12. March 2020

PFAS animal toxicity, mode of action and mixture approach

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Outline

- Animal toxicity
  - Liver effects
  - Thyroid effects
  - Reproductive and developmental effects
  - Effects on the immune system
  - Carcinogenicity and genotoxicity
- Mode of action
  - Importance of activation of PPARα
  - Other signalling pathways
  - MOA individual outcomes
- Mixture approach

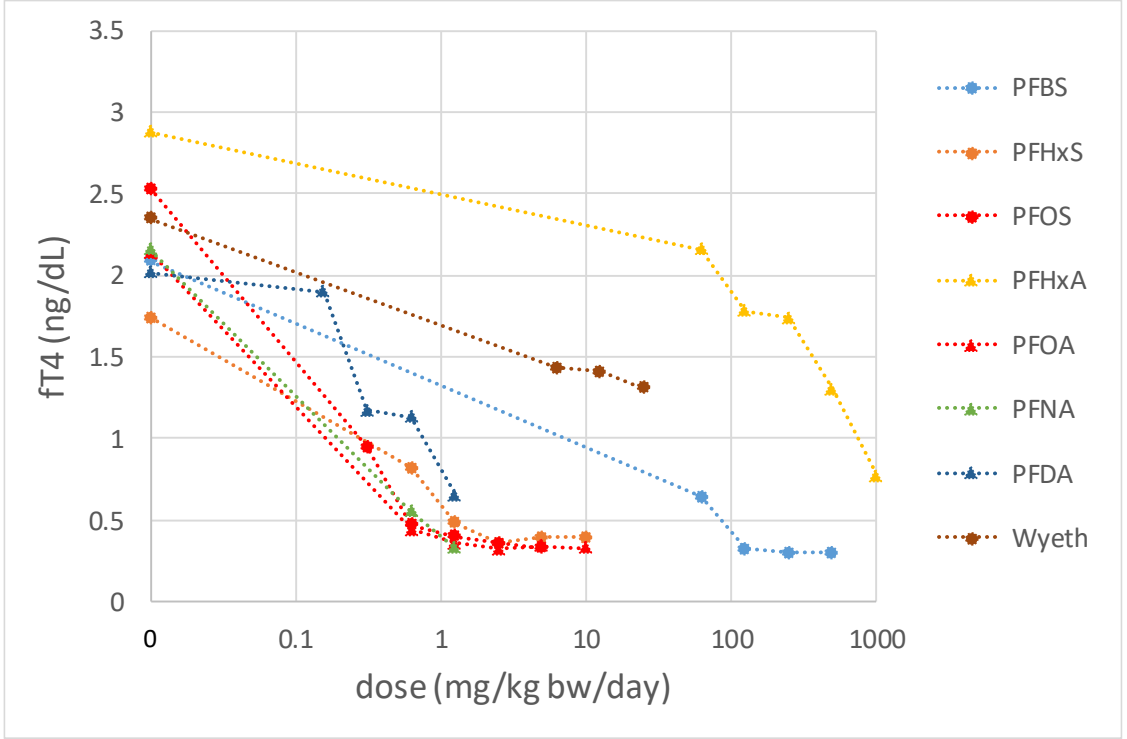
Liver effects, rats and mice

- Increased liver weight – seen with all PFASs studied
  - PFCAs: PFBA, **PFHxA**, PFHpA, **PFOA**, **PFNA**, **PFDA**, PFUnDA, PFDoDA, PFTeDA, PFHxDA and PFODA
  - PFSAs: **PFBS**, **PFHxS**, **PFOS**
  - Other PFASs: 8:2 FTOH and EtFOSE

- At higher dose levels: disturbances in lipid metabolism (steatosis), hepatotoxic effects, signs of cholestasis, necrosis, inflammation

Thyroid effects

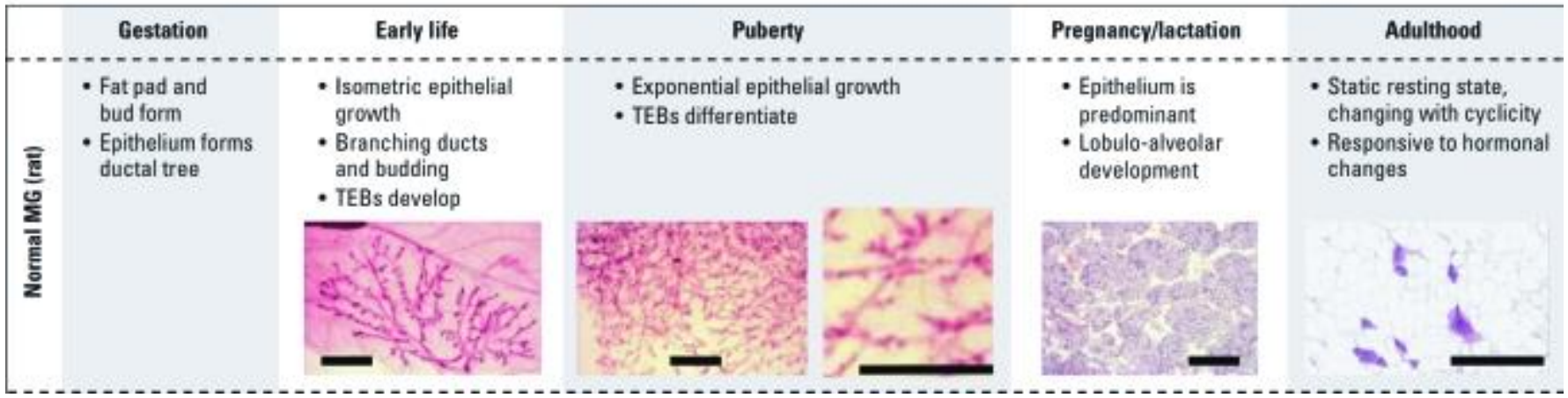
- For some PFASs: disturbed thyroid hormone levels in rodents
  - Decreased T4 and often also T3 levels
  - Often not resulting in increased TSH levels or effects on thyroid gland
  - Competition with T4 on transthyretin binding



Effects on free T4, 28 days exposure (based on results in NTP 2019a,b)

Developmental and reproductive effects

- PFOA: Impaired mammary gland development in mice
- Late gestation/via lactation
- Visible from PND 3, permanent effect (12 weeks)
- LOAEL 0.00045 mg/kg bw per day (three generation study); maternal LOAEC 66 ng/mL



Rudel et al., 2011

PFOA mammary gland development, gestational exposure

Mouse strain	Study design, exposure duration	Dosage (mg/kg bw per day)	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)	Serum or tissue levels (ng/mL)	NOAEC (ng/mL)	LOAEC (ng/mL)	Reference
CD-1	GD 1-17, GD 8-17, GD 12-17	0, 5		5	Semi-quantitative in blood of dams and pups at PND 10 and 20; quantitatively in livers of pups at PND 1, 10, 20 (data presented but not shown here).			White et al. 2007
CD-1	GD 1-17 and GD 8-17, + cross-fostering (lactation) GD 7/10/13/15-17	0, 3, 5 0, 5		3 5	Serum levels in GD 8-17 dams, 5 mg/kg bw per day: 42200 or 47900 at lactation day (LD) 1, decreasing to 16400 or 24400 at LD 10, depending on lactating control or treated pups. In pups exposed in utero GD 8-17, 66200 or 70000 at PND 1, decreasing to 20500 or 31300 at PND 10, when nursed by control or treated dams, respectively. In pups from control dams, maximum 15700 at PND 10 when nursed by treated dams. Below 1000 in all pups at PND 63 (weaning from PND 22).			White et al 2009
CD-1	GD 1-17 GD 10-17	0, 0.3, 1, 3 0, 0.01, 0.1, 1		0.3 0.01	Pup PND 7: <20, 4980, 11026, 20700 Pup PND 1: 22.6, 285, 2304, 16306 Pup PND 21: 4.1, 16.5, 132, 2025		4980 285 16.5	Macon et al 2011
CD-1	3-generations, P0 GD 1-17, +/- 5 µg/L in drinking water (0.00045 mg/kg bw per day) continuously from P0 GD 7	0, 0+5 µg/L, 1, 1+5 µg/L, 5		0+5 µg/L	F1 PND 22: 0.6, 21.3, 2444, 2744, 10045 F1 PND 63: 3.1, 66.2, 210.7, 187, 760		21.3 66.2	White et al 2011
Sv/129	GD 1-17	0, 3	3		GD 18 dam: 19000	19000		Albrecht et al. 2013
CD-1	GD 1-17	0, 0.01, 0.1, 0.3, 1		0.01	Pup PND 21: <5, 74.8, 457, 905, 3119		74.8	Tucker et al. 2015
C57Bl/6		0, 0.01, 0.1, 0.3, 1	0.1	0.3	Pup PND 21: <10, 26.1, 247, 891, 2142	247	891	

Maternal LOAEC = 66ng/mL

Bl/6 less sensitive

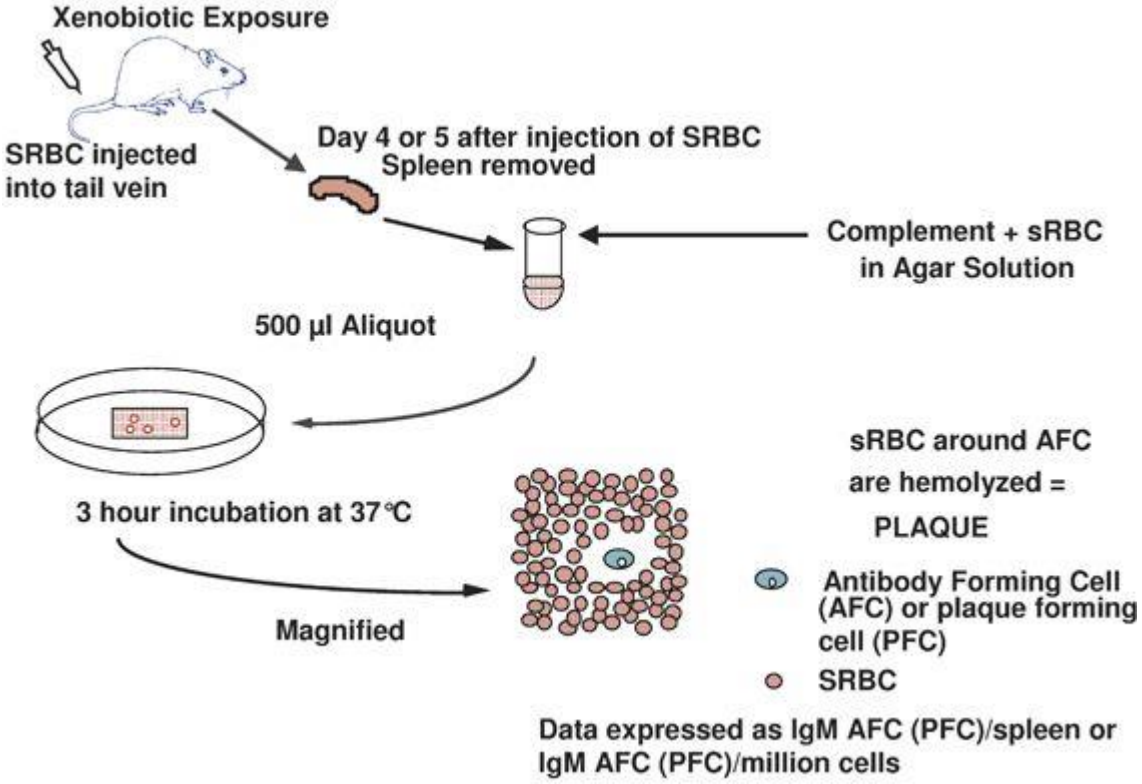
PFOA mammary gland development, gestational exposure

Mouse strain	Study design, exposure duration	Dosage (mg/kg bw per day)	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)	Serum or tissue levels (ng/mL)	NOAEC (ng/mL)	LOAEC (ng/mL)	Reference
CD-1	GD 1-17, GD 8-17, GD 12-17	0, 5		5	Semi-quantitative in blood of dams and pups at PND 10 and 20; quantitatively in livers of pups at PND 1, 10, 20 (data			White et al. 2007
CD-1	GD 1-17 17, + cro fostering (lactation GD 7/10/							hite et al 09
CD-1	GD 1-17 GD 10-1							acon et al 11
CD-1	3-genera P0 GD 1- 5 µg/L in water (0. mg/kg b continuo P0 GD 7							hite et al 11
Sv/129	GD 1-17	0, 3	3		GD 18 dam: 19000	19000		Albrecht et al. 2013
CD-1	GD 1-17	0, 0.01, 0.1, 0.3, 1		0.01	Pup PND 21: <5, 74.8, 457, 905, 3119		74.8	Tucker et al. 2015
C57Bl/6		0, 0.01, 0.1, 0.3, 1	0.1	0.3	Pup PND 21: <10, 26.1, 247, 891, 2142	247	891	

- **Not studied for other PFASs**
- **Other developmental endpoints at much higher dose levels**
  - **Increased fetal and/or neonatal mortality and reduction in fetal weight and/or postnatal growth**

Immunotoxic effects

- Antibody response to a T-cell dependent antigen
  - Immunization with sheep red blood cells (SRBC), other antigens also possible
  - Plaque forming assay (PFC)
  - Serum IgM by Enzyme-Linked Immunosorbent Assay (ELISA)
- Experimental infections



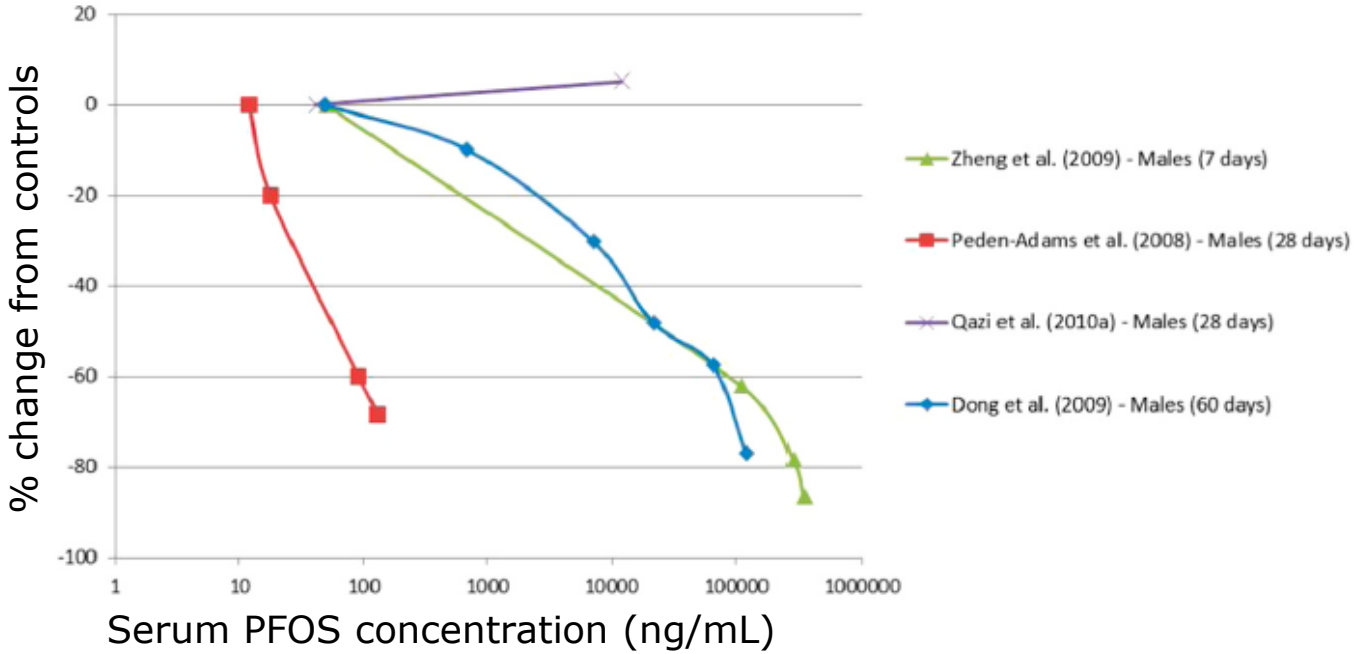
Gregory Ladics **DOI:** https://doi.org/10.1007/3-540-27806-0_117

Immunological effects of PFASs in rodents

Species	Strain	Sex	Route	duration	PFAS	NOAEL	LOAEL	NOAEC	LOAEC	Immune treatment	days before sacrifice	immune endpoint (*)	Effect	Reference
				(days)		(mg/kg perday)			(ng/mL)					
mouse	B6C3F1	male	Gavage	28	PFOS	0.000166	0.00166	18	92	SRBC	5 days	PFCs	↓	Peden-Adams et al., 2008
mouse	B6C3F1	female	Gavage	28	PFOS	0.00331	0.0166	123	666	SRBC	5 days	PFCs	↓	
mouse	B6C3F1	female	Gavage	21	PFOS	0.005	0.025	189	670	Influenza	None	survival	↓	Guruge et al., 2009
mouse	B6C3F1	male	Diet	28	PFOS	0.25	--	11600	--	SRBC	5 days	serum IgM, PFCs	--	Qazi et al., 2010
mouse	B6C3F1	male	via dams, gavage	GD 1-17	PFOS	1	5	NR	NR	SRBC	4 days	PFCs	↓	Keil et al., 2008
mouse	B6C3F1	female			PFOS	5	--	NR	--	SRBC	4 days	PFCs	--	
mouse	C57BL/6	male	Gavage	60	PFOS	0.00833	0.0833	674	7132	SRBC	4 days	PFCs	↓	Dong et al., 2009
mouse	C57BL/6	male	Gavage	60	PFOS	0.0167	0.0833	2360	10750	SRBC	7 days	serum IgM	↓	Dong et al., 2011
mouse	C57BL/6	male	Gavage	60	PFOS	0.0833	0.4167	8210	24530	None		TNF-a, IL-6	↑	Dong et al., 2012
mouse	C57BL/6	male	Gavage	7	PFOS	--	5	--	110460	SRBC	5 days	PFCs	↓	Zheng et al., 2009
mouse	C57BL/6	male	Gavage	7	PFOS	--	5	--	97250	None		non-specific IgM	↓	Zheng et al., 2011
mouse	BALB/c	female	Gavage	21	PFOS	--	20	--	NR	Ovalbumin	14 and 7 days (two injections)	serum IgM	↓	Vetvicka and Vetvickova, 2013
					PFOA	--	20	--	NR	Ovalbumin			↓	
mouse	CD-1(ICR)BR	male	Gavage	29	APFO	1	10	32000	225000	SRBC	5 days	serum IgM	↓	Loveless et al., 2008
mouse	C57BL/6	female	Gavage	15	PFOA	1.88	3.75	NR	74913	SRBC	5 days	serum IgM	↓	DeWitt et al., 2008
mouse	C57BL/6	female	Water	15	PFOA	7.5	30	NR	NR	SRBC	5 days	serum IgM	↓	DeWitt et al., 2016
rat	SD	male	Diet	28	PFOS	–	0.14	470	950	None		serum IgG1	↓	Lefebvre et al., 2008
rat	SD	female	Diet	28	PFOS	7.58	–	43200	--	None		--	--	Lefebvre et al., 2008
rat	CD(SD)IGS BR	male	Gavage	29	APFO	30	--	223000	--	SRBC	5 days	serum IgM	--	Loveless et al., 2008

NR: not reported; PFCs: plaque forming colonies in spleen cells producing anti SRBC antibodies; * In case serum IgM is mentioned as well as the time between injection of antigen and sacrifice, authors looked for antigen-specific IgMs.

Peden-Adams 2008, critical study, immunotox in animals



Comparison of percentage change from controls for PFC response studies that reported serum PFOS concentrations

B. Pachkowski et al., Environmental Research 171 (2019) 452–4

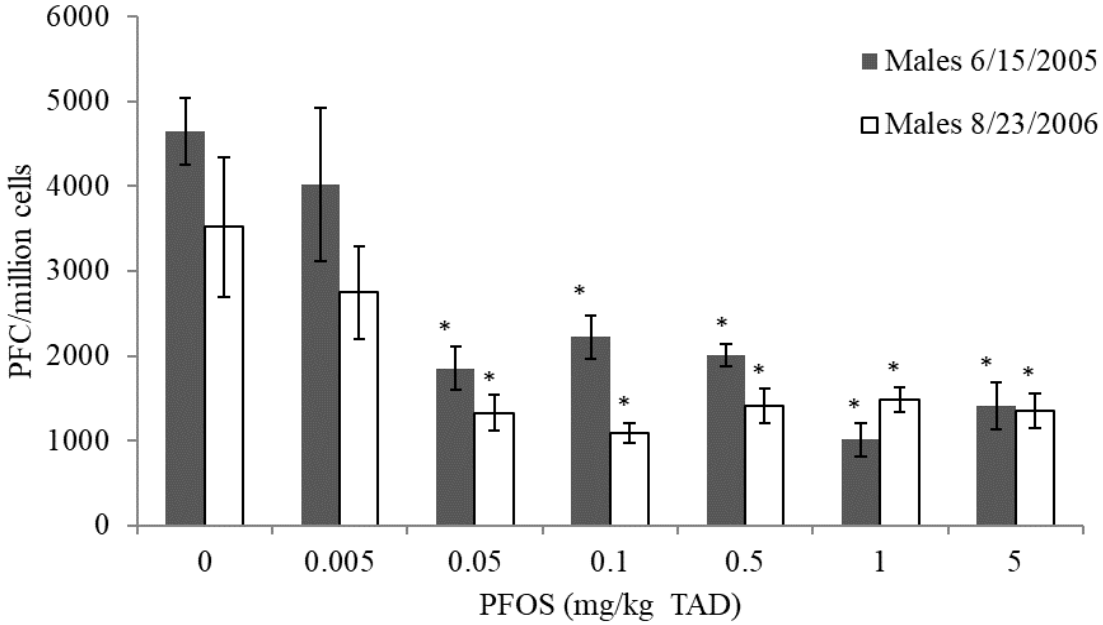


Figure H.1. PFC response in male B6C3F1 mice treated with 0, 0.005, 0.05, 0.1, 0.5, 1 or 5 mg PFOS/kg bw (TAD) for 28 days by oral gavage (n=5). * significantly different from control ($p<0.05$). (Mean and SEM). Two independent experiments.

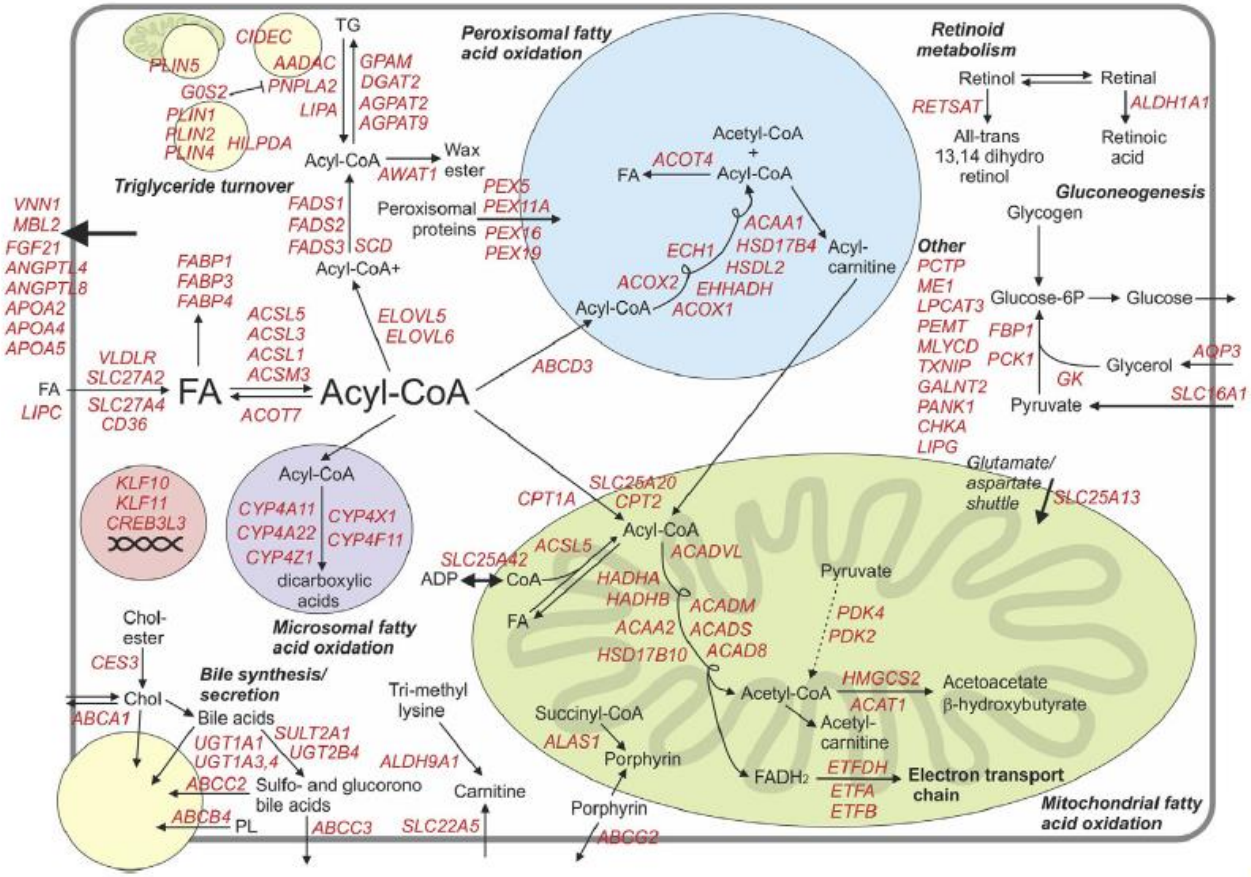
Peden-Adams, personal communication, 2020

Carcinogenicity and genotoxicity

- PFOS and PFOA are tumour promoters in rodent liver
- PFOA also induces Leydig cell tumours in rats
- For PFOS and PFOA no evidence for a direct genotoxic mode of action was identified
- For PFASs other than PFOS and PFOA, the number of studies and data are limited. Structural similarity for PFHxS and PFOS, as well as for PFNA and PFOA, indicates that also for these PFASs a direct genotoxic mode of action is unlikely

MOA – PPARα activation

- PFASs activate PPARα, with different potencies
  - peroxisomal ß-oxidation enzymes
  - mitochondrial enzymes and transporters
  - lipoprotein metabolism
  - gluconeogenesis
  - bile acid metabolism
- Rodent **AND** human liver
- Peroxisomal proliferation is rodent-specific

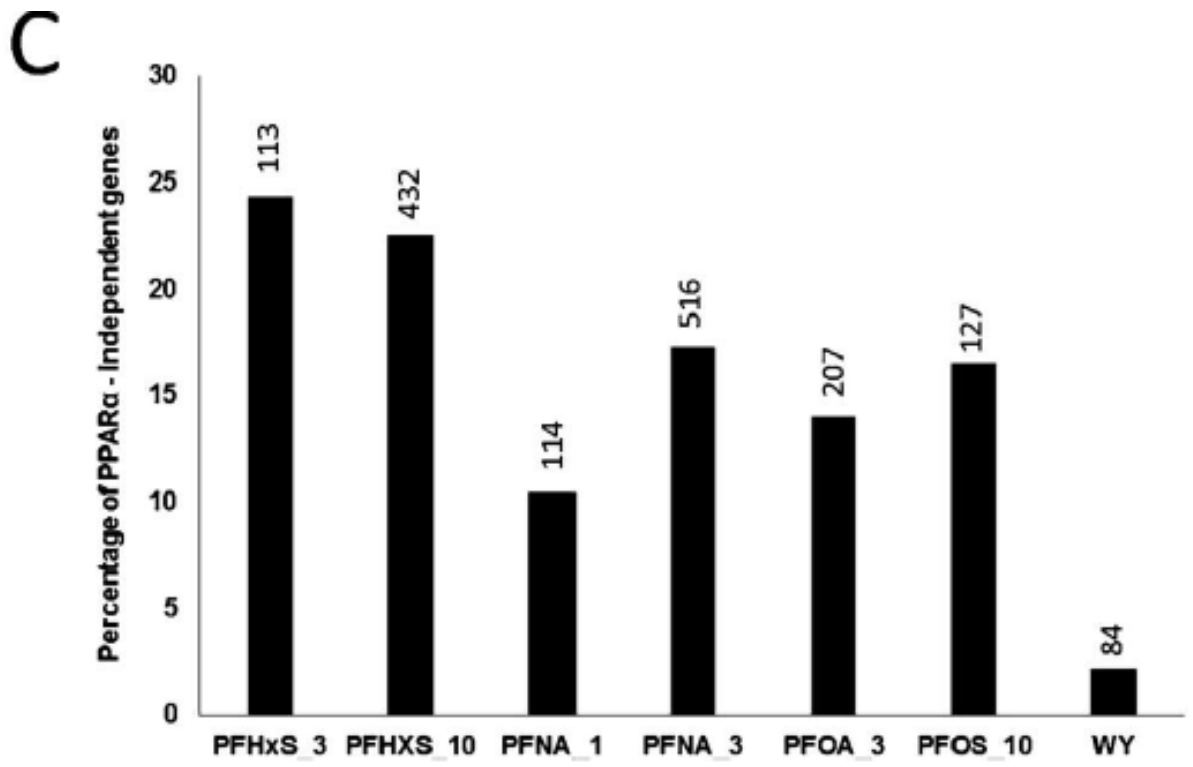


Overview map of target genes of PPARa in human liver. The map depicts the target genes of PPARa involved in metabolism excluding drug metabolism and is based on the published literature and publicly available transcriptomics datasets.
S. Kersten, R. Stienstra / Biochimie 136 (2017) 75-84

Interaction with other nuclear receptors

- Approximately 11-24% of regulated genes in liver are PPARα independent
  - Suppression of STAT5B
  - PPARγ activation
  - CAR activation
  - ERα activation

- In vitro:
  - PPARβ/δ activation
  - PXR activation



Rosen et al., 2017

MOA liver toxicity

Increased weight

- Hyperplasia (increased number of cells)
- Changed balance proliferation and apoptosis
- Rodent PPARα dependent

+

- Hypertrophy (increased volume of cells)
- Proliferation of peroxisomes, smooth ER, steatosis

- Regulation of PPARα-dependent peroxisomal ß-oxidation seems independent from control of hepatocellular proliferation
- Rodents and humans have different susceptibility towards PPARα-dependent hyperplastic liver growth
- Steatosis, and related to this, necrotic liver cells and increased serum transaminases – MoA unknown (also in PPARα KOs)

MOA thyroid hormone effects

- Competition with T4 on transthyretin (TTR) binding
  - TTR binding potencies of the most potent PFASs were 12.5–50 times lower than those of T4
- Possible increased conjugation of thyroid hormones by induction of UDP-glucuronosyltransferases (UGT), via CAR activation
- Effects occur at higher dose than immune effects and impaired mammary gland development

MOA impaired mammary gland development

- MOA is unknown
- All three major developmental stages (embryonic, puberty, lactation/involution) can be affected by PFOA
- MOA might be different in these stages
  - Steroid production in puberty?
- Postnatal exposure sufficient for permanent effect
- Early perinatal stages most sensitive
- No information on other PFASs than PFOA

MOA immunotoxicity

- Not established
- Most information from PFOS and PFOA
- Seems PPARα independent, based on KO and mutant strains
- At lower doses than decrease in body weight or thyroid hormone levels
- PPARβ/δ?
- NFκB seems involved

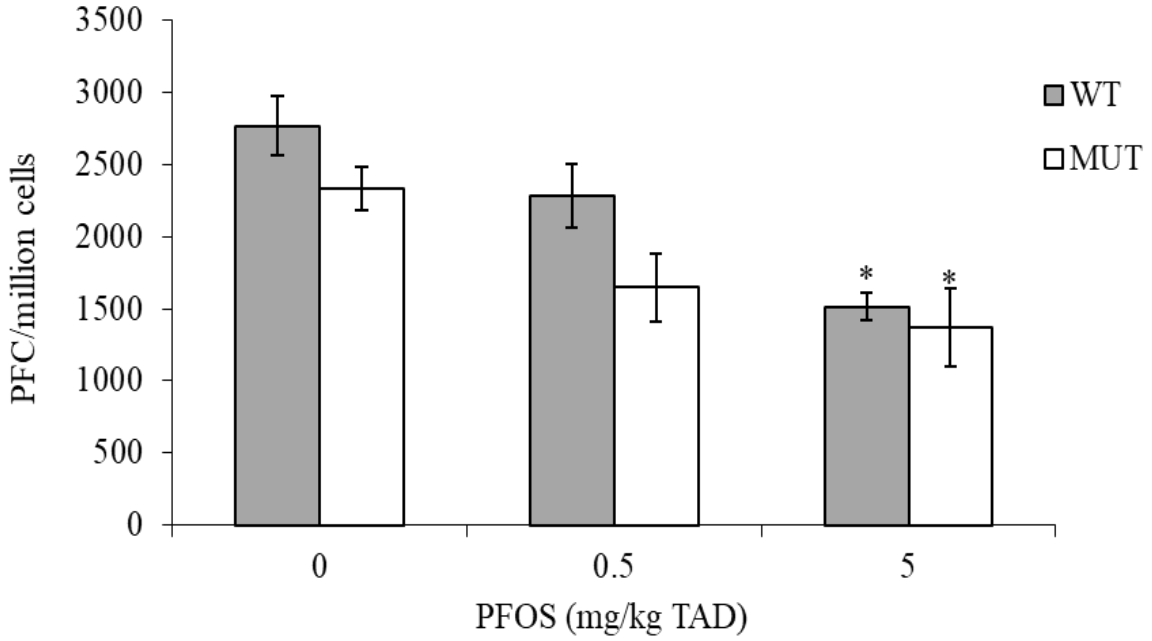


Figure H.3. PFC response in female C57Bl/6 mice (WT) and PPAR-alpha targeted mutation mouse model (MUT; Taconic), treated with PFOS for 28-d by gavage, with N=5 (mean, SEM). Samples were blinded to person reading slides. Doses of 0, 0.5 and 5 mg/kg bw (TAD).

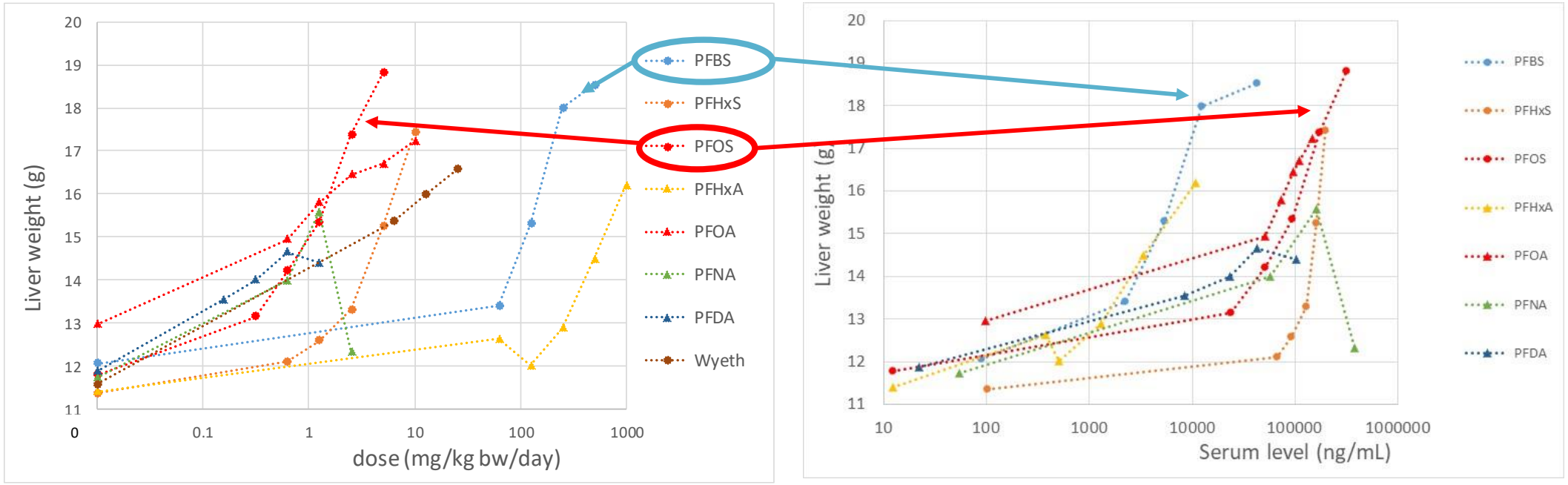
Peden-Adams, personal communication, 2020

Basis for mixture approach

- EFSA 2018: “since both toxicity as well as underlying modes of toxic action for PFOS and PFOA are not sufficiently understood and might differ, but also overlap.”
- EFSA launched a new Guidance document on how to evaluate the effects of mixtures (EFSA Scientific Committee, 2019) and it was considered that similarities in chemical properties and effects warrant a mixture approach for a number of PFASs.

NTP – 28 days studies, liver weight

- PFBS, PFHxS, PFOS, PFHxA, PFOA, PFNA, PFDA + PPARα agonist Wyeth (WY)-14,643
- Differences in the potencies, and dependent on external or internal (serum level) dose



Absolute liver weights in male rats, based on applied dose (left) or serum levels (right).

PFASs have similar effects

Table 21: Effects of PFASs in male and female rats (NTP, 2019a, b). Figures express the lowest/highest ratio compared to the controls observed for either one of the dose levels. In some cases most animals died and these dose groups were not included.

PFAS	Liver weight		Serum total T4		Serum free T4		Serum T3		Serum triglycerides		Serum cholesterol		Serum bile salts/acids		Gene expression liver					
	(g)		(µg/dL)		(ng/dL)		(ng/dL)		(mg/dL)		(mg/dL)		(µmol/mL)		Acox1		Cyp4a1		Cyp2b1	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
PFBS	1.5	1.3	0.03	0.3	0.1	0.2	0.4	0.6	0.3	---	0.2	0.8	1.9	5.3	4.8	3.1	24	4.5	63	510
PFHxS	1.5	1.2	0.3	0.7	0.2	0.6	0.6	---	0.6	---	0.7	---	---	---	2.8	---	16	1.3	27	38
PFOS	1.6	1.5	0.1	0.1	0.1	0.3	0.6	0.6	0.2	0.7	0.2	0.7	3.4	3.7	5.3	3.0	30	2.4	349	802
PFHxA	1.4	1.4	0.4	---	0.3	--	0.7	---	---	---	0.8	---	2.2	2.6	2.0	1.6	12	2.5	6	68
PFOA	1.3	1.6	0.03	0.5	0.1	0.7	0.6	---	0.6	1.6	0.6	1.2	4.5	---	5.7	3.9	30	7.6	18	71
PFNA	1.3	1.3	0.1	0.6	0.1	0.5	---	---	0.5	---	0.7	---	16.9	2.8	5.9	5.2	22	13	6.8	14
PFDA	1.2	1.3	0.7	---	0.1	0.3	---	2.1	0.5	---	0.6	0.7	13.1	7.6	9.1	5.4	60	17	10	89
Wyeth	1.4	1.6	0.8	---	0.6	1.5	---	1.3	---	---	0.8	0.9	7.1	---	6.5	5.6	43	7.1	3.7	33

Dose levels (mg/kg bw per day; males/females): PFBS (62.5-1000/62.6-1000), PFHxS (0.625-10/3.12-50), PFOS (0.312-5/0.312-5), PFHxA (62.6-1000/62.6-1000), PFOA (0.625-10/6.25-100), PFNA (0.625-10/1.56-25), PFDA (0.156-2.5/0.156-2.5), Wyeth 14,346 (6.25-25/6.25-25). Blue colour indicates significantly higher than controls, brown colour significantly lower.

Serum TSH levels decreased in males treated with PFOA, PFNA, and Wyeth 14,643, but increased in females treated with PFOA and Wyeth 14,643.

Mixture approach

- Similar potencies for critical effects?
  - There are no comparative studies that provide reliable insight in the relative potencies for immune effects.
  - For mammary gland development, only data on PFOA
- As a pragmatic approach, the CONTAM Panel decided to restrict the mixture approach to the four most abundant PFASs in human serum (PFOA, PFNA, PFHxS and PFOS)
- MOA largely unknown
- In the absence of more specific information, to assume equal potencies by default for these four PFASs on immune outcomes

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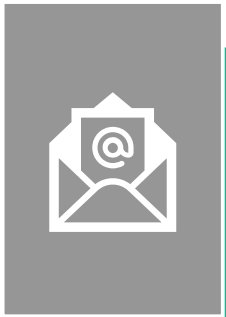

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