBL2* – no effect ↑ *CYP4A11* ↑ *CPT1A* ↑ *ANGPTL4* ↑ *HMGCS2* *ACOX1* – no effect	Not reported	No antagonist used No *P*-values reported	Rosen et al. (2013)

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – µM)	Comments	Reference
				↑ *SLC25A34*			
				↑ *PLIN2*			
			PFOS – form not stated	*PDK4* – no effect	Not reported		
			5−250 µM, 48 h	↑ *FABP1*			
				↑ *MBL2*			
				CYP4A11 – no effect			
				CPT1A – no effect			
				↑ *ANGPTL4*			
				HMGCS2 – no effect			
				ACOX1 – no effect			
				↓ *SLC25A34*			
				↑ *PLIN2*			
PPARα-mediated gene transcription	mRNA expression (RT-qPCR)	Primary human hepatocytes	PFOA – Free acid	*CYP4A11*-no effect	LEC – 25	No antagonist used	Marques et al. (2022)
			0.25, 2.5, 25 µM, 48 h	↑ *CD36*			
				SREBF1 – no effect			
				LPL – no effect			
			PFOS – K salt	*CYP4A11* – no effect	LEC – 25		
			0.25, 2.5, 25 µM, 48 h	↑ *CD36*			
				SREBF1 – no effect			
				LPL – no effect			

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End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
PPARα-mediated gene transcription	mRNA expression (microarray and RT-qPCR) and Ingenuity Pathway Analysis	Primary human hepatocytes	PFOA – form not stated 1–100 μM, 24 h	Microarray Predicted upstream regulator: PPARα ↑ pathway expression *RT-qPCR* ↑ *PLIN2*	LEC – 1		Buhrke et al. (2015)
PPARα-mediated gene transcription	mRNA expression (RNA-Seq) and Ingenuity Pathway Analysis	Spheroids composed of primary human hepatocytes and Kupffer cells	PFOA – form not stated 0.02–100 μM, 1, 4, 10 or 14 days	↑ Fatty acid β oxidation pathway	Benchmark conc: Day 1 – ~40 Day 4 – ~55 Day 10 – ~30 Day 14 – ~5	20 μM – highest concentration that did not induce cytotoxicity at any timepoint	Rowan-Carroll et al. (2021)
			PFOS – form not stated 0.02–100 μM, 1, 4, 10 or 14 days	↑ Fatty acid β oxidation pathway	Benchmark conc: Day 1 – ~10 Day 4 – ~10 Day 10 – ~10 Day 14 – ~10	20 μM – highest concentration that did not induce cytotoxicity at any timepoint	
Peroxisome proliferator-activated receptor α (PPARα) receptor– cell lines							
PPARα-mediated gene transcription	mRNA expression (microarray and RT-qPCR)	HepaRG human liver cells	PFOS – Free acid 6.25–400 μM, 24 h	↑ *PDK4* ↑ *CPT1A* ↑ *ANGPTL4* ↑ *PLIN2*	Not reported	No antagonist	Louisse et al. (2023)

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End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – µM)	Comments	Reference
PPARα-mediated gene transcription and lipid accumulation	mRNA expression (microarray and RT-qPCR) and gene set enrichment analysis, MADMAX) Gas chromatography	HepaRG human liver cells	PFOA – Free acid Microarray – 100 µM, 24 h	Microarray (most strongly induced gene sets) Fatty acid β oxidation PPARA Targets	BMC_{50} – 11–19	No antagonist	Louisse et al. (2020)
			RT-qPCR – 6.25–400 µM, 24 h	RT-qPCR ↑*ANGPTL4* (= 200µM) ↑ *PDK4 (> 100µM)* ↑ *PLIN2 (< 50µM)* *CPT1A* – no effect			
			Lipid accumulation 25–200 µM, 24 h	Lipid accumulation – no effect			
			PFOS – Free acid Microarray – 100 µM, 24 h	Microarray – no effect on Fatty acid β oxidation or PPARA targets gene sets	BMC_{50} – 28–72		
			RT-qPCR – 6.25–400 µM, 24 h	RT-qPCR *ANGPTL4* – no effect ↑ *PDK4 (= 100µM)* ↑ *PLIN2(> 50µM)* *CPT1A* – no effect			
			Lipid accumulation	↑ Lipid accumulation			

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End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
			25–100 μM, 24 h				
PPARα-mediated gene transcription	mRNA expression (microarray and RT-qPCR) and WikiPathway analysis	HepaRG human liver cells	PFOA – form not stated 10, 100 μM, 24 h	↑ *APOA2* ↑ *CYP4A11* ↑ *CPT1A* ↑ *FABP1* ↑ *ACADM* ↑ *HMGCS2* (but decrease at 250 μM)	LEC – 10		Murase et al. (2023)
PPARα-mediated gene transcription	mRNA expression (RT-qPCR)	HepG2 human liver cancer cells	PFOA – form not stated 1–250 μM, 24 h	↑ *CPT1A* ↑ *CYP2B6* ↑ *PLIN2*	LEC – 25	Similar to positive control GW7647	Behr et al. (2020b)
PPARα-mediated gene transcription	mRNA expression (RT-qPCR)	HepG2/C3a human liver cancer cells	PFOA – form not stated 0–200 μM, 24 h PFOS – form not stated 25 μM, 24 h	*ACOX1* – no effect *ACOT1* – no effect *CYP4A11*– no effect *ACOX1* – no effect *ACOT1* – no effect *CYP4A11*– no effect		Single concentration of PFOS	Bjork and Wallace (2009)
PPARα binding	Competitive binding assay	Human PPARα ligand-binding domain	PFOA – form not stated 1.14–2500 μM	Positive	IC_{50} – 371		Ishibashi et al. (2019)
			PFOS – form not stated	Positive	IC_{50} – 237		

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End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – µM)	Comments	Reference
			1.14–2500 µM				
PPARα binding	Competitive binding assay	Human PPARα ligand-binding domain (800 nM)	PFOS – form not stated 0–100 µM	Positive	IC_{50} – 248		Li et al. (2018b)
PPARα reporter activation	Reporter assay – full length human PPARα and PPRE-driven reporter	African green monkey Cos-1 fibroblasts	PFOA – form not stated 0.5–40 µM, 24 h	↑ transactivation	LEC – 1		Maloney and Waxman (1999)
PPARα reporter activation	Reporter assay – full length human PPARα and PPRE-driven reporter	African green monkey Cos-7 fibroblasts	PFOA – Free acid 0.01–100 µM, 24 h	↑ transactivation	EC_{50} – 10		Nielsen et al. (2022)
			PFOS – Free acid 0.04–40 µM, 24 h	↑ transactivation	EC_{50} – 24		
PPARα reporter activation	Reporter assay – full length human PPARα and PPRE-driven reporter	MDA-MB-231 human breast cancer cells	PFOA – Free acid 1–100 µM, 24 h	↑ transactivation	EC_{50} – 100		Sakai et al. (2022)
PPARα reporter activation	Reporter assay – full length human PPARα and PPRE-driven reporter	African green monkey Cos-1 fibroblasts	PFOS – form not stated 8–250 µM, 24 h	↑ transactivation	LEC – 16 EC_{50} – 15		Shipley et al. (2004)
PPARα reporter activation	Reporter assay – chimera of human PPARα ligand-binding domain with Gal4 DNA binding domain	Mouse 3T3-L1 fibroblasts	PFOA – NH_4 salt 1–200 µM, 24 h	↑ transactivation	LEC – 50		Vanden Heuvel et al. (2006)
PPARα reporter activation	Reporter assay – chimera of PPARa ligand-binding	African green monkey Cos-1 fibroblasts	PFOA – NH_4 salt 0.5–40 µM, 24 h	↑ transactivation	LEC – 30		Takacs and Abbott (2007)

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End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – µM)	Comments	Reference
	domain with Gal4 DNA binding domain						
			PFOS – K salt 1–250 µM, 24 h	No change	N/A		
PPARα reporter activation	Reporter assay – chimera of PPARa ligand-binding domain with Gal4 DNA binding domain	African green monkey Cos-1 fibroblasts	PFOA – NH_4 salt 0.5–100 µM, 24 h	↑ transactivation	LEC – 10 EC_{20} – 16		Wolf et al. (2008a)
			PFOS – K salt 1–250 µM, 24 h	↑ transactivation	LEC – 30		
PPARα reporter activation	Reporter assay – chimera of PPARa ligand-binding domain with Gal4 DNA binding domain	African green monkey Cos-1 fibroblasts	PFOA – NH_4 salt 0.5–100 µM, 24 h	↑ transactivation	LEC – 1 EC_{20} – 7		Wolf et al. (2012)
PPARα reporter activation	Reporter assay – chimera of PPARa ligand-binding domain with Gal4 DNA binding domain	Human promyelocytic cell line THP-1	PFOA – Free acid 1, 10 µM, 3 h	↑ transactivation	LEC – 10	Short exposure time	Corsini et al. (2012)
			PFOS – Free acid 1, 10 µM, 3 h	No change	N/A		
PPARα reporter activation	Reporter assay – chimera of PPARa ligand-binding domain with Gal4 DNA binding domain	Human kidney cell line HEK293	PFOA – form not stated 1–200 µM, 24 h	↑ transactivation	EC_{20} – 0.9		Buhrke et al. (2013)
PPARα reporter activation	Reporter assays – Cis (endogenous receptor and PPRE-driven reporter)	HepG2 human liver cancer cells	PFOA – form not stated 0.14–300 µM, 24 h	↑ transactivation	Cis EC_{50} – 31 Trans EC_{50} – 23	Potentially toxic concentrations included	Houck et al. (2021)

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End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – µM)	Comments	Reference
	Trans (chimera of PPARa ligand-binding domain with Gal4 DNA binding domain)		PFOS – form not stated 0.14–300 µM, 24 h	↑ transactivation	Cis EC_{50} – 180 Trans EC_{50} – no effect		
PPARα reporter activation	Reporter assay – chimera of PPARa ligand-binding domain with Gal4 DNA binding domain	INDIGO Reporter Cells	PFOA – NH_4 salt 1–200 µM, 24 h	↑ transactivation	EC_{20} – 81		Evans et al. (2022)
			PFOS – K salt 1–200 µM, 24 h	↑ transactivation	EC_{20} – 275		
PPARα reporter activation	Reporter assay – chimera of PPARa ligand-binding domain with a plasmid containing the upstream-activating sequence (UAS)	3T3-L1 mouse fibroblasts	PFOA – Free acid 0.3–100 µM, 22 h	↑ transactivation	LEC – 100		Rosenmai et al. (2016)
PPARα reporter activation	Reporter assay – chimera of PPARa ligand-binding domain with Gal4 DNA binding domain	HepG2 human liver cancer cells	PFOA – Free acid 10, 30, 100 µM, 24 h	↑ transactivation	LEC – 30		Rosenmai et al. (2018)
			PFOS – Free acid 10, 30, 100 µM, 24 h	No effect	N/A		
PPARα reporter activation	Reporter assay – chimera of PPARa ligand-binding domain with Gal4 DNA binding domain	Human kidney cell line HEK293T	PFOA – form not stated 25, 50, 100 µM, 24 h	↑ transactivation	LEC – 50		Behr et al. (2020b)
			PFOS – form not stated	↑ transactivation	LEC – 100		

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End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – µM)	Comments	Reference
			25, 50, 100 µM, 24 h				
CAR and PXR receptors – primary cells							
CAR-mediated gene transcription	mRNA expression (RT-qPCR)	Primary human hepatocytes	PFOA – form not stated 25 µM, 24 h	*CYP2B6* – no effect *CYP2C19* – no effect	N/A	Single concentration	Bjork et al. (2011)
			PFOS – form not stated 25 µM, 24 h	↑ *CYP2B6* ↑ *CYP2C19*	N/A		
CAR-mediated gene transcription	mRNA expression (RT-qPCR)	Primary human hepatocytes	PFOA – Free acid 0.25, 2.5, 25 µM, 48 h	↑ *CYP2B6*	LEC – 25	No antagonist used	Marques et al. (2022)
			PFOS – K salt 0.25, 2.5, 25 µM, 48 h	↑ *CYP2B6*	LEC – 25		
CAR-mediated gene transcription	mRNA expression (microarray) and Ingenuity Pathway Analysis	Primary human hepatocytes	PFOA – form not stated 1–100 µM, 24 h	Microarray Predicted upstream regulator: CAR ↑ pathway expression	LEC – 25	No antagonist used	Buhrke et al. (2015)
PXR-mediated gene transcription	mRNA expression (RT-qPCR)	Primary human hepatocytes	PFOA – form not stated 5–100 µM, 48 h	↑ *CYP3A4*	LEC – 100	No antagonist used No *P*-values reported	Rosen et al. (2013)

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End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
			PFOS – form not stated 5–250 μM, 48 h	↑ *CYP3A4*	LEC – 100		
PXR-mediated gene transcription	mRNA expression (RT-qPCR)	Primary human hepatocytes	PFOA – form not stated 25 μM, 24 h	*CYP3A4* – no effect *ABCB1* – no effect	N/A	Single concentration	Bjork et al. (2011)
			PFOS – form not stated 25 μM, 24 h	↑ *CYP3A4* *ABCB1* – no effect	N/A		
CAR and PXR receptors – cell lines							
CAR-mediated gene transcription	mRNA expression (RT-qPCR)	HepaRG human liver cells	PFOA – form not stated 30, 100 μM, 48 h	↑ *CYP2B6*	LEC – 30		Abe et al. (2017)
CAR-mediated gene transcription	mRNA expression (microarray and RT-qPCR) and WikiPathway analysis	HepaRG human liver cells	PFOA- form not stated 10, 100 μM, 24 h	↑ *CYP2B6*	LEC – 10		Murase et al. (2023)
CAR-mediated gene transcription	mRNA expression (RT-qPCR)	HepaRG human liver cells	PFOA – form not stated 0.0001–1 μM, 24, 48 h	↓ *CYP2C19*	LEC – 0.0001		Franco et al. (2020)
			PFOS – form not stated 0.0001–1 μM, 24, 48 h	↓ *CYP2C19*	LEC – 0.0001		

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End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – µM)	Comments	Reference
CAR-mediated gene transcription	mRNA expression (RT-qPCR)	HepG2 human liver cancer cells	PFOA – form not stated 1–250 µM, 24 h	↑ *CYP2B6*	LEC – 250		Behr et al. (2020b)
PXR-mediated gene transcription	mRNA expression (RT-qPCR)	HepaRG human liver cells	PFOA – form not stated 10–500 µM, 24, 48 h	↑ *CYP3A4*	LEC, 24 h – 50 LEC, 48 h – 50		Behr et al. (2020a)
			PFOS – from not stated 1–100 µM, 24, 48 h	↑ *CYP3A4*	LEC, 24 h – 25 LEC, 48 h – 1		
PXR-mediated gene transcription	mRNA expression (microarray and RT-qPCR) and WikiPathway analysis	HepaRG human liver cells	PFOA – form not stated 10, 100 µM, 24 h	↑ *CYP3A4*	LEC – 100		Murase et al. (2023)
PXR-mediated gene transcription	mRNA expression (RT-qPCR)	HepaRG human liver cells	PFOA – form not stated 0.0001–1 µM, 24, 48 h	↓ *CYP3A4* (only at 48 h)	LEC – 0.0001		Franco et al. (2020)
			PFOS – form not stated 0.0001–1 µM, 24, 48 h	↓ *CYP3A4*	LEC (24h) – 1 LEC (48h) – 0.0001		
PXR Reporter activation	Reporter assay – full length human PXR and CYP3A4 promoter-driven reporter	HepG2 human liver cancer cells	PFOA – Free acid 0.1–30 µM, 24 h	↑ transactivation	EC_{50} – 9		Zhang et al. (2017b)
			PFOS – K salt	↑ transactivation	EC_{50} – 8		

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End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
			0.1–30 μM, 24 h				
PXR Reporter activation	Reporter assays – Cis (endogenous receptor and PXRE-driven reporter)	HepG2 human liver cancer cells	PFOA – form not stated 0.14–300 μM, 24 h	↑ transactivation (PXRE only)	Trans EC_{50} – 35	Potentially toxic concentrations included	Houck et al. (2021)
	Trans (chimera of human PXR ligand-binding domain with Gal4 DNA binding domain)		PFOS – form not stated 0.14–300 μM, 24 h	↑ transactivation	Cis EC_{50} – 9 Trans EC_{50} – 18		
PXR reporter activation	Reporter assay – full length human PXR and CYP3A4 promoter-driven reporter	African green monkey Cos-7 fibroblasts	PFOA – form not stated 0.3–100 μM, 24 h	No effect	N/A		Murase et al. (2023)
PXR reporter activation	Reporter assay – chimera of PPARa ligand-binding domain with Gal4 DNA binding domain	Human kidney cell line HEK293T	PFOA – form not stated 25, 50, 100 μM, 24 h	No effect	N/A		Behr et al. (2020b)
			PFOS – form not stated 25, 50, 100 μM, 24 h	No effect	N/A		
Peroxisome proliferator-activated receptor γ (PPARγ) receptor – primary cells							
PPARγ expression	mRNA expression (RT-qPCR)	Human umbilical vein endothelial cells	PFOS – form not stated 50, 100 μM 24, 48 h	↑ *PPARG*	LEC – 100		Liao et al. (2012)

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End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
PPARγ-mediated gene transcription	mRNA expression (RT-qPCR)	Primary human hepatocytes	PFOA – Free acid 0.25, 2.5, 25 μM, 48 h	*FABP4- no effect* ↑ *CD36* *FASN* – no effect ↑ *SCD* *GPAM* – no effect ↑ *PPARG*	LEC – 25	No antagonist used	Marques et al. (2022)
			PFOS – K salt 0.25, 2.5, 25 μM, 48 h	*FABP4- no effect* ↑ *CD36* *FASN* – no effect *SCD* – no effect *GPAM* – no effect ↑ *PPARG*	LEC – 25		
PPARγ-mediated gene transcription	mRNA expression (microarray) and Ingenuity Pathway Analysis	Primary human hepatocytes	PFOA – form not stated 1–100 μM, 24 h	Microarray Predicted upstream regulator: PPARγ ↑ pathway expression	LEC – 1		Buhrke et al. (2015)
PPARγ-mediated gene transcription	mRNA expression (nanosensors)	Primary human mesenchymal stromal cells	PFOS – Free acid 0.1, 10 μM 7–21 days	↑ *PPARG* ↑ *FABP4*	LEC – 0.1		Gao et al. (2020)
PPARγ-mediated gene transcription and differentiation	mRNA expression (RT-qPCR) and adipocyte differentiation (Oil Red O)	Primary human mesenchymal stromal cells	PFOA – Free acid 0.1–10 μM,	↑ *PPARG* ↑ *FABP4*	LEC – 1		Qin et al. (2022c)

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End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – µM)	Comments	Reference
			RT-qPCR 7–14 days Differentiation 14 days	↑ Lipid accumulation			
			PFOS – K salt 0.1–10µM, RT-qPCR 7–14 days Differentiation 14 days	↑ *PPARG* ↑ *FABP4* ↑ Lipid accumulation	LEC – 1		
PPARγ-mediated gene transcription and differentiation	mRNA expression (RT-qPCR) and adipocyte differentiation (Oil Red O)	Primary human subcutaneous preadipocytes	PFOA – form not stated 0, 6, 12, 25 µM 10 days	↑ *PPARG* ↑ *FABP4* ↑ *PLIN1* ↑ Lipid accumulation	LEC – 6		Li et al. (2019b)
PPARγ-mediated differentiation	Oil Red O staining	Primary human visceral preadipocytes	PFOS – K salt 5, 50 µM 11 days	↑ Lipid accumulation	LEC – 5		Xu et al. (2016)
PPARγ-mediated differentiation	Nile Red staining	Primary human bone marrow mesenchymal stromal cells	PFOA – Free acid 0.0001–10 µM 21 days	↑ Lipid accumulation	LEC – 0.1		Bérubé et al. (2023)
			PFOS – Free acid 0.0001–10 µM	↑ Lipid accumulation	LEC – 1		

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End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
			21 days				
Peroxisome proliferator-activated receptor γ (PPARγ) receptor – cell lines							
PPARγ-mediated gene transcription	mRNA expression (RT-qPCR)	HepaRG human liver cells	PFOA – Free acid 6.25–400 μM, 24 h	↑ *FABP4*	LEC – 100		Attema et al. (2022)
PPARγ binding	Competitive binding assay	Human PPARα ligand-binding domain (800 nM)	PFOS – form not stated 0–500 μM	Positive	IC_{50} – 190		Li et al. (2018b)
PPARγ binding	Competitive binding assay	Human PPARα ligand-binding domain (800 nM)	PFOA – form not stated 0–1000 μM	Positive	IC_{50} – 370		Li et al. (2019b)
PPARγ binding	Competitive binding assay	Human PPARα ligand-binding domain (800 nM)	PFOA – form not stated 1–300 μM	Positive	IC_{50} – 44		Zhang et al. (2014b)
			PFOS – form not stated 0.1–300 μM	Positive	IC_{50} – 14		
PPARγ reporter activation	Reporter assay – full length human PPARγ and PPRE-driven reporter	HepG2 human liver cancer cells	PFOA – form not stated 1–200 μM	↑ Transactivation	LEC – 10		Zhang et al. (2014b)
			PFOS – form not stated 1–200 μM	↑ Transactivation	LEC – 10		

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End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
PPARγ reporter activation	Reporter assay – chimera of PPARγ ligand-binding domain with Gal4 DNA binding domain	Human kidney cell line HEK293T	PFOA – form not stated 25, 50, 100 μM, 24 h	No effect	N/A		Behr et al. (2020b)
			PFOS – form not stated 25, 50, 100 μM, 24 h	No effect	N/A		
PPARγ reporter activation	Reporter assay – chimera of PPARγ ligand-binding domain with Gal4 DNA binding domain	Human kidney cell line HEK293	PFOA – form not stated 3–50 μM, 24 h	↑ Transactivation	LEC – 25	No *P*-values reported	Li et al. (2019b)
PPARγ reporter activation	Reporter assay – chimera of PPARγ ligand-binding domain with Gal4 DNA binding domain	Human kidney cell line HEK293	PFOA – form not stated 1–200 μM, 24 h	↑ Transactivation	EC_{20} – 20	No *P*-values reported	Buhrke et al. (2013)
PPARγ reporter activation	Reporter assay – chimera of PPARγ ligand-binding domain with Gal4 DNA binding domain	Human epithelial cell line HeLa	PFOA – Free acid 1–100 μM, 24 h	↑ Transactivation	EC_{20} – 17	No *P*-values reported	Garoche et al. (2021)
			PFOS – Free acid 1–100 μM, 24 h	↑ Transactivation	EC_{20} – 21		
PPARγ reporter activation	Reporter assays – Cis (endogenous receptor and PPRE-driven reporter) Trans (chimera of human PPARγ ligand-binding	HepG2 human liver cancer cells	PFOA – form not stated 0.14−300 μM, 24 h	↑ transactivation	Cis EC_{50} – 31 Trans EC_{50} – 50	Potentially toxic concentrations included	Houck et al. (2021)
			PFOS – form not stated	↑ transactivation	Cis EC_{50} – 180		

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
	domain with Gal4 DNA binding domain)		0.14–300 μM, 24 h		Trans EC_{50} – 27		
PPARγ reporter activation	Reporter assay – chimera of PPARa ligand-binding domain with Gal4 DNA binding domain	INDIGO Reporter Cells	PFOA – NH_4 salt 10–3000 μM, 24 h	↑ transactivation	EC_{20} – 224		Evans et al. (2022)
			PFOS – K salt 10–300 μM, 24 h	↑ transactivation	EC_{20} – 288		
HNF4α receptor – primary cells and cell lines							
HNF4α-mediated gene transcription	mRNA expression (microarray)	Primary human hepatocytes	PFOA – form not stated 1–100 μM, 24 h	↓ *HNF1A*	LEC – 25	No follow-up with RT-qPCR	Buhrke et al. (2015)
HNF4α-mediated gene transcription	Proteome analysis (MALDI-TOF) with ingenuity pathway analysis (IPA)	HepG2 human liver cancer cells	PFOA – form not stated 25 μM, 48 h	IPA – deregulation of HNF4α pathway	N/A	Single concentration	Scharmach et al. (2012)
	Immunoblot of HNF4α and HNF1α			↓ HNF4α ↓ HNF1α	N/A		
HNF4α reporter activation	Reporter assay – endogenous HNF4α and HNF1A promoter-driven reporter	HepG2 human liver cancer cells	PFOA – form not stated 0.01–50 μM, 24 h	↓ transactivation	LEC – 1		Scharmach et al. (2012)
	Reporter assay – full length HNF4α and HNF1A promoter-driven reporter	Human kidney cell line HEK293		↓ transactivation	LEC – 1		
Thyroid hormone receptor – cell lines							

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
Thyroid peroxidase activity	Enzymatic activity assay	FTC-238 human follicular carcinoma cells	PFOA – form not stated 0.001–100 μM, 48 h	↓ TPO activity only at 1 μM	N/A		Song et al. (2012)
			PFOS – form not stated 0.001–100 μM, 48 h	↓ TPO activity	LEC – 0.001		
Transthyretin binding analysis	TTR-TRβ CALUX (assesses effect on T4 binding to transthyretin)	U2OS human osteocarcinoma cells	PFOA – form not stated 0.1–100 μM (in cell-free TTR incubation)	↓ in T4 binding to TTR	IC_{50} – 2	Assuming human TTR	Sprengel et al. (2021)
Transthyretin binding analysis	TTR-TRβ CALUX (assesses effect on T4 binding to transthyretin)	U2OS human osteocarcinoma cells	PFOA – Free acid 0.003M-109 μM (in cell-free TTR incubation)	↓ in T4 binding to TTR	IC_{50} – 1.3		Behnisch et al. (2021)
			PFOS – Free acid 0.058 μM (in cell-free TTR incubation)	↓ in T4 binding to TTR	IC_{50} – 0.6		
TRβ reporter activation	Reporter assay – chimera of TRβ ligand-binding domain with Gal4 DNA binding domain	African green monkey CV-1 fibroblasts	PFOS – form not stated 0.003–0.3 μM, 24 h	No effect alone but ↓ T3-dependent activation	LEC – 0.1	Assuming human TR-LBD	Du et al. (2013)
Androgen receptors – cell lines							
AR-mediated gene transcription	mRNA expression (RT-qPCR)	LNCaP human prostate	PFOA – form not stated	*AR*- No effect	N/A		Behr et al. (2018)

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
		adenocarcinoma cells	1–100 μM, 24 h	*PSA*- No effect			
			PFOS – form not stated	*AR*- No effect	N/A		
				PSA- No effect			
			1–100 μM, 24 h				
Testosterone secretion	Radioimmune assay for testosterone in media	H295R human adrenal carcinoma cells	PFOA – Free acid	No effect	N/A	Variability in data not reported	Rosenmai et al. (2013)
			1.6–50 μM, 48 h				
Testosterone secretion	Radioimmune assay for testosterone in media	H295R human adrenal carcinoma cells	PFOA – form not stated	No effect	N/A	Single concentration	Wang et al. (2015d)
			100 μM, 48 h				
Testosterone secretion	Enzyme immunoassay for testosterone in media	H295R human adrenal carcinoma cells	PFOS – K salt	↑ at 200 μM only	N/A		van den Dungen et al. (2015)
			30–200 μM, 48 h				
Testosterone secretion	Radioimmune assay for testosterone in media	H295R human adrenal carcinoma cells	PFOA – form not stated	No effect	N/A		Behr et al. (2018)
			1–100 μM, 48 h				
			PFOS – from not stated	No effect	N/A		
			1–100 μM, 48 h				
Testosterone secretion	Radioimmune assay for testosterone in media	H295R human adrenal carcinoma cells	PFOA – form not stated	↑ T secretion	LEC – 0.6		Kraugerud et al. (2011)
			0.006–600 μM, 48 h				
			PFOS – form not stated	↑ T secretion	LEC – 600		

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
			0.006–600 μM, 48 h				
Testosterone secretion	Radioimmune assay for testosterone in media	H295R human adrenal carcinoma cells	PFOS – form not stated 0.003–0.3 μM, 48 h	↓ T secretion	LEC – 0.1		Du et al. (2013)
AR reporter activation	Reporter assay – endogenous AR regulating an MMTV-driven reporter	MDA-kb2 human breast cancer cells	PFOS – form not stated 0.003–0.3 μM, 48 h	No effect	N/A		Du et al. (2013)
AR reporter activation	Reporter assay – endogenous AR regulating an MMTV-driven reporter	22Rv1/MMTV prostate carcinoma cells	PFOA – Free acid 0.00 001–10 μM, 24 h	No effect	N/A		Kang et al. (2016)
			PFOS – Free acid 0.00 001–10 μM, 24 h	No effect	N/A		
AR reporter activation	Reporter assay – endogenous AR regulating an MMTV-driven reporter	MDA-kb2 human breast cancer cells	PFOA – form not stated 1–100 μM, 24 h	No effect in absence or presence of T	N/A		Behr et al. (2018)
			PFOS – form not stated 1–100 μM, 24 h	No effect alone but ↑ T-dependent activation	LEC – 50		
AR reporter activation	Reporter assay – full length AR regulating an MMTV-driven reporter	CHO Chinese hamster ovary cells	PFOA – Free acid 0.2–50 μM, 24 h	No effect in absence or presence of T	N/A		Rosenmai et al. (2013)

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
AR reporter activation	Reporter assay – full length AR regulating an MMTV-driven reporter	CHO Chinese hamster ovary cells	PFOA – Free acid 0.001–100 μM, 24 h	No effect alone but ↓ T-dependent activation	IC_{50} – 10		Kjeldsen and Bonefeld-Jørgensen (2013)
			PFOS – Free acid 0.001–100 μM, 24 h	No effect alone but ↓ T-dependent activation	IC_{50} – 5		
AR reporter activation	Reporter assays – Cis (full length human AR and ARE-driven reporter) Trans (chimera of AR ligand-binding domain with Gal4 DNA binding domain)	HepG2 human liver cancer cells	PFOA – form not stated 0.14–300 μM, 24 h	No effect	N/A	Potentially toxic concentrations included AR expression is very low in TRANS assay	Houck et al. (2021)
			PFOS – form not stated 0.14–300 μM, 24 h	No effect	N/A		
Estrogen receptors – primary cells							
ERα-mediated gene transcription	mRNA expression (microarray) and Ingenuity Pathway Analysis	Primary human hepatocytes	PFOA – form not stated 1–100 μM, 24 h	Microarray Predicted upstream regulator: ERα ↓ pathway expression	LEC – 25		Buhrke et al. (2015)
ERα expression	mRNA expression (RT-qPCR)	Human umbilical vein endothelial cells	PFOS – form not stated 50, 100 μM 24, 48 h	↑ *ESR1*	LEC – 100		Liao et al. (2012)
Estradiol secretion and aromatase expression	Chemiluminescent assay for E2 in medium	Primary human placental trophoblasts	PFOS – form not stated 0.0001–1 μM, 24 h	↓ E2 secretion ↓ Aromatase expression	LEC – 0.001		Zhang et al. (2015b)

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
	Immunoblot for CYP19 expression						
Estrogen receptors – cell lines							
ERα-mediated gene transcription	mRNA expression (RT-qPCR)	MCF7 human breast carcinoma cells	PFOA – form not stated 100 μM, 48 h	TFF – No effect alone but ↓ E2-dependendent expression EGR3 – No effect alone but ↓ E2-dependendent expression	N/A	Single concentration	Li et al. (2020e)
			PFOS – form not stated 50 μM, 48 h	↑ TFF ↓ TFF E2-dependendent expression EGR3 – No effect alone but ↓ E2-dependendent expression	N/A	Single concentration	
ERα-mediated gene transcription	mRNA expression (RT-qPCR)	MCF7 human breast carcinoma cells	PFOA – form not stated 1–100 μM, 24 h	ESR1- No effect TFF1- No effect PR- No effect GREB1- No effect ERβ – No effect	N/A		Behr et al. (2018)
			PFOS – form not stated	ESR1- No effect	N/A	ERβ effect was measured by	

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
			1–100 μM, 24 h	TFF1- No effect PR- No effect GREB1- No effect ERβ – No effect		luciferase activity	
ERα-mediated gene transcription	mRNA expression (RT-qPCR)	T47D human breast carcinoma cells	PFOA – form not stated 0.001, 24 h	pS2 – No effect alone but ↑ E2-dependendent expression PR – No effect in absence or presence of E2			Sonthithai et al. (2016)
			PFOS – form not stated 0.001, 24 h	pS2 – No effect alone but ↑ E2-dependendent expression PR – No effect in absence or presence of E2			
Estradiol secretion and aromatase activity	Radioimmune assay for 17β-estradiol in media Aromatization of [1β-^{3}H] androstenedione	H295R human adrenal carcinoma cells	PFOA – form not stated 0.006–600 μM, 48 h PFOS – form not stated 0.006–600 μM, 48 h	E2 secretion – No effect ↑ Aromatase activity ↑ E2 secretion Aromatase activity – no effect	LEC – 600 LEC – 600	Data for aromatase activity not shown and information on variability not provided	Kraugerud et al. (2011)

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
Estradiol secretion	Radioimmune assay for 17β-estradiol in media	H295R human adrenal carcinoma cells	PFOA – form not stated 100 μM, 48 h	E2 secretion – No effect	N/A	Single concentration	Wang et al. (2015d)
Estradiol secretion	Radioimmune assay for 17β-estradiol in media	H295R human adrenal carcinoma cells	PFOA – Free acid 1.6–50 μM, 48 h	↓ E2 secretion – 1.6 μM ↑ E2 secretion – 50 μM	LEC – 1.6/50	Variability in data not reported	Rosenmai et al. (2013)
Estradiol secretion	Radioimmune assay for 17β-estradiol in media	H295R human adrenal carcinoma cells	PFOA – form not stated 1–100 μM, 48 h	No effect	N/A		Behr et al. (2018)
			PFOS – form not stated 1–100 μM, 48 h	No effect	N/A		
Aromatase activity	Aromatization of [1β-^{3}H] androstenedione	JEG-3 human placental carcinoma cells	PFOA – form not stated 0.01–100 μM, 24 h	↓ Aromatase activity	IC_{50} – 80		Gorrochategui et al. (2014)
			PFOS – form not stated 0.01–100 μM, 24 h			PFOS effects only at cytotoxic concentrations	
Estradiol secretion	Enzyme immunoassay for 17β-estradiol in media	H295R human adrenal carcinoma cells	PFOS – K salt 30–200 μM, 48 h	↑ E2 secretion	LEC – 200		van den Dungen et al. (2015)
Estradiol secretion	Radioimmune assay for 17β-estradiol in media	H295R human adrenal carcinoma cells	PFOS – form not stated	↑ E2 secretion	LEC – 0.03		Du et al. (2013)

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
			0.003–0.3 μM, 48 h				
ERα binding	Competitive binding assay	Human ERα ligand binding domain	PFOA – form not stated 2.5–200 μM	Positive	IC_{50} – 21		Qiu et al. (2020)
			PFOS – form not stated 2.5–200 μM	Positive	IC_{50} – 17		
ERα reporter activation	Reporter assay – endogenous ERα regulating an ERE-driven reporter	T47D human breast carcinoma cells	PFOA – NH_4 salt 10–1000 μM, 24 h	No effect	N/A		Evans et al. (2022)
			PFOS – K salt 10–1000 μM, 24 h	No effect	N/A		
ERα reporter activation	Reporter assay – endogenous ERα regulating an ERE-driven reporter	T47D human breast carcinoma cells	PFOA – form not stated 0.000001–100 μM, 24 h	No effect alone ↑ E2-mediated transactivation	LEC – 0.001		Sonthithai et al. (2016)
			PFOS – form not stated 0.000001–100 μM, 24 h	No effect alone ↑ E2-mediated transactivation	LEC – 0.0001		
ERα reporter activation	Reporter assay – endogenous ERα regulating an ERE-driven reporter	MCF7 human breast carcinoma cells	PFOA – Free acid 0.00001–10 μM, 48 h	↓ E2-mediated transactivation	LEC – 10		Kang et al. (2016)

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
			PFOS – Free acid 0.00001–10 μM, 48 h	↓ E2-mediated transactivation	LEC – 10		
ERα reporter activation	Reporter assay – endogenous ERα regulating an ERE-driven reporter	MCF7 human breast carcinoma cells (formerly known as BG1 cells)	PFOA – form not state 0.001–96 μM, 24 h	No effect	N/A		Yao et al. (2014)
ERα reporter activation	Reporter assay – endogenous ERα regulating an ERE-driven reporter	MCF7 human breast carcinoma cells	PFOA – Free acid 0.001–100 μM, 24 h	↑ transactivation	EC_{50} – 65		Kjeldsen and Bonefeld-Jørgensen (2013)
			PFOS – Free acid 0.001–100 μM, 24 h	↑ transactivation	EC_{50} – 29		
ERα reporter activation	Reporter assay – endogenous ERα regulating an ERE-driven reporter	MCF7 human breast carcinoma cells	PFOS – form not stated 0.1–100 μM, 48 h	↑ transactivation	EC_{20} – 12		Li et al. (2020e)
ERα reporter activation	Reporter assay – full length human ERα and PPRE-driven reporter	Human kidney cell line HEK293T	PFOA – Free acid 0.001–1 μM, 24 h	↑ transactivation	LEC – 0.1		Benninghoff et al. (2011)
			PFOA – K salt 0.001–1 μM, 24 h	↑ transactivation	LEC – 0.001		
ERα reporter activation	Reporter assays – Cis (full length human ERα and ERE-driven reporter)	HepG2 human liver cancer cells	PFOA – form not stated 0.14–300 μM, 24 h	↑ transactivation	Cis AC_{50} – 10 Trans AC_{50} – 8	Potentially toxic concentrations included	Houck et al. (2021)

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – µM)	Comments	Reference
	Trans (chimera of PPARa ligand-binding domain with Gal4 DNA binding domain)		PFOS – form not stated 0.14–300 µM, 24 h	↑ transactivation	Cis AC_{50} – 18 Trans AC_{50} – 39		
ER reporter activation	Reporter assay – chimera of ERα ligand-binding domain or ERβ ligand-binding domain with Gal4 DNA binding domain	Human kidney cell line HEK293T	PFOA – form not stated 1–100 µM, 24 h	ERα – No effect without or with E2 ERβ – No effect alone but increased E2-dependendent activation	LEC – 100		Behr et al. (2018)
			PFOS – form not stated 1–100 µM, 24 h	ERα – No effect alone but increased E2-dependendent activation ERβ – No effect alone but increased E2-dependendent activation	ERα LEC – 50 ERβ LEC – 50		
Glucocorticoid receptors – primary cells and cell lines							
Cortisol production	mRNA expression (RT-qPCR)	Human primary placenta decidual stromal cells	PFOS – form not stated 0.0001–1 µM, 24 h	↓ *HSD11B1*	LEC (mRNA) – 0.1		Yang et al. (2016)
	Protein expression (immunoblot)			↓ 11β-HSD1	LEC (protein) – 0.001		
Cortisol degradation	11β-HSD2 enzyme activity	Human kidney microsomes	PFOA – Free acid	↓ 11β-HSD2 activity	IC_{50} – 24	No cell-based analyses	Zhao et al. (2011b)

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
			0.01–250 μM in cell free preparation				
			PFOS – Free acid	↓ 11β-HSD2 activity	IC_{50} – 0.048		
			0.01–250 μM in cell free preparation				
Cortisol degradation	11β-HSD2 enzyme activity	Microsomes prepared from BeWo human trophoblast placental cells	PFOA – Free acid	↓ 11β-HSD2 activity	IC_{50} – 26	No cell-based analyses	Zhao et al. (2023)
			0.01–1000 μM in cell free preparation				
			PFOS – Free acid	↓ 11β-HSD2 activity	IC_{50} – 0.1		
			0.01–1000 μM in cell free preparation				
Cortisol secretion	Radioimmune assay for testosterone in media	H295R human adrenal carcinoma cells	PFOA – form not stated	No effect	N/A		Kraugerud et al. (2011)
			0.006–600 μM, 48 h				
			PFOS – form not stated	No effect	N/A		
			0.006–600 μM, 48 h				
Cortisol secretion	Radioimmune assay for cortisol in media	H295R human adrenal carcinoma cells	PFOA – form not stated	No effect	N/A	Variability in data not reported	Rosenmai et al. (2013)
			1.6–50 μM, 48 h				
Cortisol secretion	Radioimmune assay for cortisol in media	H295R human adrenal carcinoma cells	PFOA – form not stated	No effect	N/A	Single concentration	Wang et al. (2015d)
			100 μM, 48 h				

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
Cortisol secretion	Enzyme immunoassay for corticosteroids in media	H295R human adrenal carcinoma cells	PFOS – K salt 30–200 μM, 48 h	↑ cortisol	N/A		van den Dungen et al. (2015)
GR reporter activation	Reporter assay – full length GR and MMTV promoter-driven reporter	T47D human breast carcinoma cells	PFOS – form not stated 0.01–0.3 mg/ml, 24 h	No effect alone ↑ Cortisol-mediated transactivation	LEC 0.01 mg/ml	Decreases seen only at cytotoxic concentrations.	Wilson et al. (2016)
Various receptors – cell lines (not informative)							
PPARα-mediated gene transcription	mRNA expression (RT-qPCR)	MDA-MB-231 human breast cancer cells	PFOA – Free acid 50, 100 μM, 48 h	↑ *FA2H*	LEC – 100	No positive control	Sakai et al. (2022)
PPARα-mediated cytokine secretion	Cytokine secretion	Human promyelocytic cell line THP-1	PFOA – Free acid 240 μM, 3 h PFOS – Free acid 200 μM, 3 h	↓ MMP9, TNFα, IL-8 secretion (the effect of which was reduced following PPARα knockdown) PPARα-independent effects on cytokine secretion		Single concentration of PFAS; siRNA knockdown of PPARα	Corsini et al. (2011)
ERβ expression	Immunoblot for ERβ	HepG2 human liver cancer cells	PFOS – K salt 10, 100 μM, 24 h	↑ ERβ expression	↑ only at 10 μM	Quantification inconsistent with blot shown	Xu et al. (2017)
Aryl hydrocarbon dependent-gene and protein expression	mRNA expression (RT-qPCR)	HepaRG human liver cells	PFOA – form not stated 0.0001–1 μM, 24–48 h	24 h ↑ *CYP1A2*	24 h LEC – 0.001 48 h LEC – 0.01		Franco et al. (2020)

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – µM)	Comments	Reference
	Enzymatic activity			48 h ↓ *CYP1A2* Enzymatic activity – no effect			
			PFOS – form not stated 0.0001–1 µM, 24–48 h	24 h ↑ *CYP1A2* (≤ 0.01 µM) ↓ *CYP1A2* (≥ 0.01 µM) 48 h ↓ *CYP1A2* ↓ Enzymatic activity	24 h LEC – 0.0001 48 h LEC – 0.1 LEC enzymatic activity – 0.01 (only at 48h)		
Vitamin D receptor binding, receptor-dependent gene expression and activity	VDR binding	Surface plasmon resonance of human VDR	PFOA – Free acid 0.5–4 µM	↓ 1,25(OH)D binding to VDR	10% decrease of 1,25(OH)D binding at 4 µM		Di Nisio et al. (2020)
	mRNA expression (RT-qPCR)	Epithelial colorectal adenocarcinoma Caco-2 cells	PFOA – Free acid 1 µM, 24 h	↓ Vit D-induced expression of *TRPV6, CABP9K, CYP24A1*	N/A	Single concentration	
	Mineral deposition	Human osteosarcoma Saos-2 cells	PFOA 1 µM, 24 h	↓ Calcium deposition	N/A	Single concentration	
Glucocorticoid receptor pathway	mRNA expression (RT-qPCR)	THP-1 human monocyte cells	PFOS – Free acid	↓ *RACK1*	LEC – 0.2		Masi et al. (2022)

Table S4.24 End-points relevant to modulation of receptor-mediated effects in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	PFAS concentrations and Timing	Results[a]	Effective concentration (LEC, EC_{20} or EC_{50} – μM)	Comments	Reference
			0.2–20 μM, at 6 h, 16 h				
	Protein expression (immunoblot)			↓ RACK1			
	Reporter Assay – endogenous GR regulating *RACK1*-promoter			↓ transactivation, but only at 6 h			

AC_{50}, half-maximal activity concentration; AR, androgen receptor; BMC_{50}, bench mark response of 50%; CAR, constitutive androstane receptor; CYP, cytochrome P450; EC_{20}, 20% effective concentration; E2, estradiol; EC_{50}, half-maximal effective concentration; EGR3, early growth response 3; ER, estrogen receptor; ERE, estrogen responsive element; h, hour(s); IC50, half-maximal inhibitory concentration; IPA, Ingenuity pathway analysis; LEC, lowest effective concentration; MADMAX, management and analysis database for multiple omics experiments; MMP, matrix metalloproteinase; NH4 salt, ammonium salt; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight mass spectrometer; MMTV, mouse mammary tumour virus; N/A, not applicable; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR response element; PR, progesterone receptor; PXR, pregnane X receptor; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; SD, standard deviation; TFF, trefoil factor; TNF, tumour necrosis factor; TPOAb, thyroid peroxidase antibody; TTR, transthyretin; TR-LBD, TRβ ligand-binding domain; VDR, vitamin D receptor.

[a] ↓, decrease; ↑, increase.