Appendix A – Levels of PFOS, PFOA, PFNA and PFHxS in different species of fish, arranged according to the LB level for PFOS

9081

Fish species	PFOS				PFOA				PFNA				PFHxS			
	N	%LC	LB	UB	N	%LC	LB	UB	N	%LC	LB	UB	N	%LC	LB	UB
Carp (*Cyprinus*)	145	14%	14.12	14.21	149	32%	4.10	4.33	125	65%	0.84	1.47	126	97%	0.07	1.01
Eels (*Apodes*)	164	35%	9.23	9.44	177	96%	0.07	0.68	54	91%	0.98	1.66	58	98%	0.02	0.73
Roach (*Rutilus*)	8	13%	8.05	8.18	10	100%	0.00	1.00	10	100%	0.00	1.00	10	100%	0.00	1.00
Perch (*Perca*)	47	31%	6.08	6.31	49	99%	0.04	0.45	17	100%	0.00	0.95	15	100%	0.00	0.88
Bream (*Charax*)	41	49%	6.03	6.29	45	100%	0.00	0.55	16	100%	0.00	0.94	16	100%	0.00	0.94
Barbel (*Barbus*)	13	8%	5.16	5.24	14	100%	0.00	0.56	5	100%	0.01	0.60	5	100%	0.00	0.51
Sardine and pilchard (*Sardina*)	14	0%	4.73	4.73	28	64%	0.10	0.37	14	57%	0.08	0.53	14	64%	0.01	0.45
Sea catfish and wolf-fish (*Anarhichas*)	20	70%	3.04	3.46	16	94%	0.11	0.80	13	100%	0.00	0.79	13	100%	0.00	0.73
Plaice (*Pleuronectes*)	39	46%	2.95	3.29	39	97%	0.08	0.72	28	100%	0.00	0.85	5	100%	0.00	0.51
Whitefish (*Coregonus*)	18	23%	1.52	1.62	18	100%	0.00	0.29	1	100%	0.01	0.60	-	-	-	-
-Bass (*Marone*)	6	67%	1.40	1.74	6	100%	0.00	0.44	3	100%	0.01	0.60	3	100%	0.00	0.51
Grey mullet (*Mugil*)	13	8%	0.93	0.97	18	11%	0.17	0.20	8	25%	0.15	0.15	8	38%	0.02	0.04
Sprat (*Sprattus sprattus*)	51	12%	0.86	0.92	56	84%	0.05	0.35	58	84%	0.04	0.33	58	84%	0.01	0.30
Shrimps (*Crangon crangon*)	39	25%	0.74	0.76	38	76%	0.02	0.09	34	93%	0.02	0.12	19	100%	0.00	0.09
Norway lobster (*Nephrophs norvegicus*)	2	50%	0.74	0.76	2	100%	0.02	0.10	2	100%	0.03	0.13	2	100%	0.00	0.10
Crayfish (*Astacus spp.*)	2	100%	0.74	0.76	2	100%	0.02	0.10	1	100%	0.03	0.13	1	100%	0.00	0.10
Flounder (*Platichthys flesus*)	16	50%	0.72	1.04	17	100%	0.00	0.52	7	100%	0.00	0.74	1	100%	0.00	0.51
Sole (*Limanda; Solea*)	15	67%	0.70	1.08	16	88%	0.13	0.55	11	82%	0.02	0.63	3	100%	0.00	0.51
Crab (*Cancer spp.*)	16	44%	0.69	0.93	13	46%	0.38	0.54	16	50%	0.35	0.50	20	85%	0.30	0.78

Fish species	PFOS				PFOA				PFNA				PFHxS			
	N	%LC	LB	UB	N	%LC	LB	UB	N	%LC	LB	UB	N	%LC	LB	UB
Char (*Salvelinus*)	3	100%	0.58	0.98	1	100%	0.12	0.53	3	100%	0.01	0.60	2	100%	0.00	0.51
Lophiiformes (*Pediculati*)	4	50%	0.58	0.98	7	100%	0.00	0.31	7	100%	0.00	0.36	3	100%	0.00	0.51
Rays (*Hypotremata*)	2	50%	0.58	0.98	2	100%	0.12	0.53	2	100%	0.01	0.60	1	100%	0.00	0.51
Bonito (*Sarda Sarda*)	1	0%	0.58	0.98	1	100%	0.12	0.53	-	-	-	-	-	-	-	-
-Cod and whiting (*Gadus spp.*)	174	67%	0.47	1.05	145	93%	0.01	0.74	130	92%	0.02	0.78	27	100%	0.00	0.53
Mackeral (*Scomber*)	125	79%	0.36	0.93	136	81%	0.31	0.88	129	96%	0.00	0.74	122	99%	0.00	0.74
Prawns (*Palaemon serratus*)	8	38%	0.33	0.50	9	33%	0.20	0.42	2	100%	0.03	0.13	2	100%	0.00	0.10
Herring (*Clupea*)	288	74%	0.32	0.62	290	96%	0.02	0.38	243	90%	0.02	0.38	237	99%	0.00	0.38
Salmon and trout (*Salmo spp.*)	574	88%	0.31	0.83	521	95%	0.13	0.63	522	100%	0.00	0.70	365	100%	0.00	0.63
Hake (*Merluccius*)	32	11%	0.27	0.31	35	93%	0.06	0.12	19	97%	0.00	0.07	15	100%	0.00	0.03
Halibut (*Hippoglossus spp.*)	487	71%	0.26	0.81	106	99%	0.00	0.30	487	100%	0.00	0.77	487	100%	0.00	0.69
Tuna (*Thunnus*)	21	39%	0.16	0.26	34	100%	0.00	0.12	17	100%	0.00	0.13	17	100%	0.00	0.11
Mussel (*Mytilus edulis*)	55	21%	0.08	0.17	58	100%	0.00	0.14	53	100%	0.00	0.15	33	100%	0.00	0.08
Water molluscs	10	60%	0.06	0.35	10	40%	0.01	0.33	9	44%	0.00	0.34	9	100%	0.00	0.31
Squid (*Loligo vulgaris*)	4	100%	0.06	0.35	4	100%	0.01	0.33	-	-	-	-	-	-	-	-
-Cuttlefish (*Sepia officinalis*)	2	0%	0.06	0.35	2	50%	0.01	0.33	1	100%	0.00	0.34	1	100%	0.00	0.31
Cockle (*Cardium edule*)	1	100%	0.06	0.35	1	100%	0.01	0.33	1	100%	0.00	0.34	-	-	-	-
-Scallop (*Pecten spp.*)	19	83%	0.01	0.18	19	66%	0.01	0.18	19	100%	0.00	0.18	19	100%	0.00	0.16
Oyster (*Ostrea edulis*)	36	93%	0.00	0.77	37	100%	0.00	0.78	37	100%	0.00	0.78	33	100%	0.00	0.78
Queen scallop (*Chlamys opercularis*)	9	100%	0.00	0.05	9	100%	0.00	0.05	9	100%	0.00	0.06	9	100%	0.00	0.05

-: No data provided to EFSA; LB: lower bound: LC: left-censored; N: number of samples, UB: upper bound

Appendix B – Biomonitoring

9084 **Table B.1.** Time trends for PFASs in blood and breast milk

Period	Study Population	Country	PFASs	Trend	Reference
European time trend studies					
1987–2007	n = 80 plasma samples women, sampled in connection with breast reduction surgery (1987–1991), or as wives of men with cancer (2006–2007) mean age (range): 48 (36–56) years cross sectional	Sweden	PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA	PFHxS, PFOS, and PFOA peaked during the period 1990–2000. PFHxS increased during the whole period. PFOS and PFOA decreased from around 2000, even though only PFOS was significant. PFNA, PFDA and PFUnDA increased during the whole period, largest increase after the year 2000.	Axmon et al. (2014)
1972–2008	n = 20 pools of breast milk healthy native Swedish mothers at the Mothers' milk center in Stockholm cross sectional	Sweden	PFOS, PFOA, PFHxS	Concentrations of PFOS, PFOA and PFHxS increased from 1972 to the 1990s. From around 2001 to 2008 the concentrations of all three PFASs decreased, while the trend for PFHxS was not significant.	Sundström et al. (2011)
1996–2010	n = 36 pools of serum primiparous women living in Uppsala County, donated serum samples within the third week after delivery age: 19–41 years cross sectional	Sweden	PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFHxS, PFOS, PFDS, FOSA	PFBS, PFHxS, PFNA and PFDA increased during the period 1996 to 2010, while PFOS, PFDS, FOSA and PFOA decreased in the same period. For PFHpA and PFUnDA no significant trend was observed. PFHxA, PFDoDA, PFTrDA and PFTeDA were not detected in any sample above LOQ. The increasing trends of PFBS and PFHxS have later been shown to be due to contamination of drinking water with these PFASs (Gyllenhammar et al., 2013)	Glynn et al. (2012)

9085

1997–2012	n = 27 pools of serum (three pools at each time point) primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery cross sectional	Sweden	PFBS, PFHxS (branched and linear), PFOS (branched and linear), FOSA (branched and linear), EtFOSA, PFHpA, PFOA (branced and linear), PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, , 8:2/8:2 diPAP	Significant decreasing trends were observed for branched and linear PFOS, PFDS, branched and linear FOSA and for PFOA. Significant increasing trends were observed for branched and linear PFHxS, PFNA, PFDA, PFUnDA, PFDoDA and PFTrDA. No significant trends were observed for PFBS, and 8:2/8:2 diPAP. While EtFOSA, was detected in less than 60% of the samples and thus no statistical calculations were made.	Gebbink et al. (2015)
1974–2010	archived serum samples from Swedish primiparous women living in the Uppsala county (n = 36 pools between 1996 and 2010) archived American serum or plasma samples from 1974, 1989, 2000/2001, 2006 and 2010 were collected from individuals residing in the Hagerstown, Maryland (n = 60) cross sectional	Sweden and USA	PFOS, PFOS isomers and 1-m PFOS enantiomers	Swedish population: For ΣPFOS; No significant trend was seen upto 2000, but between 2000 and 2010 a downward trend was observed. For % branched PFOS; no significant trend was found between 1996 and 2000, but a significant upward trend was seen between 2000 and 2010. For the enantiomeric fraction of 1-m PFOS; a significant decreasing temporal trend was observed between 1996 and 2000, but no significant trend was seen between 2000 and 2010. American population: For ΣPFOS; No significant temporal trend was found for from 1974 to 2000/2001, but in the 2000/2001–2010 period the downward trend was seen. For % branched PFOS; no significant trend was found for the period 1974 to 2010. For the enantiomeric fraction of 1-m PFOS; No significant temporal trend was observed.	Liu et al. (2015)

9086

1996–2004	n = 9 pools from Swedish primiparous women with a median age ranging from 27 to 30 years for the various pools cross sectional	Sweden	PFHxS, PFOS, FOSA, PFOA and PFNA	PFOA and FOSA were not observed above LOQ in any samples while PFNA was only found in three samples in similar concentrations. Quite stable concentrations were observed for both PFHxS and PFOS between 1996 and 2000, while a slight decrease was observed in 2003–2004 for PFHxS and in 2002–2004 for PFOS.	Kärrman et al. (2007)
2001–2014	n = 579, men and women, 70 years (2001–2004), 75 years (2006–2009) and 80 years (2011–2014) at the three sampling time points, serum longitudinal sampling	Sweden	Fourteen PFASs, whereof trends were evaluated for the following: PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFOS, FOSA	The concentrations of all PFASs except FOSA and PFOS increased from the first to the second sampling. Between the two last samplings the concentrations decreased for all PFASs.	Stubleski et al. (2016)
1972–2016	n = 20 pools from Stockholm (9–116 individuals per pool), women, breast milk	Sweden	PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFPeDA, PFBS, PFHxS, PFOS, PFDS, FOSA, MeFOSAA, EtFOSAA	PFOS, PFOA, PFHxS and PFNA increased from 1972 but decreased from 1988, 2000, 2004 and 2010, respectively. PFUnDA increased throughout the whole period, while PFTrDA, PFBS and PFHxA decreased from 1972 to 2004, 2011 and 2011, respectively, before increasing until 2016. No trends were observed for PFHpA and PFDoDA.	Nyberg et al. (2018)
2007–2015	n= 11 pools from Gothenburg (5–11 individuals per pool), women, breast milk	Sweden	PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFPeDA, PFBS, PFHxS, PFOS, PFDS, FOSA, MeFOSAA, EtFOSAA	PFOS and PFDoDA decreased significantly from 2007 to 2015. None of the other PFASs showed any statistically significant trends	Nyberg et al. (2018)

9087

1977–2006	n = 24 pools of serum males 40–50 years cross sectional	Norway	Nineteen PFASs, whereof trends were evaluated for the following 13: PFPeA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFHxS, PFHpS, PFOS, FOSA	PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFHpS and PFOS all increased from the mid-1970s up to the mid-1990s, while FOSA reached a plateau between 1985 and 1993. The concentrations of PFOS, PFOA and PFHpS decreased from around 2000, while for FOSA the decrease started somewhat earlier. No particular trends were observed for PFNA, PFDA and PFUnDA after around 2000. The concentrations of PFPeA, PFHpA, PFDoDA and PFTrDA were quite stable during the whole study period.	Haug et al. (2009)
1979–2007	n = 254 serum samples from 53 males from Northern Norway median age at first and last sampling was 43 and 71 years, respectively repeated sampling and measurements at up to five time points (1979, 1986, 1994, 2001 and 2007) longitudinal sampling	Norway	Ten PFASs were quantified, from which trends were assessed for eight: PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFHpS, PFOS, FOSA	Increasing trends were observed for all the eight PFAS from 1979 and onwards. For PFOS, PFOA and FOSA significant decreasing concentrations were observed from 2001 to 2007. In contrast, increasing concentrations were observed throughout the whole study period for PFNA, PFDA and PFUnDA, while only the trends for PFNA and PFDA were statistically significant for all time periods. Similar concentrations of PFHxS were observed in 2001 and 2007. Somewhat lower concentrations of PFHpS were observed in 2007 when compared to 2001, but this trend was not significant.	Nøst et al. (2014)

9088

2008–2013	n = 1,533 serum samples pregnant nulliparous women from the Aarhus Birth Cohort Biobank most participants gave a blood sample between 11 weeks and 14 weeks of gestation median age 29 years cross sectional	Denmark	Sixteen PFASs; whereof trends were assessed for PFASs detected in >50% of the samples, i.e. PFHxS, PFHpS, PFOS, PFOA, PFNA, PFDA, PFUnDA	All seven PFASs decreased in the period 2008 to 2013.	Bjerregaard-Olesen et al. (2016)
1982–2010	n = 258 plasma samples from the German Environmental Specimen Bank males and females, approximately 50% of each age range 20–29 years cross sectional	Germany	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFHxS, PFHpS, PFOS, PFDS	PFTrDA, PFTeDA, PFBS, PFDS not found in any samples. For PFBA, PFPeA, PFHxA, PFUnDA and PFDoDA only very few samples had concentrations above LOQ. PFHpA, PFDA and PFHpS were quantifiable in 20–30% of the samples. PFOS increased from 1982 to 1986, and remained quite stable up to 2001. From 2001 to 2010 a steadily decrease has been observed. PFOA concentrations also increased from 1982 to 1986, were quite stable up to 2008 and decreased from 2008 to 2010. PFHxS increased from 1982 to 2001, was stable up to 2005 and decreased after that. For PFNA no trend was observed.	Schröter-Kermani et al. (2013)
1982–2009	n = 420, students, male and female 20-29 years from Halle and Munster cross sectional	Germany	PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA,PFDoDA, PFTrDA, PFTeDA, 8:2 diPAP	From 2000 to 2009 the concentrations of PFOA decreased, while the concentrations of PFNA, PFDA and PFUnDA increased in the same period. No significant trend was observed for 8:2 di-PAPs throughout the same period.	Yeung et al. (2013a)

9089

1982–2009	n = 420, students, male and female 20–29 years from Halle and Münster cross sectional	Germany	PFOS, PFHxS	For some of the compounds increasing concentrations were observed during the first years of the study. From around 1995 to 2009 decreasing concentrations were observed for PFOS, while no clear trend was seen for PFHxS.	Yeung et al. (2013b)
1977–2004	n = 30, students, 19 males and 11 female, 20–29 years from Arnsberg cross sectional	Germany	PFBS, PFHxA, PFPeA, PFOA, PFOS, PFHxS	PFOS and PFOA levels remained fairly stable throughout the period, while PFHxS concentrations increased during the whole study period.	Wilhelm et al. (2009)
Examples of time trend studies in other countries					
2003–2013	n = 71 persons. 81% of the participants were female Gullah African Americans participating in the SLEIGH study serum The age ranged from 6.1 to 77.6 years at the first visit longitudinal study; two time points	USA	Trends reported for PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA	At a population level an overall decrease in the concentration of PFOA, PFOS, PFHxS and PFUnDA was seen. No significant trend was observed for PFDA. Some individuals had a considerable increase of PFHxS concentration from the first to the second blood sample, but for the overall population the PFHxS concentration decreased.	Gribble et al. (2015)
2003–2011	30 random samples from 2003, 2005, 2007, 2009 and 2011 (n = 150) pregnant women, between 28 and 32 weeks of gestation, who were enrolled in a prospective birth cohort study in Hokkaido (the Hokkaido Study on Environment and Children's Health) mean age: 30.3 years	Japan	PFHxS, PFOS PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA	Concentrations of PFOS and PFOA decreased during the period 2003–2011, while the levels of PFNA and PFDA increased in the same period. The trends for the remaining compounds were not significant.	Okada et al. (2013)

	cross sectional				
2002–2011	n = 158 pools of serum males and females, aged from 0–>60 years four time points 2002/2003, 2006/2007, 2008/2009 and 2010/2011 cross sectional	Australia	PFDA, PFHxS, PFNA, PFOA, PFOS, FOSA	Decreasing trends were observed for PFOS and PFOA, while more variable trends were observed for the other PFASs and the various age groups.	Toms et al. (2014)
2002–2013	n = 54 pools from 4,920 individuals, men and women, 0–>60 years, serum cross sectional	Australia	8:2 diPAP, EtFOSA, EtFOSE, PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxDA, PFODA, PFHxS, PFOS, PFDS	Decreasing trends were observed throughout the entire period for PFHxS, PFHpS, PFOS and PFOA, while PFNA, PFDA and PFUnDA concentrations started to decrease from 2006. PFDoDA increased from 2006 and onwards.	Eriksson et al. (2017)
1999–2008	n = 7,876 samples from the NHANES study age: ≥12 years, males and females cross sectional	USA	PFOS, PFHxS, PFOA, PFNA	The PFOS concentrations decreased significantly during the period, while the PFNA concentrations had a significant upward trend. The highest concentrations of PFOA were observed in 1999–2000, while the concentrations were quite stable from 2003 to 2008. Decreasing concentrations were observed for PFHxS in the period 1999 to 2006, but an increase has been observed in 2007–2008.	Kato et al. (2011)

9090

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9093 **Table B.2.** Concentrations of PFASs in general European adult populations with serum or plasma samples collected from 2007–2008 and onwards

Year	Country	Study population	Median (ng/mL)	Geometric mean (ng/mL)	Aritmetric mean (ng/mL)	Min (ng/mL)	Max (ng/mL)	Reference
PFBA								
2012	Sweden	n = 3 pools primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery. Serum.	NR	NR	NR	<LOQ (0.3)[(b)]	<LOQ (0.3)[(b)]	Gebbink et al. (2015)
2015	Czech Republic	n = 300, men and women, mean age: 40.8 years. Serum.	<LOQ (0.013)	<LOQ (0.013)	NR	<LOQ (0.013)	<LOQ (0.013)	Sochorová et al. (2017)
2015–2016	Italy	n = 250, men and women, age: 20–51 years. Background exposed population. Serum.	<LOQ (NR)	NR	NR	<LOQ (NR)	0.35	Ingelido et al. (2018)
2015–2016	Italy	n = 257, men and women, age: 20–51 years. Exposed to elevated PFAS concentrations through drinking water. Serum.	<LOQ (NR)	NR	NR	<LOQ (NR)	3.59	Ingelido et al. (2018)
2015	UK	n = 29, women, mean age: 32.9 years, Controls. Serum. n = 30, women, mean age 32.9 years, Cases with polycystic ovary syndrome. Serum.	NR	<LOQ (0.5)	NR	<LOQ (0.5)	<LOQ (0.5)	Heffernan et al. (2018)
PFPeA								
2012	Sweden	n = 3 pools primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery. Serum.	NR	NR	NR	<LOQ (0.1)[(b)]	<LOQ (0.1)[(b)]	Gebbink et al. (2015)

9094

2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66) years. Serum	<LOQ (0.09)	NR	<LOQ (0.09)	<LOQ (0.09)	<LOQ (0.09)	Poothong et al. (2017)
2008–2013	Denmark	n =1,533 samples, pregnant nulliparous women from the Aarhus Birth Cohort Biobank Most participants gave a blood sample between 11 weeks and 14 weeks of gestation. Median age 29 years. Serum.	NR	NR	NR	<LOQ (0.19)	<20% of samples above LOQ	Bjerregaard-Olesen et al. (2016)
2015	Czech Republic	n = 300, men and women, mean age 40.8 years. Serum.	<LOQ (0.013)	<LOQ (0.013)	NR	<LOQ (0.013)	<LOQ (0.013)	Sochorová et al. (2017)
2015–2016	Italy	n = 250, men and women, 20–51 years. Background exposed population. Serum.	<LOQ (NR)	NR	NR	<LOQ (NR)	0.22	Ingelido et al. (2018)
2015–2016	Italy	n = 257, men and women, 20–51 years. Exposed to elevated PFAS concentrations through drinking water. Serum.	<LOQ (NR)	NR	NR	<LOQ (NR)	0.46	Ingelido et al. (2018)
2015	UK	n = 29, women, mean age 32.9 years, Controls. Serum. n = 30, women, mean age 32.9 years, Cases with polycystic ovary syndrome. Serum.	NR	<LOQ (0.5)	NR	<LOQ (0.5)	2.02	Heffernan et al. (2018)
PFHxA								
2012	Sweden	n = 3 pools primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery. Serum.	NR	NR	NR	<LOQ (0.05) [(b)]	<LOQ (0.05)[(b)]	Gebbink et al. (2015)
2013–2014	Norway	n = 61, men and women, median age 41 years (range: 20–66 years). Serum	<LOQ (0.045)	NR	<LOQ (0.045)	<LOQ (0.045)	<LOQ (0.045)	Poothong et al. (2017)

9095

2008–2013	Denmark	n= 1,533, samples pregnant nulliparous women from the Aarhus Birth Cohort Biobank Most participants gave a blood sample between 11 weeks and 14 weeks of gestation. Median age 29 years. Serum.	NR	NR	NR	<LOQ (0.19)	<LOQ (0.19)	Bjerregaard-Olesen et al. (2016)
2007–2009	Germany	n = 30, students, male and female, age: 20–29 years from Münster. Plasma.	NR	NR	NR	<LOQ (0.0003)	0.0617	Yeung et al. (2013a)
2007–2009	Germany	n = 30, students, male and female, age: 20–29 years from Halle. Plasma.	NR	NR	NR	<LOQ (0.0014-0.005)	1.58	Yeung et al. (2013a)
2015	Czech Republic	n = 300, men and women, mean age: 40.8 years. Serum.	<LOQ (0.013)	<LOQ (0.013)	NR	<LOQ (0.013)	<LOQ (0.013)	Sochorová et al. (2017)
2015–2016	Italy	n = 250, men and women, age: 20–51 years. Background exposed population. Serum.	<LOQ (NR)	NR	NR	<LOQ (NR)	0.26	Ingelido et al. (2018)
2015–2016	Italy	n = 257, men and women, 20-51 years. Exposed to elevated PFAS concentrations through drinking water. Serum.	<LOQ (NR)	NR	NR	<LOQ (NR)	0.68	Ingelido et al. (2018)
2015	UK	n = 29, women, mean age 32.9 years, Controls. Serum. n = 30, women, mean age 32.9 years, Cases with polycystic ovary syndrome. Serum.	NR	<LOQ (0.5)	NR	<LOQ (0.5)	<LOQ (0.5)	Heffernan et al. (2018)
PFHpA								
2008–2011	Sweden	n= 150, primiparous women, within the third week after delivery. The County has a known contamination of the drinking water with PFAS. Mean (range) age: 30.2 (21–40 years). Serum.	0.045[(b)]	NR	0.059[(b)]	<LOQ (0.04) [(b)]	0.296[(b)]	Gyllenhammar et al. (2015)

9096

2010	Sweden	n = 36 pools of serum. Primiparous women living in Uppsala county, donated serum samples within the third week after delivery. Age 19–41 years.	NR	NR	NR	0.073	0.11	Glynn et al. (2012)
2012	Sweden	n = 3 pools primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery. Serum.	NR	NR	NR	0.022[(b)]	0.030[(b)]	Gebbink et al. (2015)
2011–2014	Sweden	n = 579, men and women, 80 years	0.03	0.02	0.04	0.01	0.70	Stubleski et al. (2016)
2007–2008	Norway	n = 41, women, mean age: 36.7 years (range 25–45 years). Serum.	<LOQ (0.05)	NR	<LOQ (0.05)	<LOQ (0.05)	0.10	Haug et al. (2011)
2007–2009	Norway	n = 391, pregnant women, mean (range) age: 31 (18–43) years. Serum.	NR	NR	NR	<LOQ (0.03)	0.45	Berg et al. (2014)
2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66) years. Serum.	<LOQ (0.045)	NR	0.06	<LOQ (0.045)	0.34	Poothong et al. (2017)
2010–2013	Greenland	n = 207, pregnant women age >18 years, Inuits. Serum.	0.03	0.03	0.04	0.03	0.26	Long et al. (2015)
2011–2014	Greenland	n = 128, women, median age around 50 years, breast cancer cases and controls. Serum.	0.14	NR	NR	NR	NR	Wielsøe et al. (2017)
2015	Czech Republic	n = 300, men and women, mean age: 40.8 years. Serum.	NR	NR	NR	<LOQ (0.013)	0.52	Sochorová et al. (2017)
2015–2016	Italy	n = 250, men and women, age: 20–51 years. Background exposed population. Serum.	<LOQ (NR)	NR	NR	<LOQ (NR)	0.26	Ingelido et al. (2018)
2015–2016	Italy	n = 257, men and women, age: 20–51 years. Exposed to elevated PFAS concentrations through drinking water. Serum.	<LOQ (NR)	NR	NR	<LOQ (NR)	0.42	Ingelido et al. (2018)

9097

2015	UK	n = 29, women, mean age 32.9 years, Controls. Serum. n = 30, women, mean age 32.9 years, Cases with polycystic ovary syndrome. Serum.	NR	<LOQ (0.1)	NR	<LOQ (0.1)	0.70	Heffernan et al. (2018)
PFNA								
2007	Sweden	n = 10, women, mean (range) age: 48 (36–56 years). Serum.	0.87	NR	NR	NR	NR	Axmon et al. (2014)
2008–2010	Sweden	n = 153, males, mean (range) age: 67 (53–79) years. Whole blood.	0.58	0.56	0.64	0.09	1.6	Bao et al. (2014)
2010–2011	Sweden	n = 270, women, median (range) age: 50 (22–75 years). Serum	0.80	NR	NR	0.35 (P5)	1.66 (P95)	Bjermo et al. (2013)
2007–2011	Sweden	n = 201, men with prostate cancer. Median (range) age: 67 (49–79) years. Serum	0.612	NR	0.679	0.05	4.6	Hardell et al. (2014)
2007–2011	Sweden	n = 186, men without prostate cancer. Median (range) age: 67 (50–79) years. Serum	0.572	NR	0.631	0.0850	2.1	Hardell et al. (2014)
2008–2011	Sweden	n = 150, primiparous women, within the third week after delivery. The County has a known contamination of the drinking water with PFAS. Mean (range) age: 30.2 (21–40) years. Serum.	0.46[(b)]	NR	0.52[(b)]	0.064[(b)]	2.2[(b)]	Gyllenhammar et al. (2015)
2010	Sweden	n = 36 pools of serum. Primiparous women living in Uppsala county, donated serum samples within the third week after delivery. Age: 19–41 years.	NR	NR	NR	0.59	0.86	Glynn et al. (2012)
2012	Sweden	n = 3 pools primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery. Serum.	NR	NR	NR	0.48[(b)]	0.56[(b)]	Gebbink et al. (2015)
2011–2014	Sweden	n = 579, men and women, 80 years	0.87	0.89	1.1	0.07	6.9	Stubleski et al. (2016)
2007–2008	Norway	n = 41, women, mean (range) age: 36.7 (25–45) years. Serum.	0.63	NR	0.64	0.28	1.3	Haug et al. (2011)

2007–2008	Norway	n = 123, pregnant women. Plasma.	0.34	NR	0.4	<LOQ (0.05)	2.18	Gützkow et al. (2012)
2007–2008	Norway	n = 99, pregnant women. Plasma.	0.3	NR	0.3	<LOQ (0.05)	0.9	Granum et al. (2013)
2007–2009	Norway	n = 391, pregnant women, mean (range) age: 31 (18–43) years. Serum.	0.56	NR	0.67	0.15	4.36	Berg et al. (2014)
2012–2014	Norway	n = 74, 59 consumers (HC) and 15 non-consumers (NC) of fish from AFFF-affected waters. Median (range) age: 58.5 (32–79) years. Serum.	1.72 (HC)	1.57 (HC)	2.18 (HC)	0.58 (HC, P10)	5.22 (HC, P90)	Hansen et al. (2016)
2012–2014	Norway	n = 74, 59 consumers (HC) and 15 non-consumers (NC) of fish from AFFF-affected waters. Median (range) age: 58.5 (32–79) years. Serum.	0.80 (NC)	0.80 (NC)	0.95 (NC)	0.30 (NC, P10)	1.88 (NC, P10)	Hansen et al. (2016)
2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66) years. Serum	0.94	NR	1.06	0.19	2.73	Poothong et al. (2017)
2008–2009	Denmark	n = 247, adult men. Mean age: 19.6 years. Serum.	1.07	NR	1.23	0,64 (P5)	2.41 (P95)	Joensen et al. (2013)
2011	Denmark	n = 200 samples of serum from pregnant women. Serum.	0.61	NR	0.69	0.18	4.4	Vorkamp et al. (2014)
2010–2012	Denmark	n = 392, newly pregnant women. Serum.	0.72	NR	NR	0.18 (P5)	4.40 (P95)	Jensen et al. (2015)
2011	Denmark	n = 145, women, mean (range) age: 41 (31–52) years. Plasma.	0.64	NR	0.75	0.26	2.55	Mørck et al. (2015)

9098

2008–2013	Denmark	n = 1,507 pregnant women, primiparous, median age between 29 and 31 years for the four quartiles of participants. Serum.	0.8	NR	NR	0.6 (IQR)	1.0 (IQR)	Bach et al. (2016)
2010–2012	Denmark	n = 649 pregnant women, mean age: 30.7 years. Serum.	NR	NR	0.7	0.5 (IQR)	0.9 (IQR)	Lind et al. (2017)
2007–2009	Faroe Islands	n = 487, pregnant women, mean age: 30.6 years. Serum.	0.66	NR	NR	0.52 (IQR)	0.86 (IQR)	Timmermann et al. (2016
2010–2013	Greenland	n = 207, pregnant women age: >18 years, Inuits. Serum.	1.30	1.29	1.49	0.41	7.71	Long et al. (2015)
2011–2014	Greenland	n = 128, women, median age around 50 years, breast cancer cases and controls. Serum.	2.64	NR	NR	NR	NR	Wielsøe et al. (2017)
2008	France	n = 478, age: 18 and 75 years, males and females, had current residence in targeted areas and had a fishing license. Serum.	1.3	1.4	1.6	0.2	8	Denys et al. (2014)
2010–2013	France	n = 100, pregnant women, median (range) age: 32 (20–46) years. Serum.	0.430	NR	0.519	<LOQ (0.3)	3.29	Cariou et al. (2015)
2007–2009	Germany	n = 44, pregnant women, mean (range) age: 33 (21–43 years), samples collected during pregnancy. Plasma.	0.6	NR	0.8	NR	NR	Fromme et al. (2010)
2009	Germany	n = 60, men and women, age: 18–67 years. Plasma (rural town, near fluorpolymer production plant)	0.8	NR	1.1	NR	8.6	Fromme et al. (2017)
2015	Germany	n = 26, men and women, age: 18–67 years. Plasma (rural town, near fluorpolymer production plant)	0.5	NR	0.7	NR	3.8	Fromme et al. (2017)
2009	Germany	n = 60, men and women, age: 18–67 years. Plasma (potential contamination of drinking water)	0.8	NR	0.8	NR	2.9	Fromme et al. (2017)
2015	Germany	n = 50, men and women, age: 18–67 years. Plasma (potential contamination of drinking water)	0.5	NR	0.5	NR	2.6	Fromme et al. (2017)
2014	Germany	n = 42, men and women, age: 18–67 years. Plasma.	0.4	NR	0.4	NR	0.8	Fromme et al. (2017)

2016	Germany	n = 158, men and women, age: 18–67 years. Plasma.	0.4	NR	0.4	NR	1.5	Fromme et al (2017)
2009–2010	Spain	n = 46, males and females, age: 19–53 years. Whole blood.	NR	NR	0.12	<LOQ (0.05)	2.94	Gómez-Canela et al. (2015)
2009–2010	Spain	n = 755, men and women, age: 18–65 years. Serum	0.92	0.96	1.11	0.51 (P10)	2.55 (P95)	Bartolome et al. (2017)
2010–2012	Slovakia	n = 120, pregnant women. Age range: 18–45 years. Plasma.	0.44	0.44	0.54	<LOQ (NR)	1.7	Uhl (2016)
2010–2012	Austria	n = 114, pregnant women. Age range: 18–45 years. Plasma.	0.38	0.41	0.45	<LOQ (NR)	1.1	Uhl (2016)
2015	Czech Republic	n = 300, men and women, mean age: 40.8 years. Serum.	0.325	0.300	NR	<LOQ (0.013)	6.55	Sochorová et al. (2017)
2015–2016	Italy	n = 250, men and women, age: 20–51 years. Background exposed population. Serum.	0.58	NR	NR	0.03	7.72	Ingelido et al. (2018)
2015–2016	Italy	n = 257, men and women, age: 20–51 years. Exposed to elevated PFAS concentrations through drinking water. Serum.	0.61	NR	NR	<LOQ (NR)	2.46	Ingelido et al. (2018)
2015	UK	n = 29, women, mean age: 32.9 years, Controls. Serum. n = 30, women, mean age: 32.9 years, Cases with polycystic ovary syndrome. Serum.	NR	0.57	NR	0.2	1.79	Heffernan et al. (2018)

9099

PFDA								
2007	Sweden	n = 10, women, mean (range) age: 48 (36–56) years. Serum.	0.35	NR	NR	NR	NR	Axmon et al. (2014)
2010–2011	Sweden	n = 270, women, median (range) age: 50 (22–75) years. Serum.	0.39	NR	NR	0.19 (P5)	0.84 (P95)	Bjermo et al. (2013)
2008–2010	Sweden	n = 153 males, mean (range) age: 67 (53–79) years. Whole blood.	0.27	0.23	0.29	<LOQ (0.2)	1.0	Bao et al. (2014)
2007–2011	Sweden	n = 201, men with prostate cancer. Median (range) age: 67 (49–79) years. Serum.	0.301	NR	0.338	0,0300	1.2	Hardell et al. (2014)
2007–2011	Sweden	n = 186, men without prostate cancer. Median (range) age: 67 (50–79) years. Serum.	0.269	NR	0.291	0,0244	1.0	Hardell et al. (2014)
2008–2011	Sweden	n = 150, primiparous women, within the third week after delivery. The County has a known contamination of the drinking water with PFAS. Mean (range) age: 30.2 (21–40) years. Serum.	0.26[(b)]	NR	0.28[(b)]	<LOQ (0.05) [(b)]	1.1[(b)]	Gyllenhammar et al. (2015)
2010	Sweden	n = 36 pools of serum. Primiparous women living in Uppsala county, donated serum samples within the third week after delivery. Age: 19–41 years.	NR	NR	NR	0.28	0.42	Glynn et al. (2012)
2012	Sweden	n = 3 pools primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery. Serum.	NR	NR	NR	0.27[(b)]	0.29[(b)]	Gebbink et al. (2015)
2011–2014	Sweden	n = 579, men and women, age: 80 years.	0.34	0.34	0.40	0.03	2.0	Stubleski et al. (2016)
2007–2008	Norway	n=41, women, mean (range) age: 36.7 (25–45) years. Serum.	0.23	NR	0.25	0.10	0.59	Haug et al. (2011)

9100

2007–2008	Norway	n = 123, pregnant women. Plasma.	0.07	NR	0.10	<LOQ (0.05)	1.14	Gützkow et al. (2012)
2007–2009	Norway	n = 391, pregnant women, mean (range) age: 31 (18–43) years. Serum.	0.23	NR	0.26	0.05	2.34	Berg et al. (2014)
2012–2014	Norway	n = 74, 59 consumers (HC) and 15 non-consumers (NC) of fish from AFFF-affected waters. Median (range) age: 58.5 (32–79) years. Serum.	0.47 (HC)	0.43 (HC)	0.59 (HC)	0.11 (HC, P10)	1.40 (HC, P90)	Hansen et al. (2016)
2012–2014	Norway	n = 74, 59 consumers (HC) and 15 non-consumers (NC) of fish from AFFF-affected waters. Median (range) age: 58.5 (32–79) years. Serum.	0.37 (NC)	0.22 (NC)	0.31 (NC)	0.04 (NC, P10)	0.66 (NC, P90)	Hansen et al. (2016)
2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66 years). Serum	0.37	NR	0.40	0.15	1.07	Poothong et al. (2017)
2008–2009	Denmark	n = 247, adult men. Mean age: 19.6 years. Serum.	0.35	NR	0.38	0.22 (P5)	0.61 (P95)	Joensen et al. (2013)
2011	Denmark	n = 200 samples of pregnant women. Serum.	0.27	NR	0.31	0.088	1.7	Vorkamp et al. (2014)
2010–2012	Denmark	n = 392, newly pregnant women. Serum.	0.27	NR	NR	0.07 (P5)	1.75 (P95)	Jensen et al. (2015)
2011	Denmark	n = 145, women, mean (range) age: 41 (31–52) years. Plasma.	0.28	NR	0.33	0.08	1.14	Mørck et al. (2015)
2008–2013	Denmark	n = 1,507, pregnant women, primiparous, median age between 29 and 31 for the four quartiles of participants. Serum.	0.3	NR	NR	0.2 (IQR)	0.4 (IQR)	Bach et al. (2016)
2010–2012	Denmark	n = 649 pregnant women, mean age: 30.7 years. Serum	NR	NR	0.3	0.2 (IQR)	0.3 (IQR)	Lind et al. (2017)

9101

2007–2009	Faroe Islands	n = 487, pregnant women, mean age: 30.6 years. Serum	0.26	NR	NR	0.19 (IQR)	0.35 (IQR)	Timmermann et al. (2016)
2010–2013	Greenland	n = 207, pregnant women age: >18 years, Inuits. Serum.	0.72	0.78	0.99	0.12	7.84	Long et al. (2015)
2011–2014	Greenland	n = 128, women, median age around 50 years, breast cancer cases and controls. Serum.	1.34	NR	NR	NR	NR	Wielsøe et al. (2017)
2008	France	n = 478, age: 18 and 75 years, males and females, had current residence in targeted areas and had a fishing license. Serum.	0.5	0.6	0.7	<LOQ (NR)	11.2	Denys et al. (2014)
2010–2013	France	n = 100, pregnant women, median (range) age: 32 (20–46) years. Serum.	<LOQ (0.4)	NR	0.277	<LOQ (0.4)	1.99	Cariou et al. (2015)
2007–2009	Germany	n = 44, pregnant women, mean (range) age: 33 (21–43) years, samples collected during pregnancy. Plasma.	<LOQ (0.4)	NR	<LOQ (0.4)	NR	NR	Fromme et al. (2010)
2009	Germany	n = 60, men and women, age: 18–67 years. Plasma (rural town, near fluorpolymer production plant)	0.5	NR	1.1	NR	19.2	Fromme et al. (2017)
2015	Germany	n = 26, men and women, age: 18–67 years. Plasma (rural town, near fluorpolymer production plant).	NR	NR	<LOQ (0.4)	NR	3.3	Fromme et al. (2017)
2009	Germany	n = 60, men and women, age: 18–67 years. Plasma (potential contamination of drinking water).	0.3	NR	0.4	NR	1.3	Fromme et al. (2017)
2015	Germany	n = 50, men and women, age: 18–67 years. Plasma (potential contamination of drinking water)	0.2	NR	0.3	NR	2.6	Fromme et al. (2017)

9102

2014	Germany	n = 42, men and women, age: 18–67 years. Plasma.	NR	NR	NR	NR	0.5	Fromme et al. (2017)
2016	Germany	n = 158, men and women, age: 18–67 years. Plasma	NR	NR	NR	NR	1.0	Fromme et al. (2017)
2009–2010	Spain	n = 755, men and women, age: 18–65 years. Serum	0.36	0.42	0.49	<LOQ (0.20, P10)	0.99 (P95)	Bartolome et al. (2017)
2010–2012	Slovakia	n = 120, pregnant women. Age range: 18–45 years. Plasma.	0.27	0.29	0.36	<LOQ (NR)	1.1	Uhl (2016)
2010–2012	Austria	n = 114, pregnant women. Age range: 18–45 years. Plasma.	0.18	0.18	0.20	<LOQ (NR)	0.5	Uhl (2016)
2015	Czech Republic	n = 300, men and women, mean age: 40.8 years. Serum	0.145	0.141	NR	0.013	1.81	Sochorová et al. (2017)
2015–2016	Italy	n = 250, men and women, age: 20–51 years. Background exposed population. Serum.	0.32	NR	NR	<LOQ (NR)	3.07	Ingelido et al. (2018)
2015–2016	Italy	n = 257, men and women, age: 20–51 years. Exposed to elevated PFAS concentrations through drinking water. Serum.	0.33	NR	NR	<LOQ (NR)	1.96	Ingelido et al. (2018)
2015	UK	n = 29, women, mean age: 32.9 years, Controls. Serum. n = 30, women, mean age: 32.9 years, Cases with polycystic ovary syndrome. Serum.	NR	0.31	NR	<LOQ (0.2)	1.17	Heffernan et al. (2018)
PFUnDA								
2007	Sweden	n = 10, women, mean (range) age: 48 (36–56) years. Serum.	0.24	NR	NR	NR	NR	Axmon et al. (2014)
2010–2011	Sweden	n = 270, women, median (range) age: 50 (22–75 years). Serum	0.33	NR	NR	0.11 (P5)	0.86 (P95)	Bjermo et al. (2013)

9103

2008–2010	Sweden	n = 153 males, mean (range) age: 67 (53–79) years. Whole blood.	0.25	0.20	0.28	<LOQ (0.2)	1.5	Bao et al. (2014)
2007–2011	Sweden	n = 201, men with prostate cancer. Median (range) age: 67 (49–79 years). Serum.	0.264	NR	0.308	0.0150	1.3	Hardell et al. (2014)
2007–2011	Sweden	n = 186, men without prostate cancer. Median (range) age: 67 (50–79) years. Serum.	0,254	NR	0,285	0.0250	1.5	Hardell et al. (2014)
2008–2011	Sweden	n = 150, primiparous women, within the third week after delivery. The County has a known contamination of the drinking water with PFAS. Mean (range) age: 30.2 (21–40 years). Serum.	0.23[(b)]	NR	0.26[(b)]	<LOQ (0.05) [(b)]	0.91[(b)]	Gyllenhammar et al. (2015)
2010	Sweden	n = 36 pools of serum. Primiparous women living in Uppsala county, donated serum samples within the third week after delivery. Age: 19–41 years.	NR	NR	NR	0.19	0.31	Glynn et al. (2012)
2012	Sweden	n = 3 pools primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery. Serum.	NR	NR	NR	0.23[(b)]	0.27[(b)]	Gebbink et al. (2015)
2011–2014	Sweden	n = 579, men and women, 80 years	0.36	0.36	0.42	0.12	1.8	Stubleski et al. (2016)
2007–2008	Norway	n = 41, women, mean (range) age: 36.7 (25–45 years). Serum.	0.42	NR	0.44	0.080	1.1	Haug et al. (2011)
2007–2008	Norway	n = 123, pregnant women. Plasma.	0.16	NR	0.19	<LOQ (0.05)	0.54	Gützkow et al. (2012)
2007–2009	Norway	n = 391, pregnant women, mean (range) age: 31 (18–43) years. Serum.	0.26	NR	0.30	0.03	1.46	Berg et al. (2014)

9104

2012–2014	Norway	n = 74, 59 consumers (HC) and 15 non-consumers (NC) of fish from AFFF-affected waters. Median (range) age: 58.5 (32–79) years. Serum.	0.71 (HC)	0.66 (HC)	1.10 (HC)	0.12 (HC, P10)	3.01 (HC, P90)	Hansen et al. (2016)
2012–2014	Norway	n = 74, 59 consumers (HC) and 15 non-consumers (NC) of fish from AFFF-affected waters. Median (range) age: 58.5 (32–79) years. Serum.	0.21 (NC)	0.25 (NC)	0.45 (NC)	0.03 (NC, P10)	1.05 (NC, P90)	Hansen et al. (2016)
2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66) years. Serum.	0.37	NR	0.43	0.05	1.67	Poothong et al. (2017)
2008–2013	Denmark	n = 1,507 pregnant women, primiparous, median age between 29 and 31 for the four quartiles of participants. Serum.	0.3	NR	NR	0.2 (IQR)	0.4 (IQR)	Bach et al. (2016)
2010–2013	Greenland	n = 207, pregnant women age >18 years, Inuits. Serum.	1.60	1.68	2.58	0.18	18.2	Long et al. (2015)
2011–2014	Greenland	n = 128, women, median age around 50 years, breast cancer cases and controls. Serum.	2.49	NR	NR	NR	NR	Wielsøe et al. (2017)
2010–2013	France	n = 100, pregnant women, median (range) age: 32 (20–46) years. Serum.	<LOQ (0.35)	NR	0.21	<LOQ (0.35)	2.60	Cariou et al. (2015)
2010–2012	Slovakia	n = 120, pregnant women. Age range: 18–45 years. Plasma.	0.61	0.58	0.62	<LOQ (NR)	0.9	Uhl (2016)
2010–2012	Austria	n = 114, pregnant women. Age range: 18–45 years. Plasma.	0.40	0.37	0.37	<LOQ (NR)	0.4	Uhl (2016)
2015	Czech Republic	n = 300, men and women, mean age: 40.8 years. Serum	0.058	0.055	NR	<LOQ (0.013)	0.417	Sochorová et al. (2017)
2015–2016	Italy	n = 250, men and women, age: 20–51 years. Background exposed population. Serum.	0.18	NR	NR	< LOQ (NR)	1.35	Ingelido et al. (2018)

9105

2015–2016	Italy	n = 257, men and women, age: 20–51 years. Exposed to elevated PFAS concentrations through drinking water. Serum.	0.16	NR	NR	<LOQ (NR)	1.02	Ingelido et al. (2018)
2015	UK	n = 29, women, mean age 32.9 years, Controls. Serum. N = 30, women, mean age 32.9 years, Cases with polycystic ovary syndrome. Serum.	NR	<LOQ (0.2)	NR	<LOQ (0.2)	0.47	Heffernan et al. (2018)
PFDoDA								
2008–2011	Sweden	n = 150, primiparous women, within the third week after delivery. The County has a known contamination of the drinking water with PFAS. Mean (range) age: 30.2 (21–40) years. Serum.	<LOQ (0.05)[(b)]	NR	NR	<LOQ (0.05)[(b)]	0.25[(b)]	Gyllenhammar et al. (2015)
2012	Sweden	n = 3 pools primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery. Serum.	NR	NR	NR	0.027[(b)]	0.033[(b)]	Gebbink et al. (2015)
2007–2008	Norway	n = 41, women, mean (range) age: 36.7 (25–45 years). Serum.	<LOQ (0.05)	NR	0.050	<LOQ (0.05)	0.14	Haug et al. (2011)
2007–2009	Norway	n = 391, pregnant women, mean (range) age: 31 (18–43) years. Serum.	0.03	NR	0.04	<LOQ (0.03)	0.20	Berg et al. (2014)
2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66) years. Serum	0.07	NR	0.07	<LOQ (0.0036)	0.26	Poothong et al. (2017)
2010–2013	Greenland	n = 207, pregnant women age >18 years, Inuits. Serum.	0.21	0.31	0.39	0.20	1.85	Long et al. (2015)
2011–2014	Greenland	n = 128, women, median age around 50 years, breast cancer cases and controls. Serum	0.54	NR	NR	NR	NR	Wielsøe et al. (2017)

9106

2009	Germany	n = 60, men and women, age: 18–67 years. Plasma (rural town, near fluorpolymer production plant)	NR	NR	<LOQ (0.4)	NR	3.9	Fromme et al. (2017)
2015	Germany	n = 26, men and women, age: 18–67 years. Plasma (rural town, near fluorpolymer production plant)	NR	NR	<LOQ (0.4)	NR	0.8	Fromme et al. (2017)
2009	Germany	n = 60, men and women, age: 18–67 years. Plasma (potential contamination of drinking water)	NR	NR	NR	NR	<LOQ (0.4)	Fromme et al. (2017)
2015	Germany	n = 50, men and women, age: 18–67 years. Plasma (potential contamination of drinking water)	NR	NR	NR	NR	<LOQ (0.4)	Fromme et al. (2017)
2014	Germany	n = 42, men and women, age: 18–67 years. Plasma	NR	NR	NR	NR	<LOQ (0.4)	Fromme et al. (2017)
2016	Germany	n = 158, men and women, age: 18–67 years. Plasma	NR	NR	NR	NR	<LOQ (0.4)	Fromme et al. (2017)
2015	Czech Republic	n = 300, men and women, mean age: 40.8 years. Serum	NR	NR	NR	<LOQ (0.013)	0.196	Sochorová et al. (2017)
2015–2016	Italy	n = 250, men and women, age: 20–51 years. Background exposed population. Serum.	0.04	NR	NR	<LOQ (NR)	1.67	Ingelido et al. (2018)
2015–2016	Italy	n = 257, men and women, age: 20–51 years. Exposed to elevated PFAS concentrations through drinking water. Serum.	<LOQ (NR)	NR	NR	<LOQ (NR)	1.33	Ingelido et al. (2018)
2015	UK	n = 29, women, mean age 32.9 years, Controls. Serum. n = 30, women, mean age 32.9 years, Cases with polycystic ovary syndrome. Serum.	NR	<LOQ (0.5)	NR	<LOQ (0.5)	<LOQ (0.5)	Heffernan et al. (2018)

9107

PFTrDA								
2012	Sweden	n = 3 pools primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery. Serum.	NR	NR	NR	0.030[(b)]	0.038[(b)]	Gebbink et al. (2015)
2007–2008	Norway	n = 41, women, mean (range) age: 36.7 (25–45 years). Serum.	<LOQ (0.05)	NR	0.056	<LOQ (0.05)	0.22	Haug et al. (2011)
2007–2008	Norway	n = 123, pregnant women. Plasma.	<LOQ (0.05)	NR	0.06	< LOQ (0.05)	0.23	Gützkow et al. (2012)
2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66) years. Serum.	<LOQ (0.009)	NR	<LOQ (0.009)	<LOQ (0.009)	0.05	Poothong et al. (2017)
2010–2013	Greenland	n = 207, pregnant women age >18 years, Inuits. Serum.	0.21	0.21	0.21	0.21	0.90	Long et al. (2015)
2015	Czech Republic	n = 300, men and women, mean age: 40.8 years. Serum	NR	NR	NR	<LOQ (0.013)	0.094	Sochorová et al. (2017)
PFTeDA								
2012	Sweden	n = 3 pools of serum primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery. Serum.	NR	NR	NR	<LOQ (0.002) [(b)]	<LOQ (0.002) [(b)]	Gebbink et al. (2015)
2007–2009	Germany	n = 30, students, male and female, age: 20–29 years from Münster. Plasma.	NR	NR	NR	<LOQ (0.004)	0.0169	Yeung et al. (2013a)
2007–2009	Germany	n = 30, students, male and female, age: 20–29 years from Halle. Serum.	NR	NR	NR	<LOQ (0.0103-0.0304)	0.0077	Yeung et al. (2013a)
2015	Czech Republic	n = 300, men and women, mean age: 40.8 years. Serum.	NR	NR	NR	<LOQ (0.013)	0.029	Sochorová et al. (2017)

9108

PFBS (total)								
2008–2011	Sweden	n = 150, primiparous women, within the third week after delivery. The County has a known contamination of the drinking water with PFAS. Mean (range) age: 30.2 (21–40 years). Serum.	0.027[(b)]	NR	0.055[(b)]	<LOQ (0.01)[(b)]	0.80[(b)]	Gyllenhammar et al. (2015)
2010	Sweden	n = 36 pools of serum. Primiparous women living in Uppsala county, donated serum samples within the third week after delivery. Age: 19–41 years.	NR	NR	NR	0.070	0.10	Glynn et al. (2012)
2012	Sweden	n = 3 pools primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery. Serum.	NR	NR	NR	<LOQ (<0.009) [(b)]	0.02[(b)]	Gebbink et al. (2015)
2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66 years). Serum	0.04	NR	0.05	<LOQ (0.009)	0.20	Poothong et al. (2017)
2009–2010	Spain	n = 46, males and females, age: 19–53 years. Whole blood.	NR	NR	0.20	<LOQ (0.04)	0.43	Gómez-Canela et al. (2015)
2009	Germany	n = 60, men and women, age: 18–67 years. Plasma (rural town, near fluorpolymer production plant)	NR	NR	NR	NR	<LOQ (0.4)	Fromme et al. (2017)
2015	Germany	n = 26, men and women, age: 18–67 years. Plasma (rural town, near fluorpolymer production plant)	NR	NR	NR	NR	<LOQ (0.4)	Fromme et al. (2017)
2009	Germany	n = 60, men and women, age: 18–67 years. Plasma (potential contamination of drinking water)	NR	NR	NR	NR	<LOQ (0.4)	Fromme et al. (2017)
2015	Germany	n = 50, men and women, age: 18–67 years. Plasma (potential contamination of drinking water)	NR	NR	NR	NR	<LOQ (0.4)	Fromme et al. (2017)
2014	Germany	n = 42, men and women, age: 18–67 years. Plasma	NR	NR	NR	NR	<LOQ (0.4)	Fromme et al. (2017)

2016	Germany	n = 158, men and women, age: 18–67 years. Plasma	NR	NR	NR	NR	<LOQ (0.4)	Fromme et al. (2017)
2015	Czech Republic	n = 300, men and women, mean age: 40.8 years. Serum	NR	NR	NR	<LOQ (0.006)	0.057	Sochorová et al. (2017)
2015–2016	Italy	n = 250, men and women, age: 20–51 years. Background exposed population. Serum.	<LOQ (NR)	NR	NR	<LOQ (NR)	0.36	Ingelido et al. (2018)
2015–2016	Italy	n = 257, men and women, age: 20–51 years. Exposed to elevated PFAS concentrations through drinking water. Serum.	<LOQ (NR)	NR	NR	<LOQ (NR)	4.26	Ingelido et al. (2018)
2015	UK	n = 29, women, mean age 32.9 years, Controls. Serum. n = 30, women, mean age 32.9 years, Cases with polycystic ovary syndrome. Serum.	NR	<LOQ (0.2)	NR	<LOQ (0.2)	0.46	Heffernan et al. (2018)
PFHxS (total)								
2007	Sweden	n = 10, women, mean (range) age: 48 (36–56) years. Serum.	0.93	NR	NR	NR	NR	Axmon et al. (2014)
2010–2011	Sweden	n = 270, women, median (range) age: 50 (22–75) years, serum	1.95	NR	NR	0.73 (P5)	10.29 P95)	Bjermo et al. (2013)
2008–2010	Sweden	n = 153, males, mean (range) age: 67 (53–79) years. Whole blood.	0.88	0.86	0.96	0.19	2.8	Bao et al. (2014)
2007–2011	Sweden	n = 201, men with prostate cancer. Median (range) age: 67 (49–79) years. Serum.	0.909	NR	1.1	0.0876	16	Hardell et al. (2014)
2007–2011	Sweden	n = 186, men without prostate cancer. Median (range) age: 67 (50–79) years. Serum.	0.865	NR	0.940	0.154	3.0	Hardell et al. (2014)

9109

2008-2011	Sweden	n = 150, primiparous women, within the third week after delivery. The County has a known contamination of the drinking water with PFAS. Mean (range) age: 30.2 (21–40 years). Serum.	3.7[(b)]	NR	5.4[(b)]	0.32[(b)]	34[(b)]	Gyllenhammar et al. (2015)
2010	Sweden	n = 36 pools of serum. Primiparous women living in Uppsala county, donated serum samples within the third week after delivery. Age 19–41 years.	NR	NR	NR	5.6	8.0	Glynn et al. (2012)
2012	Sweden	n = 3 pools primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery. Serum.	NR	NR	NR	2.0[(b)]	5.0[(b)]	Gebbink et al. (2015)
2014–2016	Sweden	n = 3,418, men and women from Ronneby municipality where drinking water was highly contaminated for many years, wide age range from children to elderly, plasma	152	NR	228	<LOQ (0.5)	1790	Li et al. (2018a)
2014–2016	Sweden	n = 242, men and women in a wide age range from children to elderly, plasma	0.84	NR	1.91	<LOQ (0.5)	60.1	Li et al. (2018a)
2011–2014	Sweden	n = 579, men and women, age: 80 years	2.9	3.9	7.5	0.14	77	Stubleski et al. (2016)
2007–2008	Norway	n = 41, women, mean (range) age: 36.7 (25–45 years). Serum.	0.39	NR	0.57	0.16	4.1	Haug et al. (2011)
2007–2008	Norway	n = 123, pregnant women. Plasma.	0.28	NR	0.34	0.04	1.64	Gützkow et al. (2012)
2007–2008	Norway	n = 99, pregnant women. Plasma.	0.3	NR	0.3	<LOQ (0.05)	2.8	Granum et al. (2013)
2007–2009	Norway	n = 391 pregnant women, mean (range) age: 31 (18–43) years. Serum.	0.44	NR	0.61	<LOQ (0.06)	14.8	Berg et al. (2014)

9110

2012-2014	Norway	n=74, 59 consumers (HC) and 15 non-consumers (NC) of fish from AFFF-affected waters. Median age 58.5 years (range 32-79 years). Serum.	2.15 (HC)	2.66 (HC)	4.07 (HC)	0.86 (HC, P10)	14.0 (HC, P90)	Hansen et al. 2016
2012–2014	Norway	n = 74, 59 consumers (HC) and 15 non-consumers (NC) of fish from AFFF-affected waters. Median age 58.5 years (range 32-79 years). Serum.	1.06 (NC)	0.89 (NC)	1.29 (NC)	0.15 (NC, P10)	3.03 (NC, P90)	Hansen et al. (2016)
2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66 years). Serum	0.78	NR	0.95	0.23	2.34	Poothong et al. (2017)
2008–2009	Denmark	n = 247, adult men. Mean age: 19.6 years. Serum.	0.67	NR	0.81	0.37 (P5)	1.58 (P95)	Joensen et al. (2013)
2011	Denmark	n = 200 samples from pregnant women. Serum.	0.22	NR	0.25	<LOQ (0.03)	0.75	Vorkamp et al. (2014)
2010–2012	Denmark	n = 392, newly pregnant women. Serum.	0.29	NR	NR	0.02 (P5)	7.28 (P95)	Jensen et al. (2015)
2011	Denmark	n = 145, women, mean (range) age: 41 (31–52 years). Plasma.	0.32	NR	0.39	0.08	1.74	Mørck et al. (2015)
2008–2013	Denmark	n = 1,507, pregnant women, primiparous, median age between 29 and 31 for the four quartiles of participants. Serum	0.5	NR	NR	0.4 (IQR)	0.6 (IQR)	Bach et al. (2016
2010–2012	Denmark	n = 649, pregnant women, mean age 30.7 years. Serum	NR	NR	0.3	0.2 (IQR)	0.4 (IQR)	Lind et al. (2017)
2007–2009	Faroe Islands	n = 487, pregnant women, mean age: 30.6 years. Serum	0.20	NR	NR	0.13 (IQR)	0.31 (IQR)	Timmermann et al. (2016)
2010–2013	Greenland	n = 207, pregnant women age >18 years, Inuits. Serum.	0.70	0.69	0.81	0.13	4.48	Long et al. (2015)

9111

2011–2014	Greenland	n = 128, women, median age around 50 years, breast cancer cases and controls. Serum	1.11	NR	NR	NR	NR	Wielsøe et al. (2017)
2008	France	n = 478, age: 18 and 75 years, males and females, had current residence in targeted areas and had a fishing license. Serum.	2.3	2.3	2.8	0.1	14.5	Denys et al. (2014)
2010–2013	France	n = 100, pregnant women, median (range) age: 32 (20–46) years. Serum.	0.619	NR	2.28	<LOQ (0.3)	31	Cariou et al. (2015)
2007–2009	Germany	n = 44 pregnant women, mean (range) age: 33 (21–43) years, samples collected during pregnancy. Plasma.	0.5	NR	0.6	NR	NR	Fromme et al. (2010)
2010	Germany	n = 18, male and female, age: 20–29 years. Plasma	0.86	0.82	NR	0.25	1.39	Schrôter-Kermani et al. (2013)
2009	Germany	n = 60, men and women, age: 18–67 years. Plasma (rural town, near fluorpolymer production plant)	0.4	NR	0.5	NR	2.3	Fromme et al. (2017)
2015	Germany	n = 26, men and women, age: 18–67 years. Plasma (rural town, near fluorpolymer production plant)	0.4	NR	0.4	NR	1.1	Fromme et al. (2017)
2009	Germany	n = 60, men and women, age: 18–67 years. Plasma (potential contamination of drinking water)	0.7	NR	0.8	NR	5.7	Fromme et al. (2017)
2015	Germany	n = 50, men and women, age: 18–67 years. Plasma (potential contamination of drinking water)	0.3	NR	0.4	NR	1.0	Fromme et al. (2017)
2014	Germany	n = 42, men and women, age: 18–67 years. Plasma	0.2	NR	0.2	NR	0.6	Fromme et al. (2017)
2016	Germany	n = 158, men and women, age: 18–67 years. Plasma	0.5	NR	0.7	NR	11.6	Fromme et al. (2017)
2009–2010	Spain	n = 46, males and females, age: 19–53 years. Whole blood.	NR	NR	0.26	<LOQ (0.07)	0.87	Gómez-Canela et al. (2015)

2009–2010	Spain	n = 755, men and women, age: 18–65 years. Serum	0.82	0.91	1.18	<LOQ (0.34. P10)	2.85 (P95)	Bartolome et al. (2017)
2010–2012	Slovakia	n = 120, pregnant women. Age range: 18–45 years. Plasma.	0.35	0.37	0.38	<LOQ (NR)	0.7	Uhl (2016)
2010–2012	Austria	n = 114, pregnant women. Age range: 18–45 years. Plasma.	0.36	0.38	0.43	<LOQ (NR)	1.3	Uhl (2016)
2015	Czech Republic	n = 300, men and women, mean age: 40.8 years. Serum	0.184	0.171	NR	0.008	1.39	Sochorová et al. (2017)
2015–2016	Italy	n = 250, men and women, age: 20–51 years. Background exposed population. Serum.	2.49	NR	NR	<LOQ (NR)	9.14	Ingelido et al. (2018)
2015–2016	Italy	n = 257, men and women, age: 20–51 years. Exposed to elevated PFAS concentrations through drinking water. Serum.	2.98	NR	NR	<LOQ (NR)	43.43	Ingelido et al. (2018)
2015	UK	n = 29, women, mean age 32.9 years, Controls. Serum. n = 30, women, mean age 32.9 years, Cases with polycystic ovary syndrome. Serum.	NR	1.04	NR	0.2	10.2	Heffernan et al. (2018)
PFHpS (total)								
2007–2008	Norway	n = 41, women, mean age: 36.7 years (range 25-45 years). Serum.	0.079	NR	0.083	<LOQ (0.05)	0.19	Haug et al. (2011)
2007–2009	Norway	n = 391, pregnant women, age: mean 31 (18-43 years). Serum.	0.10	NR	0.12	<LOQ (0.06)	1.10	Berg et al. (2014)
2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66 years). Serum	0.20	NR	0.25	0.06	0.63	Poothong et al (2017)
2008-2009	Denmark	n=247, adult men. Mean age: 19.6 years. Serum.	0.26	NR	0.29	0.15 (P5)	0.52 (P95)	Joensen et al. (2013)
2008–2013	Denmark	n = 1,507, pregnant women, primiparous, median age between 29 and 31 for the four quartiles of participants. Serum,	0.2	NR	NR	0.1 (IQR)	0.2 (IQR)	Bach et al. (2016)
2010–2013	Greenland	n = 207, pregnant women age: >18 years, Inuits. Serum.	0.19	0.19	0.23	0.06	1.44	Long et al. (2015)

2008	France	n = 478, age 18 and 75 years, males and females, had current residence in targeted areas and had a fishing license. Serum.	0.6	0.6	0.8	<LOQ (NR)	13.3	Denys et al. (2014)
2010–2013	France	n = 100, pregnant women, median (range) age: 32 (20–46) years. Serum.	<LOQ (0.4)	NR	0.182	<LOQ (0.4)	0.808	Cariou et al. (2015)
PFDS (total)								
2008-2011	Sweden	n = 150, primiparous women, within the third week after delivery. The County has a known contamination of the drinking water with PFAS. Mean (range) age: 30.2 (21–40 years). Serum.	<LOQ (0.005) [(b)]	NR		<LOQ (0.005) [(b)]	0.13 [(a),(b)]	Gyllenhammar et al. (2015)
2010	Sweden	n = 36 pools of serum. Primiparous women living in Uppsala county, donated serum samples within the third week after delivery. Age: 19–41 years.	NR	NR	NR	0.011	0.025	Glynn et al. (2012)
2012	Sweden	n = 3 pools primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery. Serum.	NR	NR	NR	<LOQ (0.005)[(b)]	<LOQ (0.005) [(b)]	Gebbink et al. (2015)
2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66 years). Serum	0.06	NR	0.06	<LOQ (0.0018)	0.17	Poothong et al. (2017)

9112

2015	Czech Republic	n = 300, men and women, mean age: 40.8 years. Serum.	NR	NR	NR	<LOQ (0.006)	0.245	Sochorová et al. (2017)
2015	UK	n = 29, women, mean age: 32.9 years, Controls. Serum. n = 30, women, mean age: 32.9 years, Cases with polycystic ovary syndrome. Serum.	NR	<LOQ (0.5)	NR	<LOQ (0.5)	<LOQ (0.5)	Heffernan et al. (2018)
PFOSI								
2008–2009	Denmark	n = 247, adult men. Mean age: 19.6 years. Serum.	NR	NR	NR	<LOQ (0.15)	NR	Joensen et al. (2013)
FOSA								
2008–2010	Sweden	n = 9 pools primiparous women living in Uppsala County, donated serum samples within the third week after delivery. Age: 19–41 years. Serum.	NR	NR	NR	<LOQ (0.040)[(b)]	0.049[(b)]	Glynn et al. (2012)
2008–2012	Sweden	n = 3 pools primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery. Serum.	NR	NR	NR	0.002[(b)]	0.004[(b)]	Gebbink et al. (2015)
2010	Sweden	n = 36 pools of serum. Primiparous women living in Uppsala county, donated serum samples within the third week after delivery. Age: 19–41 years.	NR	NR	NR	<LOQ (0.040)	<LOQ (0.040)	Glynn et al. (2012)
2011–2014	Sweden	n = 579, men and women, age: 80 years.	0.02	0.03	0.04	0.01	0.52	Stubleski et al. (2016)
2007–2008	Norway	n = 41, women, mean (range) age: 36.7 (25–45) years. Serum.	<LOQ (0.035)	NR	<LOQ (0.035)	<LOQ (0.035)	0.10	Haug et al. (2011)
2007–2009	Norway	n = 391 pregnant women, mean (range) age: 31 (18–43) years. Serum.	NR	NR	NR	<LOQ (0.01)	0.38	Berg et al. (2014)
2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66) years. Serum.	0.03	NR	0.03	< LOQ (0.0018)	0.05	Poothong et al. (2017)
2008–2013	Denmark	n = 1,533 serum samples pregnant nulliparous women from the Aarhus Birth Cohort Biobank	NR	NR	NR	<LOQ (1.19)	<LOQ (1.19)	Bjerregaard-Olesen et al. (2016)

		Most participants gave a blood sample between 11 weeks and 14 weeks of gestation. Median age: 29 years.						
2015	Czech Republic	n = 300, men and women, mean age: 40.8 years. Serum.	<LOQ (0.007)	<LOQ (0.007)	NR	<LOQ (0.007)	<LOQ (0.007)	Sochorová et al. (2017)
8:2 monoPAP								
2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66 years). Serum.	<LOQ (0.045)	NR	<LOQ (0.045)	<LOQ (0.045)	<LOQ (0.045)	Poothong et al. (2017)
8:2 diPAP								
2008–2012	Sweden	n = 9 pools (three pools at each time point) primiparous women living in Uppsala County, donated serum samples within the fourth week after delivery. Serum.	NR	NR	NR	<LOQ (0.0005)[(b)]	0.0183[(b)]	Gebbink et al. (2015)
2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66) years. Serum	<LOQ (0.009)	NR	0.03	<LOQ (0.009)	0.11	Poothong et al. (2017)
2007–2009	Germany	n = 30, students, male and female, age: 20–29 years from Münster. Serum.	NR	NR	NR	<LOQ (0.001)	0.0721	Yeung et al. (2013a)
2007–2009	Germany	n = 30, students, male and female, age: 20–29 years from Halle. Serum.	NR	NR	NR	<LOQ (0.0008-0.002)	0.0131	Yeung et al. (2013a)

9113

EtFOSA								
2007–2008	Norway	n = 41, women, mean (range) age: 36.7 (25–45) years. Serum.	NR	NR	NR	<LOQ (0.05)	<LOQ (0.05)	Haug et al. (2011)
2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66) years. Serum	<LOQ (0.045)	NR	<LOQ (0.045)	<LOQ (0.045)	<LOQ (0.045)	Poothong et al. (2017)
2015	Czech Republic	n = 300, men and women, mean age: 40.8 years old. Serum.	<LOQ (0.004)	<LOQ (0.004)	NR	<LOQ (0.004)	<LOQ (0.004)	Sochorová et al. (2017)
EtFOSAA								
2009–2010	Spain	n = 755, men and women, age: 18–65 years old. Serum	NR	NR	NR	< LOQ (0.27, P10)	< LOQ (0.27, P95)	Bartolome et al. (2017)
FC-807								
2007–2009	Germany	n = 30, students, male and female, age: 20–29 years from Münster. Serum.	NR	NR	NR	<LOQ (0.0004-0.0009)	<LOQ (0.0004-0.0009)	Yeung et al. (2013b)
2007–2009	Germany	n = 30, students, male and female, age: 20–29 years from Halle. Serum.	NR	NR	NR	<LOQ (0.0004-0.0007)	<LOQ (0.0004-0.0007)	Yeung et al. (2013b)

The term LOQ has been used for both limit of quantification, limit of detection and method detection limit.
P5: 5th percentile. P10: 10th percentile. P90: 90th percentile. P95: 95th percentile. HC: high consumers. NC: nonconsumers. IQR: Interquartile range. NR: not reported. LOQ: limit of quantification.
(a): Linear PFDS.
(b): Reported in ng/g.

9118 **Table B.3.** Concentrations of PFHxA and FOSA in general European adult populations with whole blood samples collected from 2007–2008 and onwards

Year	Country	Study population	Median, ng/mL	Geometric mean (ng/mL)	Aritmetric mean (ng/mL)	Min (ng/mL)	Max (ng/mL)	Reference
PFHxA								
2013–2014	Norway	n = 61, men and women, median (range) age: 41 (20–66) years. Whole blood.	0.62	NR	0.68	0.14	1.65	Poothong et al. (2017)
FOSA								
2013–2014	Norway	N = 61, men and women, median (range) age: 41 (20–66) years. Whole blood.	0.14	NR	0.22	0.05	2.35	Poothong et al. (2017)

9119 NR: not reported.

9120

9121 **Table B.4.** Concentrations of PFASs in general European children populations with serum or plasma samples collected from 2007–2008 and onwards

Year	Country	Study population	Median (ng/mL)	Geometric mean (ng/mL)	Aritmetric mean (ng/mL)	Min (ng/mL)	Max (ng/mL)	Reference
PFBA								
2011	Faroe Islands	n = 51, boys and girls, age: 13 years. Serum.	<LOQ (0.1)	NR	NR	<LOQ (0.1) (IQR)	<LOQ (0.1) (IQR)	Dassuncao et al. (2018)
2012	Faroe Islands	n = 51, boys and girls, age: 5 years. Serum.	<LOQ (0.1)	NR	NR	<LOQ (0.1) (IQR)	<LOQ (0.1) (IQR)	Dassuncao et al. (2018)
PFPeA								
2011	Faroe Islands	n = 51, boys and girls, age: 13 years. Serum.	<LOQ (0.05)	NR	NR	<LOQ (0.05) (IQR)	<LOQ (0.05) (IQR)	Dassuncao et al. (2018)
2012	Faroe Islands	n = 51, boys and girls, age: 5 years. Serum.	<LOQ (0.05)	NR	NR	<LOQ (0.05) (IQR)	<LOQ (0.05) (IQR)	Dassuncao et al. (2018)
PFHxA								
2010–2011	Norway	n = 940, boys and girls, age: 15–19 years. Serum.	0.08	NR	0.11	0.03	1.34	Averina et al. (2018)
2011	Faroe Islands	n = 51, boys and girls, age: 13 years. Serum.	<LOQ (0.05)	NR	NR	<LOQ (0.05) (IQR)	<LOQ (0.05) (IQR)	Dassuncao et al. (2018)
2012	Faroe Islands	n = 51, boys and girls, age: 5 years. Serum.	<LOQ (0.05)	NR	NR	<LOQ (0.05) (IQR)	<LOQ (0.05) (IQR)	Dassuncao et al. (2018)
PFHpA								
2010–2011	Norway	n = 112, toddlers, age: 3 years. Serum.	0.15	NR	NR	0.05	0.82	Papadopoulou et al. (2016)

2010–2011	Norway	n = 940, boys and girls, age: 15–19 years. Serum.	0.11	NR	0.14	0.07	1.47	Averina et al. (2018)
2011	Faroe Islands	n = 51, boys and girls, age 13 years. Serum.	0.06	NR	NR	0.05 (IQR)	0.08 (IQR)	Dassuncao et al. (2018)
2012	Faroe Islands	n = 51, boys and girls, age 5 years. Serum.	<LOQ (0.03)	NR	NR	<LOQ (0.03) (IQR)	<LOQ (0.03) (IQR)	Dassuncao et al. (2018)
PFNA								
2011	Denmark	n = 145, children, mean (range) age: 8.7 (6–11) years. Plasma.	0.82	NR	0.88	0.28	2.16	Mørck et al. (2015)
2007–2009	Germany	n = 44, infants, age: 6 months. Plasma.	1.0	NR	1.1	NR	2.3 (P95)	Fromme et al. (2010)
2007–2009	Germany	n = 24, infants, age: 19 months. Plasma.	0.6	NR	0.7	NR	1.4 (P95)	Fromme et al. (2010)
2007–2008	Germany	n = 112, children, mean ag:e 6.6 years. Plasma.	0.79	NR	0.84	0.42	2.38	Wilhelm et al. (2015)
2009–2010	Germany	n = 101, children, mean age: 8.5 years. Plasma.	0.68	NR	0.70	<LOQ (0.4)	1.59	Wilhelm et al. (2015)
2010–2011	Norway	n = 112, toddlers, age: 3 years. Serum.	2.13	1.31	NR	0.17	23.96	Papadopoulou et al. (2016)
2010–2011	Norway	n = 940, boys and girls, age: 15–19 years. Serum.	0.50	NR	0.60	0.12	5.35	Averina et al. (2018)
2012–2014	Faroe Island	n = 349, children, age: 5 years. Serum	1.1	NR	NR	0.8 (IQR)	1.6 (IQR)	Grandjean et al. (2017)

2010–2013	Faroe Island	n = 587, children, age: 13 years. Serum.	0.7	NR	NR	0.6 (IQR)	0.9 (IQR)	Grandjean et al. (2017b)
PFDA								
2011	Denmark	n = 145, children, mean (range) age: 8.7 (6–11) years. Plasma.	0.32	NR	0.34	0.11	0.75	Mørck et al. (2015)
2007–2009	Germany	n = 44, infants, age: 6 months. Plasma.	<LOQ (0.4)	NR	<LOQ (0.4)	NR	0.7 (P95)	Fromme et al. (2010)
2007–2009	Germany	n = 24, infants, age: 19 months. Plasma.	<LOQ (0.4)	NR	<LOQ (0.4)	NR	<LOQ (P95)	Fromme et al. (2010)
2010–2011	Norway	n = 112, toddlers, age: 3 years. Serum.	0.13	NR	NR	0.05	0.55	Papadopoulou et al(. 2016)
2010–2011	Norway	n = 940, boys and girls, age: 15–19 years. Serum.	0.21	NR	0.26	0.05	1.89	Averina et al. (2018)
2012–2014	Faroe Island	n = 349, children, age: 5 years. Serum.	0.3	NR	NR	0.2 (IQR)	0.5 (IQR)	Grandjean et al. (2017)
2010–2013	Faroe Island	n = 587, children, age: 13 years. Serum.	0.3	NR	NR	0.2 (IQR)	0.4 (IQR)	Grandjean et al. (2017b)
PFUnDA								
2010–2011	Norway	n = 112, toddlers, age: 3 years. Serum.	0.21	0.14	NR	<LOQ (0.05)	1.08	Papadopoulou et al. (2016)
2010–2011	Norway	n = 940, boys and girls, age: 15–19 years. Serum.	0.16	NR	0.18	0.03	0.85	Averina et al. (2018)
2011	Faroe Islands	n = 51, boys and girls, age: 13 years. Serum.	0.29	NR	NR	0.16 (IQR)	0.45 (IQR)	Dassuncao et al. (2018)

2012	Faroe Islands	n = 51, boys and girls, age: 5 years. Serum.	0.15	NR	NR	0.1 (IQR)	0.26 (IQR)	Dassuncao et al. (2018)
PFDoDA								
2010–2011	Norway	n = 112, toddlers, age: 3 years. Serum.	0.08	NR	NR	0.07	0.11	Papadopoulou et al. (2016)
2010–2011	Norway	n = 940, boys and girls, age: 15–19 years. Serum.	0.04	NR	0.06	0.01	0.24	Averina et al. (2018)
2011	Faroe Islands	n = 51, boys and girls, age: 13 years. Serum.	<LOQ (0.05)	NR	NR	<LOQ (0.05) (IQR)	<LOQ (0.05) (IQR)	Dassuncao et al. (2018)
2012	Faroe Islands	n = 51, boys and girls, age: 5 years. Serum.	<LOQ (0.05)	NR	NR	<LOQ (0.05) (IQR)	<LOQ (0.05) (IQR)	Dassuncao et al. (2018)
PFTrDA								
2010–2011	Norway	n = 112, toddlers, age: 3 years. Serum.	0.10	NR	NR	0.05	0.17	Papadopoulou et al. 2016)
PFBS								
2011	Faroe Islands	n = 51, boys and girls, age: 13 years. Serum.	<LOQ (0.1)	NR	NR	<LOQ (0.1) (IQR)	<LOQ (0.1) (IQR)	Dassuncao et al. 2018)
2012	Faroe Islands	n = 51, boys and girls, age: 5 years. Serum.	<LOQ (0.1)	NR	NR	<LOQ (0.1) (IQR)	<LOQ (0.1) (IQR)	Dassuncao et al. 2018)

9122

PFHxS								
2011	Denmark	n = 145, children, mean age: 8.7 years (range 6–11 years). Plasma.	0.34	NR	0.44	<LOQ (0.03)	3.68	Mørck et al. (2015)
2007–2009	Germany	n = 44, infants, age: 6 months. Plasma.	0.6	NR	0.7	NR	1.6 (P95)	Fromme et al. (2010)
2007–2009	Germany	n = 24, infants, age: 19 months. Plasma.	0.6	NR	0.7	NR	1.2 (P95)	Fromme et al. (2010)
2007–2008	Germany	n = 112, children, mean age: 6.6 years. Plasma.	0.81	NR	0.91	0.27	4.73	Wilhelm et al. (2015)
2009–2010	Germany	n = 101, children, mean age: 8.5 years. Plasma.	0.75	NR	0.83	0.32	2.92	Wilhelm et al. (2015)
2010–2011	Norway	n = 112, toddlers, age: 3 years. Serum.	0.55	0.60	NR	0.16	6.73	Papadopoulou et al. (2016)
2010–2011	Norway	n = 940, boys and girls, age: 15–19 years. Serum.	0.71	NR	1.53	0.18	84.7	Averina et al. (2018)
2012–2014	Faroe Island	n = 349, children, age: 5 years. Serum	0.3	NR	NR	0.2 (IQR)	0.4 (IQR)	Grandjean et al. (2017)
2010–2013	Faroe Island	n = 587, children, age: 13 years. Serum	0.4	NR	NR	0.3 (IQR)	0.5 (IQR)	Grandjean et al. (2017b)
PFHpS								
2010–2011	Norway	n = 112, toddlers, age: 3 years. Serum.	0.12	0.11	NR	<LOQ (0.05)	0.48	Papadopoulou et al. (2016)
2010–2011	Norway	n = 940, boys and girls, age: 15–19 years. Serum.	0.15	NR	0.17	0.03	7.62	Averina et al. (2018)

2011	Faroe Islands	n = 51, boys and girls, age: 13 years. Serum.	0.19	NR	NR	0.14 (IQR)	0.24 (IQR)	Dassuncao et al. (2018)
2012	Faroe Islands	n = 51, boys and girls, age: 5 years. Serum.	0.07	NR	NR	0.04 (IQR)	0.11 (IQR)	Dassuncao et al. (2018)
PFDS								
2011	Faroe Islands	n = 51, boys and girls, age: 13 years. Serum.	<LOQ (0.03)	NR	NR	<LOQ (0.03) (IQR)	<LOQ (0.03) (IQR)	Dassuncao et al. (2018)
2012	Faroe Islands	n = 51, boys and girls, age: 5 years. Serum.	<LOQ (0.03)	NR	NR	<LOQ (0.03) (IQR)	0.04 (IQR)	Dassuncao et al. (2018)
FOSA								
2010–2011	Norway	n = 112, toddlers, age: 3 years. Serum	0.11	NR	NR	<LOQ (0.05)	0.16	Papadopoulou et al. 2016
2011	Faroe Islands	n = 51, boys and girls, age: 13 years. Serum.	<LOQ (0.03)	NR	NR	<LOQ (0.03) (IQR)	0.05 (IQR)	Dassuncao et al. (2018)
2012	Faroe Islands	n = 51, boys and girls, age: 5 years. Serum.	<LOQ (0.03)	NR	NR	<LOQ (0.03) (IQR)	<LOQ (0.03) (IQR)	Dassuncao et al. (2018)
ETFOSAA								
2011	Faroe Islands	n = 51, boys and girls, age: 13 years. Serum.	<LOQ (0.03)	NR	NR	<LOQ (0.03) (IQR)	0.03 (IQR)	Dassuncao et al. (2018)
2012	Faroe Islands	n = 51, boys and girls, age: 5 years. Serum.	<LOQ (0.03)	NR	NR	<LOQ (0.03) (IQR)	0.02 (IQR)	Dassuncao et al. (2018)

9123

MeFOSAA								
2011	Faroe Islands	n = 51, boys and girls, age: 13 years. Serum.	0.05	NR	NR	0.03 (IQR)	0.08 (IQR)	Dassuncao et al. (2018)
2012	Faroe Islands	n = 51, boys and girls, age: 5 years. Serum.	<LOQ (0.03)	NR	NR	<LOQ (0.03) (IQR)	0.02 (IQR)	Dassuncao et al. (2018)

The term LOQ was used for both limit of quantification, limit of detection and method detection limit.
P5: 5th percentile. P95: 95th percentile. NR: not reported. LOQ: limit of quantification. IQR: interquartile range.

Appendix C – Toxicokinetics in Experimental Animals

During the previous decades, most of the information on the fate of PFASs was based on PFOS and PFOA (EFSA CONTAM Panel, 2008, 2018). These compounds have been shown to be readily absorbed in the gastrointestinal tract in mammals, including humans, and to distribute predominantly to the plasma and liver. PFOS and PFOA are not metabolised and are excreted in both urine and faeces. They may be subject to extensive enterohepatic recirculation. For PFOS, the serum elimination half-lives in rats and mice were slightly higher than one month, whereas in rabbits and monkeys, the serum elimination half-life was 3–4 months. Significant sex differences in the elimination of PFOA are observed in some species such as rats, for which half-lives may vary from few hours (in females) to several days (in males). Differences in biological half-lives between species for both PFOS and PFOA and between sexes for PFOA are mainly due to differences in renal clearance. For both PFOS and PFOA, maternal transfer occurs prenatally to the fetus through placental transfer and postnatally through the consumption of maternal milk.

C.1 PFCAs

In the past ten years, a significant amount of data was published on the toxicokinetics of shorter chain PFCAs such as PFBA, PFHxA and PFHpA, as well as on longer chain perfluorinated compounds, including PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA. Oral exposure of experimental animals to PFCAs having a perfluorinated carbon chain length of 3 to 11 was shown to result in an estimated absorption fraction greater than 95 % of the administered dose (ATSDR, 2018). None of the experimental studies observed the formation of metabolites, suggesting, as previously reported for PFOA (EFSA CONTAM Panel, 2018), that the biotransformation of PFCAs is unlikely in mammals, irrespective of their chain length. As it is known that transport proteins play a key role in PFCA elimination, in particular in renal tubular reabsorption, information on this process is given at the end of section C.1.

No data were identified regarding the toxicokinetics of PFPeA, PFPeDA, PFHxDA and PFODA.

Regarding PFOA, some papers and reports published recently (Gomis et al., 2018; NTP, 2019a; Pizzuro et al., 2019), confirm that this PFAS is well absorbed following oral exposure (>90%), is not biotransformed by xenobiotic metabolizing enzymes and is preferentially distributed to the liver and serum in most species. The NTP study showed that in male Sprague Dawley rats administered once daily by gavage, for 28 days to PFOA doses from 0.625 to 10 mg/kg bw per day, the liver/plasma ratios ranged from 0.87 to 1.07 (NTP, 2019a). Although the ratio was not provided for females, the plasma concentrations found in males were 2 or 3 levels of magnitude higher than in females exposed to similar doses. The reasons for such sex differences in tissue distribution is unclear, but could be related to longer half-lives of PFOA in male versus female rats (Pizzurro et al., 2019). The half-life of PFOA in male and female Sprague Dawley rats was found to range from 3 to 14 hours in females and from 8 to 12 days in males (Dzierlenga et al., 2019, cited by NTP, 2019a). Table C.2 summarises selected TK parameters for PFOA.

The toxicokinetics of **PFBA** was investigated in male and female Sprague-Dawley rats given either single oral doses by gavage (3, 10, 30, 100, 300 mg/kg bw) or a single i.v. dose (30 mg/kg bw) of ammonium PFBA (Chang et al., 2008). Gastrointestinal absorption was estimated to be rapid (mean Tmax ranging from 0.63 h in females to 1.25 h in males for the 30 mg/kg bw dose) and almost complete in both sexes (similar Cmax values obtained after oral or i.v. dose). For male rats, 24 h after oral dosing, mean liver concentrations ranged from 22 to 27% of the mean serum levels, depending on the dose. Values in females were not reported and concentrations in other tissues were not measured. Clearance for the 30 mg/kg bw oral dose (corresponding to 140 µmol/kg bw) varied from 444 mL/kg per day in males to 1718 mL/kg per day in females and mean serum elimination half-life was 9.2 h in males and 1.8 h in females (Table C.3). PFBA was mainly excreted in urine ranging from 51% to 64% of the administered

dose (for doses ranging from 3 to 100 mg/kg bw) in males at 24 h and representing 100% of the administered dose in females. Within the first 24 h, fecal excretion was between 0.1 and 3% in males. There was no evidence indicating that PFBA was metabolised in rats. A similar study was carried out in CD1 mice by the same authors (Chang et al., 2008). Male and female animals were given by gavage single oral doses of ammonium PFBA (10, 30, 100, 300 mg/kg bw). At the 30 mg/kg bw dose (corresponding to 140 µmol/kg bw), clearance varied from 254 mL/kg per day in males to 835 mL/kg per day in females and the serum elimination half-life value was 16.3 and 3.1 h in males and females, respectively (Table C.3). The percent of dose recovered in urine of female mice after 24 h (ranging from 65 to 68%) was approximately twice that recovered in urine of male mice. A small proportion of the ingested dose of PFBA (4-11%) was found in feces at 24 h, suggesting incomplete absorption and/or biliary elimination. PFBA toxicokinetics was also investigated in male and female cynomolgus monkeys given a single i.v. dose (10 mg/kg bw) (Chang et al., 2008). In both males and females, the clearance was approximately 1700 mL/kg per day, whereas elimination in urine at 24 h was 42 and 36% of the administered dose, respectively. The serum elimination half-lives were similar in males and females (approximately 40 h, Table C.3).

Butenhoff et al. (2012) carried out 28-day and 90-day studies in male and female Sprague Dawley rats exposed by gavage to ammonium PFBA at doses up to 150 and 30 mg/kg bw per day, respectively. Each study also included recovery groups that were sacrificed 3 weeks after the end of the dosing period. Serum and liver concentrations of PFBA were measured at the end of the treatment and recovery periods. At the end of both 28-day and 90-day dosing periods, males and females had mean serum PFBA concentrations that increased with dose. For the 30 mg/kg bw per day dose, concentrations measured in the serum of males were approximately 38 and 52 µg/mL at the end of the 28-day and 90-day dosing periods, respectively. These values decreased to approximately 0.2 and 0.5 µg/mL at the end of the 3 weeks recovery periods, respectively. At the same dose, in females mean serum PFBA concentrations at the end of the exposure period ranged from 1.7 µg/mL (28 day) to 5.2 µg/mL (90 day), and concentrations at the end of recovery were approximately 2-4% of those at the end of the dosing period. PFBA levels were also measured in the liver. In males exposed during 28 and 90 days at a dose of 30 mg/kg bw, hepatic concentrations were 17.4 and 16.1 µg/g, respectively, whereas females had mean concentrations of 0.4 and 0.9 µg/g, respectively. At the end of the recovery period concentrations of PFBA in the liver were close to or below the LOQ (0.050 µg/g).

Chengelis et al. (2009) investigated the toxicokinetic parameters of **PFHxA** in rats and cynomolgus monkeys. In a first experiment, male and female Sprague–Dawley rats were given a single iv dose of 10 mg PFHxA/kg bw (corresponding to 31.8 µmol/kg bw). The mean half-life of PFHxA in serum was shorter for female than for male rats (0.4 h compared to 1.0 h, Table C.3). In a second experiment, male and female Sprague–Dawley rats were given daily by gavage during 26 days 50, 150, or 300 mg PFHxA/kg bw (corresponding to 159.2, 477.7, 955.4 µmol/kg bw). No significant differences were observed between the mean serum concentrations of PFHxA measured 24 h after the first oral dose compared to the concentrations measured 24 h after the last dose. The half-life of PFHxA in serum was about 2–3 h regardless of dosage level, sex, or number of doses (Table C.3). Approximately 90% of the administered daily dose of PFHxA was recovered in the urine of male rats during 24 h post-dosing; in the same conditions, urinary elimination in female rats was about 80% of the administered dose. There were no significant sex differences in the toxicokinetics of PFHxA in monkeys following a single iv dose of 10 mg/kg bw (Table C.3). The average half-life in serum ranged from 2.4 to 5.3 h.

The toxicokinetics of sodium ^{14}C-PFHxA was investigated in rats and mice following a single dose (2 or 100 mg/kg bw by gavage) or after 14 consecutive doses (2 mg/kg unlabelled PFHxA by gavage) followed by a single oral dose of ^{14}C-PFHxA (Gannon et al., 2011). Absorption was almost complete and rapid (T_{max} between 15 and 30 min after dosing in both species) and bioavailability was nearly 100% at both levels in both species. In rats the plasma elimination half-life was slightly longer in males (1.5-1.7 h) than in females (0.5-0.7 h). Half-lives were not calculated in mice. In all tissues except skin, PFHxA was not quantifiable 24 h after dosing in both sexes of the two species. The primary route of elimination

was via urine (approximately 99% of the dose) in male and female rats and mice, irrespective of the administered dose. The route and extent of elimination was unchanged after 14 days of daily dosing. No metabolites were observed in plasma, urine or feces samples. The absence of biotransformation was confirmed *in vitro* (incubation of ^{14}C-PFHxA with rat and mouse hepatocytes) in the same study (Gannon et al., 2011).

The rapid and extensive absorption of PFHxA in rodents as well as its efficient elimination via urine were confirmed by Iwai (2011) in a study carried out in rats and mice in which animals of both sexes were orally administered single or daily doses (50 mg/kg bw) of ammonium ^{14}C-PFHxA.

Based on the data from Gannon et al. (2011), Russell et al. (2013) estimated the plasma clearance of PFHxA at 1957 and 6654 mL/kg per day for male and female rats, respectively.

In a paper dealing with the toxicokinetics of PFHxS in different species, Sundström et al. (2014) reported, as unpublished data, half-life values of PFHxA in monkeys (1.5 and 0.8 days for males and females, respectively).

Fujii et al. (2015) investigated the toxicokinetics of 8 PFCAs with six (C6) to fourteen (C14) carbon atoms, in male and female FVB/NJcl mice, administered by iv injection (0.31 µmol/kg bw, single dose, corresponding to 0.1 mg/kg bw) or by gavage (3.13 µmol/kg bw, single dose, corresponding to 1 mg/kg bw), each PFAS separately. After gavage administration or iv injection, PFHxA was not detected in the serum at any sampling time (LOD = 0.2 nmol/g).

Iwabuchi et al. (2017) administered PFHxA to male rats either as a single dose (gavage, 100 µg/kg bw) or in drinking water (1, 5 or 25 µg/L) for one or three months. PFHxA was very rapidly and completely absorbed, distributed and eliminated from the body with a half-life ranging from 2 to 4 hours (Table C.3). No tissue accumulation was observed in the chronic study and the steady state was reached within a day.

More recently, PFHxA was evaluated in male and female Sprague Dawley rats administered by gavage doses of 62.6, 125, 250, 500 and 1000 mg/kg bw perday for 28 days (NTP, 2019a). At the end of the experiment, males generally had a higher (1.6 to 3-fold) plasma concentration compared to females. The liver/plasma ratios, calculated in males only, were less than 1 (0.5 or lower) for animals treated with the three highest doses (hepatic concentrations > LOQ).

Kudo et al. (2001) investigated the elimination of different PFCAs in rats. In a first experiment, male and female Wistar rats were administered a single i.p. dose (20 mg/kg bw) of **PFHpA** and urine and feces were collected up to 5 days. In a second experiment rats were bile duct-cannulated prior to administration of a single i.v. dose (25 mg/kg bw) of PFHpA. Bile samples were collected up to 5 h after the injection. PFHpA was rapidly eliminated in urine, >90% of the dose within 120 minutes in both males and females. In contrast, fecal elimination was limited, (<2% for both sexes). Part of fecal elimination was due to biliary excretion of PFHpA, with a significantly faster biliary elimination rate in females compared to males.

The toxicokinetics of several PFCAs, including among others PFHpA, were studied by Ohmori et al. (2003) in male and female Wistar rats administered with a single i.v. dose (48.63 µmol/kg bw, corresponding to approximately 25 mg/kg bw) of the perfluorinated substance. Half lives in male and female rats were calculated to be 0.10 and 0.05 days, respectively; total clearances were 1604 and 3070 mL/kg per day, respectively, and distribution volumes were approximately 200 mL/kg in both sexes (Table C.3).

The toxicokinetics of PFHpA was investigated in male and female mice by Fujii et al. (2015, see above). Estimated gastrointestinal absorption was > 94% of the orally administered dose (3.13 µmol/kg, corresponding to approximately 1.14 mg/kg bw) in both sexes. In males, 24 h post-dosing via gavage, PFHpA was not quantifiable in the sampled tissues (serum, liver, kidney, brain, adipose tissue), whereas in females 1.8% and 0.2% of the administered dose was found in liver and kidney, respectively. The

major route of elimination was via urine (approximately 46% of the dose at 24 h) whereas fecal excretion represented less than 8% of the dose during the same period for both sexes. In animals exposed to PFHpA via gavage, total clearances were found to be 293 and 190 mL/kg per day in males and females, respectively (Table C.3).

After intraperitoneal injection of **PFNA** (20 mg/kg bw) in Wistar rats (Kudo et al., 2001, see above), elimination in urine within 5 days post-dosing amounted to 2.0% and 52% of the dose in males and females, respectively; during the same period, fecal elimination was <5% of the dose in males and <2% of the dose in females. Biliary excretion was investigated in male and female rats injected intravenously with PFNA at a dose of 25 mg/kg bw and was found to occur at a higher extent in females compared to males (approximately 0.4% of the dose eliminated within 5 hours after injection in females compared to less than 0.1% in males), suggesting a more efficient re-absorption mechanism of biliary-excreted PFNA in females. At the end of the experiment, the concentrations of PFNA for males were approximately 45 µg/mL and 90 µg/g in serum and liver, respectively; in females these levels were approximately 18 and 8 times lower, respectively.

Toxicokinetic parameters of PFNA injected intravenously to Wistar rats at a dose of 48 µmol/kg bw (corresponding to 22.3 mg/kg bw) were reported by Ohmori and co-workers (2003). Half-lives in male and female rats were calculated to be 29.5 and 2.44 days, respectively (Table C.3). A significant sex-related difference was observed for total clearance (6.9 and 105.7 mL/kg per day, in males and females respectively) and this disparity was mainly due to a significantly lower renal clearance in males compared to females. Protein binding, estimated *in vitro* using plasma protein from male and female rats, was over 98%.

The toxicokinetics of PFNA was investigated in Sprague-Dawley rats and CD-1 mice by Tatum-Gibbs et al. (2011). Male and female rats were given a single dose of PFNA by oral gavage at 1, 3, or 10 mg/kg bw, and blood was collected for analysis at 1, 2, 3, 4, 7, 16, 21, 28, 35, 42 and 50 days after treatment. In addition, PFNA concentrations in liver and kidney were measured at the end of the experiment. For the 10 mg/kg dose, the Cmax was 89.8 and 68.4 µg/mL in males and females, respectively. For the 3 mg/kg bw dose, corresponding to 6.47 µmol/kg bw, an average estimated half-life of 23.6 and 32.0 days for males and females, respectively, was reported (Table C.3). PFNA was found to be stored preferentially in the liver. Male and female CD-1 mice were given a single oral dose (1 or 10 mg/kg, corresponding to 2.16 or 21.6 µmol/kg bw) of PFNA, and animals were killed at time intervals similar to the rat experiment; blood, liver and kidney were collected. In the mouse, the rates of PFNA serum elimination were slightly faster in females than males, with estimated serum half-life of 25.7-68.8 days and 34.4-228 days, respectively, depending on the dose (Table C.3). PFNA was found to be stored preferentially in the mouse liver. A significantly higher hepatic retention of PFNA was observed in male mice than in females. The liver/serum concentration ratios ranged from 5 to 15, while kidney/serum ratios typically range between 0.2 and 0.4.

The toxicokinetics of PFNA was also investigated by Fujii et al. (2015) using male and female FVB/NJcl mice as models (see PFHxA sub-section for experimental details). Gastrointestinal absorption was found to be complete in both sexes. After iv injection, total clearance was 3.9 mL/kg per day in males and 5.1 mL/kg per day in females, whereas after gavage administration the values were 4.0 and 2.4 mL/kg per day, respectively (Table C.3). The distribution volumes reported for mice injected with PFNA were 220 and 150 mL/kg in males and females, respectively. Twenty-four hours after iv injection, a limited portion of the administered dose was eliminated in urine (1.3% for males and 2.2% for females) and even less was excreted in faeces (<1 % for both sexes). The majority of the dose was retained in serum (27% of the dose for males and 32% for females) and liver (69% for males and 46 % for females). For males and females treated by gavage, urinary elimination and faecal excretion were close to, or below 1% of the administered dose and the distribution pattern was similar to that found in animals exposed by iv injection.

Iwabuchi et al. (2017) administered PFNA to male rats either as a single dose (gavage, 50 µg/kg bw) or in drinking water (1, 5 or 25 µg/L) for one or three months. After a 3-month exposure period, PFNA was found to accumulate mainly in the liver: at the highest dose tested (25 µg/L) the concentration in this organ was 2.4 mg/kg. The estimated plasmatic half-life ranged from 18 to 64 days (Table C.3), but for the liver, the average estimated half-life was 160 days.

In a 28 day study, Sprague Dawley rats were given repeated oral gavage doses of PFNA ranging from 0.625 to 10 mg/kg bw per day in males and from 1.56 to 25 mg/kg bw per day in females. At similar doses, PFNA plasma concentrations were generally five- to ninefold higher in males compared to females. The liver/plasma ratios (calculated in males only) ranged from 0.9 to 2.6 (NTP, 2019a).

Kim et al. (2019) investigated the tissue distribution and excretion of PFNA in rats i.v. administered PFNA at a dose of 3 mg/kg bw. PFNA distributed preferentially in the liver and the kidney. The liver distribution of PFNA in male rats was about 2.5 times higher than that in female rats. In males, the cumulative excretion of PFNA in urine and faeces was 14.33 ± 9.30% and 1.28 ± 0.45% of the dose, respectively, whereas corresponding values in females were 34.56 ± 2.21% and 3.13 ± 2.18%, respectively, showing that urine is the major excretion route of PFNA in rats and that elimination occurs at a higher extent in females than in males. Based on the i.v. study and using a one-compartment model, the serum elimination half-lives in male and female rats were estimated to be 40.2 and 4.4 days, respectively, and the clearances were 7.4 and 16.6 mL/kg per day, respectively (Table C.3).

Disposition of PFAS was investigated in rats by Kudo et al. (2001, see above). In males and females, the concentrations of PFDA in the serum and the liver at the end of the experiment were approximately 37 µg/mL and 130 µg/g, respectively.

Ohmori et al. (2003) investigated the toxicokinetics of PFDA in rats (see above experimental conditions for PFHpA). Half-lives in male and female rats were found to be 40 and 59 days, respectively; distribution volumes were 348 and 441 mL/kg, respectively and total clearances were approximately 5 mL/kg per day in both sexes (Table C.3).

Toxicokinetic parameters of PFDA in mice were also reported by Fujii et al. (2015, see PFHxA sub-section for experimental details). Gastrointestinal absorption was found to be almost 100% of the administered dose for both sexes. After iv injection, total clearance was 2.2 mL/kg per day in males and 2.8 mL/kg per day in females, whereas after gavage administration the values were 3.9 and 2.2 mL/kg per day, respectively (Table C.3). The distribution volumes reported for mice injected with PFNA were 250 and 200 mL/kg in males and females, respectively. Irrespective of the route of administration and of the sex, 0-24 h urinary and faecal excretions were close to, or below 1% of the dose and most of the administered PFDA was retained in the liver.

Male and female Sprague Dawley rats were given for 28 days oral gavage doses of PFDA ranging from 0.156 to 2.5 mg/kg bw per day. PFDA plasma concentrations were slightly higher (30 % or less) in females compared to males. The liver/plasma ratios (calculated in males only) decreased with dose from 5.3 to 1.6 (NTP, 2019a). The toxicokinetics of PFDA wererecently examined in male and female rats by Kim et al. (2019). After i.v. administration of PFDA to rats at a dose of 1 mg/kg bw, PFDA was mainly distributed to the liver, followed by the kidney, with slightly higher values in males compared to females. The cumulative excretion of PFDA in urine and faeces was 11.22 ± 2.96% and 18.25±2.72% in male rats respectively, and 22.17 ± 5.28% and 16.44 ± 0.70% in female rats, respectively. Based on the i.v. study and using a one-compartment model, the serum elimination half-lives in male and female rats were estimated to be 109 and 50 days, respectively, and the clearances were 0.76 and 0.81 mL/kg per day, respectively (Table C.3).

The toxicokinetics of **PFUnDA, PFDoDA, PFTrDA** and **PFTeDA** were studied by Fujii et al. (2015) in mice (see experimental details in PFHxA sub-section). The gastrointestinal absorption of these perfluorinated compounds having between 11 to 14 perfluorinated carbon atoms was at or near 100% for both males and females. The analyses of the tissues and organs 24 h after i.v. injection or gavage administration showed that most of the perfluorinated compounds were distributed to the liver and, to a lesser extent to the serum in both males and females. For C11 to C14 compounds, urinary elimination during the 0-24 h period was ≤ 0.1% of the dose, irrespective of the route of administration and of the sex. During the same period, fecal excretion was approximately 1% of the dose for C11 to C14 injected intravenously. When administration occurred by gavage, fecal excretion was slightly higher than via i.v. for PFTrDA (1.7 – 3.1% of the dose, depending on the sex) and PFTeDA (3.0 – 6.1 % of the dose, depending on the sex). Most of the C11 to C14 compounds were retained in the liver (64-78% for males, 47-53% for females). After i.v. injection, total clearance varied from 2.8 mL/kg per day for PFUnDA to 10.4 mL/kg per day for PFTeDA, whereas after gavage administration the values were from 3.1 mL/kg per day for PFUnDA to 106.3 mL/kg per day for PFTeDA (Table C.3). When the total clearances of gavage- and i.v.-administered mice are compared, disparities exist in the long-chain perfluroalkyl carboxylic acids (C13 and C14), suggesting for these compounds that bile is an important elimination route.

There were no marked differences between sexes. The distribution volumes reported for mice injected with PFUnDA, PFDoDA, PFTrDA and PFTeDA, varied from 280 to 430 mL/kg in males and from 330 to 580 mL/kg in females.

Interactions with binding proteins, including carriers and transporters

Interactions with proteins, namely serum albumin, liver fatty acid binding proteins (L-FABP), and organic anion transporters influence the toxicokinetics of PFASs.

Although several authors investigated binding of PFASs (mainly PFCAs) to albumin, few studies tested a series of perfluorinated compounds using the same methodology. Using *in vitro* approaches PFNA and PFDA were found to bind to bovine serum albumin (BSA) at levels greater than 99% and 80%, respectively (Bischel et al., 2010; Vanden Heuvel et al., 1992). The association constants are on the order of $10^5 M^{-1}$ for PFNA, PFDA and PFUnDA (Bischel et al., 2010; McManus-Spencer et al., 2010). Bischel et al. (2011) investigated the associations, at low ligand concentrations, of PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA and PFDoDA with BSA. All the PFCAs tested were highly bound (> 95%) to BSA, except PFDoDA (80%). Affinity of PFCAs for BSA, estimated on the basis of the protein-water distribution coefficient (K_{PW}) increases with PFCAs hydrophobicity, but decreases from 8 to 11 perfluorinated carbons, probably due to steric hindrances associated with longer and more rigid perfluoroalkyl chains. Log K_{PW} ranges from 3.3 to 4.3. Additional observations at pH-induced changes in binding affinity support evidence that short- and long-chain PFCAs bind at different locations on BSA.

Liver fatty acid binding protein (L-FABP) is a lipid binding protein highly expressed in the liver as well as in the intestine and the kidney. L-FABP appears to have a higher affinity for PFCAs than albumin. Using purified recombinant rat liver L-FABP, Woodcroft et al. (2010) tested its interaction with PFCAs having perfluorinated carbons chain length of 4 to 8. They found an increasing affinity from PFPA to PFNA. Additional data on interaction of PFCAs with L-FABP are presented in section 3.3.1.2.

In rat as in human, PFCA renal active excretion and reabsorption are mediated through an organic anion transport system (Table C.1). The roles of the five rat renal organic anion transporters (OAT1, OAT2, OAT3, URAT1, and OATP1A1) in transporting PFCAs with different chain lengths (C2–C18) were

investigated by Weaver et al. (2010). OAT1 and OAT3 reside in the basolateral membrane of the proximal tubular cells, and would facilitate PFCA renal tubular secretion. In contrast, due to their expression in the apical membrane of the proximal tubular cells, OATP1A1, OAT2, OAT4, and URAT1 would be the transporters involved in PFCA renal tubular reabsorption (Han et al., 2012). Inhibition of uptake of model substrates was measured for the different anion transporters. PFHxA, PFHpA and PFOA inhibited OAT1-mediated *p*-aminohippurate transport, with PFHpA being the strongest inhibitor. PFOA and PFNA were the strongest inhibitors for OAT3-mediated estrone-3-sulfate transport, while OATP1A1-mediated estradiol-17β-glucuronide uptake was inhibited by PFNA, PFDA, and PFUnDA, with PFDA giving the strongest inhibition. No strong inhibitors were found for OAT2 or URAT1. Kinetic analysis was performed for the strongest inhibitors. OAT1 transported PFHpA with a K_m value of 50.5 µM, OAT3 transported PFNA with a K_m value 174.5 µM. OATP1A1-mediated transport yielded K_m values of 20.5 (PFNA), and 28.5 µM (PFDA). These data suggest that OAT1 and OAT3 are involved in renal secretion of PFHpA and PFNA, whereas OATP1A1 can contribute to the renal reabsorption of PFNA and PFDA.

Table C.1 Uptake transporters that have been reported to mediate PFAS membrane transport in renal tubular cells, hepatocytes and enterocytes (extracted from Weaver et al., 2010; Han et al., 2012; Zhao et al., 2015, 2017)

Cell types	Excretion		(re-)absorption	
	Human	Rat	Human	Rat
Renal proximal tubular cells	OAT1[a] (SLC22A6) OAT2[a] (SLC22A7) OAT3[a] (SLC22A8)	Oat1[a] (Slc22a6) Oat3[a] (Slc22a8)	OAT4[b] (SLC22A11) URAT1[b](SLC22A12)	Oat2[b] (Slc22a7) Urat1[b] (Slc22a12) Oatp1a1[b] (Slco1a1)
Hepatocytes			OATP1B1[a] (SLCO1B1) OATP1B3[a] (SLCO1B3) OATP2B1[a] (SLCO2B1) NTCP[a] (SLC10A1)	Oatp1a1[a] (Slco1a1) Oatp1a5[a] (Slco1a5) Oatp1b2[a] (Slco1b2) Oatp2b1[a] (Slco2b1) NTCP[a] (Slc10a1)
Enterocytes			OATP2B1[b] (SLCO2B1) ASBT[b] (SLC10A2) OSTα/β [b] (SLC51A/SLC51B)	Oatp1a5[b] (Slco1a5) Oatp2b1[b] (Slco2b1)

a: expressed at the basolateral membrane (exchanges with blood);
b: expressed at the apical membrane (exchanges with urine or bile or intestinal lumen).
Note that uptake transporters may be capable of being bi-directional (uptake and efflux).
OAT = organic anion transporter,
SLC = solute carrier family gene,
URAT = uric acid transporter, a member of the OAT family,
OATP = organic anion transporting polypeptide,
NTCP = Na^+/taurocholate cotransporting polypeptide,
ASBT = apical sodium-dependent bile salt tranporter,
OST = organic solute transporter.

C.2 PFSAs

PFOS, the most investigated PFSA, is known to be well absorbed in the gastrointestinal tract, to be resistant to biotransformation and to be mainly retained in liver and plasma (EFSA 2008, 2018). These properties are confirmed in articles and reports published recently (Chou and Lin, 2019, Gomis et al.,

2018; Huang et al., 2019; NTP, 2019b; Pizzuro et al., 2019). The NTP study showed that in male and female Sprague Dawley rats administered once daily by gavage, for 28 days, to PFOS doses from 0.312 to 5 mg/kg bw per day, plasma concentrations were similar in males and females and the liver/plasma ratios, (measured in males only) ranged from 2.7 to 3.8 (NTP, 2019b). After a single gavage administration of 2 mg/kg PFOS to male and female Sprague Dawley rats, Huang and co-workers found a liver/plasma ratio of 3-4 in females, whereas in males, the ratio increased from ca 5 (1 d post-dose) to 30 (140 d post-dose); a half-life of about 38 days was calculated in both sexes (Huang et al., 2019). Table C.2 summarises selected TK parameters for PFOS.

To date, only limited data were published on the toxicokinetics of PFSAs other than PFOS. PFBS and PFHxS were investigated in rodents and cynomolgus monkeys, but no data were identified regarding the toxicokinetics of PFHpS and PFDS. Based on animal experiments, the gastrointestinal absorption of investigated PFSAs is estimated between 50% of the dose for PFBS to almost 100% for PFHxS (ATSDR, 2018). As previously reported for PFOS, there are no indications that PFSAs are metabolised.

A series of studies was undertaken by Olsen et al. (2009) to evaluate the toxicokinetics of **PFBS** in rats and monkeys. Male and female Sprague–Dawley rats were given a single dose of 30 mg potassium PFBS/kg bw by either iv injection or oral gavage. In male and female rats dosed orally, mean Tmax values were 0.4 and 0.3 h, respectively, suggesting a rapid gastrointestinal absorption of PFBS. The mean serum PFBS concentrations at 24 h were significantly higher in males (0.38±0.07 µg/mL) than in females (0.02±0.01 µg/mL). At 96 h, the amount of PFBS found in the liver of males and females corresponded to approximately 0.03 and 0.05% of the administered dose, respectively. The comparison of the Area Under the Curve (AUC) values obtained from the i.v. and oral studies resulted in a bioavailability of 100% in females, and 55% in males. In orally treated animals, approximately 69% and 74% of the administered dose were found in urine after 24 h in males and females, respectively, whereas fecal excretion was ≤ 0.5% in both sexes. The mean serum half-life values were significantly shorter in males (4.7 h) than females (7.4 h) for animals orally exposed, but no difference was observed for animals intravenously injected (Table C.3).

In cynomolgus monkeys given a single iv dose of 10 mg/kg potassium PFBS, there were no statistically significant differences between males and females for any of the toxicokinetic parameters (Olsen et al., 2009). Mean serum concentration at 24 h was approximately 8 µg/mL. The percentage of administered dose recovered in urine from 0 to 24 h ranged from 33.8 to 86.8%. Mean serum elimination half-lives in male and female monkeys were 95 h and 83 h, respectively (Table C.3).

Chengelis et al. (2009) investigated the toxicokinetic parameters of PFBS in rats and monkeys, using the same experimental design as for PFHxA (see section C.1). In Sprague–Dawley rats given a single iv dose of 10 mg PFBS/kg bw, the half-life of PFBS in serum was shorter for female than male rats (0.64 h compared to 2.1 h) and apparent clearance from the serum was approximately 7- to 8-fold higher for female rats than for male rats (Table C.3). Approximately 70% of the administered dose of PFBS was recovered in the urine of male and female rats during the 0-24 h period. In monkeys given a single iv dose of 10 mg/kg bw, the half-life of PFBS in serum ranged from 8.1 to 15 h.

Adult male C57/BL6 mice were dietary exposed for 1–5 days to 16 mg/kg bw per day) of ^{35}S-PFBS (Bogdanska et al., 2014). PFBS was found to distribute to most of the twenty tissues examined. The tissue levels increased from 1 to 3 days of exposure but appeared thereafter to level-off in most cases. After 5 days of treatment the highest PFBS levels were detected in liver, gastrointestinal tract, blood, kidney, cartilage, whole bone, lungs and thyroid gland.

Recently, PFBS was evaluated in male and female Sprague Dawley rats administered by repeated gavage doses of 62.6, 125, 250, 500 and 1,000 mg/kg bw per day for 28 days (NTP, 2019b). Males generally had higher (5- to 18-fold) plasma concentrations compared to females across all dose groups. The liver/plasma ratios (calculated in males only) ranged from 0.4 to 0.6 across the doses.

Huang et al. (2019a) investigated the toxicokinetics of PFBS in male and female Sprague Dawley rats. After a single i.v. or gavage administration, concentrations were measured in the plasma, liver, kidney and brain. In all tissues, concentrations decreased slightly over time, with females having a faster decrease than males, and were as follows: liver > kidney > brain. In males, the liver/plasma ratio was generally above 1, dropping below 1 at 12h, whereas in females the ratio was 1.5-2 times lower than in males. After i.v. administration, plasma half-lives were 0.4 and 2.3 h in females and males, respectively, whereas after gavage these values averaged 1.3 and 3.3 h (Table C.3).

Butenhoff et al. (2009) exposed by oral gavage male and female Sprague Dawley rats to potassium **PFHxS** at dose levels of 0.3, 1, 3, and 10 mg for 2 weeks prior to mating and during mating, gestation and lactation (postnatal day 22) for parental females as well as during 6 weeks for males. The mean serum PFHxS concentrations in parent males at the end of the exposure period ranged from 44 µg/mL at 0.3 mg/kg to 201 µg/mL at 10 mg/kg. In pooled pup serum from postnatal day 22, serum PFHxS concentrations ranged from 9 µg/mL at 0.3 mg/kg to 94 µg/mL at 10 mg/kg. At the end of gestation, maternal serum PFHxS ranged from 3 µg/mL at 0.3 mg/kg to 60 µg/mL at 10 mg/kg. At doses of 1.0 mg/kg per day or higher, PFHxS-treated rats appeared to have reached serum steady state by after 2 weeks as their serum PFHxS concentrations were not statistically different between 2 and 6 weeks. Mean liver to serum PFHxS concentration ratio determined after 6 weeks of exposure ranged from approximately 1 to 3 for parent males, depending on dose, whereas in females and pups, this ratio never exceeded 0.4.

Comparative toxicokinetics of PFHxS in Sprague Dawley rats, CD-1 mice and cynomolgus monkeys were reported by Sundström et al. (2012). After a single oral dose of potassium PFHxS (10 mg/kg bw/d), given to male and female rats either by gavage or i.v., the toxicokinetics parameters were estimated using a two-compartment model. Based on the iv study, the serum elimination half-lives in male and female rats were 6.83 and 1.83 days, respectively, and the clearances were 40.3 and 119 mL/kg per day, respectively (Table C.3). At 24 h, the PFHxS mean serum concentrations after oral administration were 61 and 30 µg/mL, for males and females respectively. For females, the comparison of the AUC value obtained from the iv and oral studies resulted in a bioavailability of 50%. The female Tmax value after oral dosing was estimated to be at approximately 30 min, suggesting a rapid gastro-intestinal absorption process. Due to the short duration of the observation period (24 h) and hence to the lack of significant serum elimination over this period, the estimation of most of the parameters was considered as poorly reliable, especially for males. A second experiment was conducted in male and female rats given a single iv dose of potassium PFHxS (10 mg/kg bw per day) followed up for 10 weeks. The toxicokinetics parameters were estimated using a two-compartment model for males and one-compartment model for females. At the end of the experiment, the mean serum PFHxS concentration in males was approximately 6 µg/mL, whereas it was below the LOQ (0.01 µg/mL) in females. The half-life was estimated to be approximately 29 days in male rats, but only 1.6 days in females (Table C.3), indicating strong sex-related differences. The clearances were 6.71 and 53.35 mL/kg per day in males and females, respectively. A third experiment was designed to investigate the distribution and elimination routes of PFHxS in male and female Sprague Dawley rats. The percentage of the PFHxS administered dose recovered in serum, liver, urine, and feces 96 h after a single oral dose of either 1, 10, or 100 mg potassium PFHxS/kg bw was determined. Regardless of sex, mean serum PFHxS concentrations were non-linearly related to dose. Female serum and liver concentrations were considerably lower than those of males given an equivalent dose. For instance, for males, at the lowest dose tested (1 mg/kg bw), approximately 18 and 31% of the dose was found in serum and liver, respectively, whereas for females these values were 7 and 2%, respectively. Urine was the major route of excretion in male and female rats. Within 96 h following a single oral dose at 1, 10, and 100 mg PFHxS/kg bw, females excreted 35%, 28%, and 41% of the dose in urine, respectively, whereas urinary excretion for males was only about 6–7% of the dose at the 1 and 10 mg/kg dose level, but 30% at the

100 mg/kg dose level. Fecal excretion was limited (<1 % of administered dose), irrespective of the dose or the sex.

Additional studies were carried out in male and female mice given a single oral dose of potassium PFHxS (1 or 20 mg/kg bw) and followed for 23 weeks (Sundström et al., 2012). Regardless of sex, dose or sampling time, mean PFHxS concentrations were highest in serum followed by liver and then kidney. As indicated in Table C.3, mean serum half-life values were quite similar between male and female mice (approximately 30 versus 25 days for 1 mg/kg bw and 28 versus 27 days for 20 mg/kg bw for males and females, respectively). Clearances were similar between sexes and were approximately 3 and 4 mL/kg bw per day for the doses of 1 and 20 mg/kg bw, respectively. Urinary elimination predominated, but was slow and no indication of a clear sex-related difference was observed. Based on the data provided by the authors, a urinary elimination of about 30% of the administered dose within 2 months may be estimated. During the same period, total fecal excretion was likely close to or below 1% of the administered dose.

In male and female cynomolgus monkeys given a single i.v. dose of 10 mg potassium PFHxS/kg bw and followed during 24 weeks, mean serum elimination half-lives were less for females (87 ± 27 days) than males (141 ± 30 days), however this difference was not statistically significant (Sundström et al., 2012).

The toxicokinetics of PFHxS in female and male Sprague Dawley rats after a single i.v. or oral administration of 10 mg/kg bw were investigated by Kim et al. (2016). They found that PFHxS was almost completely absorbed in both sexes and was more rapidly absorbed in the female rats (Tmax of 1.37 h) than in the male rats (3.11 days). The measurement of PFHxS concentrations in different tissues at the end of the experiment (72 days in males, 14 days in females) showed that the highest values were found in the liver and the kidney. Based on the i.v. study and using a two-compartment model, the serum elimination half-lives in male and female rats were 20.7 and 0.88 days, respectively, and the clearances were 9.0 and 227.9 mL/kg per day, respectively (Table C.3). The calculated excreted percentage of the i.v. dose of PFHxS in female and male rats was 28.02 and 8.26% in urine, respectively, showing a significant sex difference, probably due to differences in the transport and renal re-absorption process of PFHxS between male and female rats.

Male and female Sprague Dawley rats were given for 28 days oral gavage doses of PFHxS ranging from 0.625 to 2.5 mg/kg bw per day (males) or from 3.12 to 50 mg/kg bw per day (females). Although females were administered doses five times higher than those administered to males, the female plasma concentrations were about half of male concentrations. The liver/plasma ratios (calculated in males only) ranged from 0.6 to 1.2 (NTP, 2019b). The toxicokinetic parameters of PFHxS after a single i.v. or gavage administration in male and female Sprague Dawley rats were reported recently by Huang et al. (2019). In both sexes, concentrations of PFHxS were highest in the liver, around 1- to 3-fold less in the kidney and 40-fold less in the brain. Liver/plasma ratios ranged from 0.5 to 0.82 and from 0.29 to 0.55 in males and females, respectively. Based on the i.v. study and using a two-compartment model, the serum elimination half-lives in female and male rats were 0.7 and 13 days, respectively. After gavage administration with the lower dose tested (4 mg/kg bw), the half-life based on a one compartment model was 17.6 days in males whereas it was 7.5-fold shorter in females (Table C.3).

Interactions with binding proteins including carrier and transporters

Bischel et al. (2011) investigated the associations, at low ligand concentrations, of PFBS, PFHxS and PFOS with BSA. All the PFSAs tested were highly bound (> 99%) to BSA. PFBS exhibits higher affinity for BSA than the equivalent chain-length PFCA (PFPA).

Zhao et al. (2015) demonstrated that the uptake of PFBS, PFHxS and PFOS into freshly isolated rat and human hepatocytes is mediated by sodium-dependent mechanisms through Na^+/taurocholate co-transporting polypeptide (Ntcp), a bile salt transporter expressed at the sinusoidal membrane of hepatocytes (See Table C.1). More recently, the same team found that rat organic anion transporting polypeptides OATP1A1, OATP1A5, OATP1B2 and OATP2B1 which are expressed in hepatocytes and

enterocytes can transport PFBS, PFHxS and PFOS (Zhao et al., 2017). Thus, both Na^+/taurocholate co-transporting polypeptide and the transporters of the OATP family could contribute to the enterohepatic circulation of PFSAs in rodents. It is also plausible that these transporters play a role in the accumulation of PFSAs in the liver.

9592 Table C.2 Selected TK parameters for PFOS and PFOA in animals

PFAS	Species (sex)	Route[j]	Dose (µmol/kg)	Half-life	Clearance$_{tot}$ (mL/kg per day)	Volume of distribution (mL/kg)	Reference
PFOS	Rat (F)	G	3.72	62.30±2.09 d	5.4±0.2	484±24	Chang et al. (2012)
	Rat (M)	G	3.72	38.31±2.32 d	22.2±0.3	1228±97	Chang et al. (2012)
	Rat (F)	G	27.84	71.13±11.25 d	4.9±0.5	468±25	Chang et al. (2012)
	Rat (M)	G	27.84	41.19±2.01 d	11.3±0.6	666±21	Chang et al. (2012)
	Rat (F)	i.v.	4	24.80±1.52 d	9.8±0.2	352±19	Kim et al. (2016)
	Rat (M)	i.v.	4	28.70±1.85 d	9.2±0.4	383±18	Kim et al. (2016)
	Rat (F)	G	4	23.50±1.75 d	8.5±0.4	289±16	Kim et al. (2016)
	Rat (M)	G	4	28.70±1.85 d	7.3±0.6	280±17	Kim et al. (2016)
	Rat (F)	i.v.	4	23.0±3.7 d[a]	9.0±0.6	297±43[b]	Huang et al. (2019a)
	Rat (M)	i.v.	4	22.0±2.1 d[a]	13.1±0.7	417±31[b]	Huang et al. (2019a)
	Rat (F)	G	4	28.4±11.0 d[a]	5.4±0.3	222±84[b]	Huang et al (2019a)
	Rat (M)	G	4	19.9±3.8 d[a]	9.7±0.7	280±48[b]	Huang et al (2019a)
	Rat (F)	G	20	18.0±3.1 d[a]	4.5±0.3	417±31[b]	Huang et al (2019a)
	Rat (M)	G	20	14.5±2.1 d[a]	6.4±0.5	417±31[b]	Huang et al (2019a)
	Mice (F)	G	1.86	37.80 d	4.7	258	Chang et al. (2012)
	Mice (M)	G	1.86	42.81 d	4.7	290	Chang et al. (2012)
	Mice (F)	G	37.2	30.45 d	6.0	261	Chang et al. (2012)
	Mice (M)	G	37.2	36.42 d	5.0	263	Chang et al. (2012)
	Monkey (F)	i.v.	3.72	110±15 d	1.65±0.04	274±28	Chang et al. (2012)
	Monkey (M)	i.v.	3.72	132±7 d	1.10±0.06	202±13	Chang et al. (2012)
n-PFOS	Rat (M)	G	5.4	33.7 d	NR	NR	Benskin et al. (2009)
	Rat (M)	Oral, 3m	0.04	82 (66-107) d	NR	NR	De Silva et al. (2009)
	Rat (F)	Oral, 3m	0.04	83 (73-90) d	NR	NR	De Silva et al. (2009)
iso-PFOS	Rat (M)	G	0.8	23.4 d	NR	NR	Benskin et al. (2009)
	Rat (M)	Oral, 3m	0.004	65 (47-107) d	NR	NR	De Silva et al. (2009)
	Rat (F)	Oral, 3m	0.004	38 (25-81) d	NR	NR	De Silva et al. (2009)
1m-PFOS	Rat (M)	G	<1.5	102 d	NR	NR	Benskin et al. (2009)
	Rat (M)	Oral, 3m	<0.006	103 (63-288) d	NR	NR	De Silva et al. (2009)
PFOA	Rat (F)	i.v.	48.6	0.08±0.03 d	2233±805	211±28	Kudo et al. (2002)
	Rat (M)	i.v.	48.6	5.68±0.99 d	50.4±14.4	346±57	Kudo et al. (2002)
	Rat (F)	i.v.	48.6	0.08±0.03 d	2233±805	211±28	Ohmori et al. (2003)
	Rat (M)	i.v.	48.6	5.63±1.20 d	50±17	339±68	Ohmori et al. (2003)
	Rat (F)	i.v.	2.42	0.19±0.01 d	612.8±32.5	171±11	Kim et al. (2018)
	Rat (M)	i.v.	2.42	1.64±0.44 d	47.4±3.4	112±29	Kim et al. (2018)
	Rat (F)	G	2.42	0.15±0.01 d	645.1±43.4	154±9	Kim et al. (2018)
	Rat (M)	G	2.42	1.83±0.47 d	40.3±3.4	106±9	Kim et al. (2018)
	Rat (M)	G	1 (*n*-PFOA)	13.4 d	NR	NR	Benskin et al. (2009)
	Rat (M)	G	0.1 (*iso*-PFOA)	8.1 d	NR	NR	Benskin et al. (2009)
	Rat (M)	Oral, 3m	0.05 (*n*-PFOA)	9.1 (5.3–33) d	NR	NR	De Silva et al. (2009)
	Rat (M)	Oral, 3m	0.005 (*iso*-PFOA)	6.3 (4.3–12) d	NR	NR	De Silva et al. (2009)
	Mice (F)	i.v.	0.31	NR	11.8±6.1	150±40	Fujii et al. (2015)
	Mice (M)	i.v.	0.31	NR	14.2±8.4	180±40	Fujii et al. (2015)
	Mice (F)	G	3.13	NR	9.0±1.5	NR	Fujii et al. (2015)
	Mice (M)	G	3.13	NR	13.1±7.4	NR	Fujii et al. (2015)
	Monkey (F)	i.v.	24.15	32.6±8.0 d	NR	198±69	Butenhoff et al. (2004)
	Monkey (M)	i.v.	24.15	20.9±12.5 d	NR	181±12	Butenhoff et al. (2004)
	Monkey (M)	Oral, 6m	24.15	19.5 d	NR	NR	Butenhoff et al. (2004)

9593 NR = Not Reported

9594 ([a])= elimination half life based on k_{10} calculation ([b])= Volume of distribution for the central compartment

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9597 Table C.3 Selected TK parameters for PFASs other than PFOS and PFOA in animals

PFAS	Species (sex)	Route[j]	Dose (µmol/kg)	Half-life	$Clearance_{tot}$ (mL/kg per day)	Volume of distribution (mL/kg)	Reference
PFBA	Rat (F)	i.v.	140	1.03±0.03 h	3318±66[a]	187±3	Chang et al. (2008)
	Rat (M)	i.v.	140	6.4±0.54 h	766±55[b]	253±6	Chang et al. (2008)
	Rat (F)	G	140	1.8±0.3 h	1718±163[a]	173±21	Chang et al. (2008)
	Rat (M)	G	140	9.2±0.8 h	444±27[b]	209±10	Chang et al. (2008)
	Mice (F)	G	140	3.1±0.3 h	835±38[c]	134	Chang et al. (2008)
	Mice (M)	G	140	16.3±7.2 h	254±549[d]	296	Chang et al. (2008)
	Mice (F)	G	47	2.9±0.3 h	730±29[c]	107	Chang et al. (2008)
	Mice (M)	G	47	13.3±4.6 h	240±63[d]	152	Chang et al. (2008)
	Monkey (F)	i.v.	47	41.0±4.7 h	1792±152[e]	443±59	Chang et al. (2008)
	Monkey (M)	i.v.	47	40.3±2.4 h	1694±209[f]	526±68	Chang et al. (2008)
PFHxA	Rat (F)	i.v.	31.8	0.4 h	18600	466	Chengelis et al. (2009)
	Rat (M)	i.v.	31.8	1.0 h	2784	175	Chengelis et al. (2009)
	Rat (F)	G	159.2	2.6 h	NR	NR	Chengelis et al. (2009)
	Rat (M)	G	159.2	2.2 h	NR	NR	Chengelis et al. (2009)
	Rat (F)	G (25d)	159.2	2.7 h	-	-	Chengelis et al. (2009)
	Rat (M)	G (25d)	159.2	2.2 h	-	-	Chengelis et al. (2009)
	Rat (M)	G	0.32	2.9 (2.1-4.3) h	NR	760	Iwabuchi et al. (2017)
	Monkey (F)	i.v.	31.8	2.4±1.7 h	3264±528	474±349	Chengelis et al. (2009)
	Monkey (M)	i.v.	31.8	5.3±2.5 h	2928±576	989±579	Chengelis et al. (2009)
PFHpA	Rat (F)	i.v.	48.6	0.05±0.01 d	3070±781	201±29	Ohmori et al. (2003)
	Rat (M)	i.v.	48.6	0.10±0.05 d	1604±558	196±19	Ohmori et al. (2003)
	Mice (F)	i.v.	0.31	NR	257±124	80±20	Fujii et al. (2015)
	Mice (M)	i.v.	0.31	NR	347±86	70±10	Fujii et al. (2015)
	Mice (F)	G	3.13	NR	190±22	NR	Fujii et al. (2015)
	Mice (M)	G	3.13	NR	293±154	NR	Fujii et al. (2015)
PFNA	Rat (F)	i.v.	48.6	2.44±0.41 d	106±31	243±49	Ohmori et al. (2003)
	Rat (M)	i.v.	48.6	29.6±2.3 d	6.9±0.6	287±13	Ohmori et al. (2003)
	Rat (F)	G	2.16	NR	NR	125(86-164)	Tatum-Gibbs et al. (2011)
	Rat (M)	G	2.16	42.1(33-56) d	NR	113(67-158)	Tatum-Gibbs et al. (2011)
	Rat (F)	G	6.47	32.0(3-119) d	NR	171(104-238)	Tatum-Gibbs et al. (2011)
	Rat (M)	G	6.47	23.6(20-28) d	NR	139(82-196)	Tatum-Gibbs et al. (2011)
	Rat (F)	G	21.6	NR	NR	146(90-201)	Tatum-Gibbs et al. (2011)
	Rat (M)	G	21.6	28.0(25-32) d	NR	110(65-154)	Tatum-Gibbs et al. (2011)
	Rat (M)	G	0.11	29(18-64) d	NR	880	Iwabuchi et al. (2017)
	Rat (F)	i.v.	6.47	4.4±0.2 d	16.6±1.3	45.9±3.7	Kim et al. (2019)
	Rat (M)	i.v.	6.47	40.2±18.7 d	7.4±1.4	363±183	Kim et al. (2019)
	Rat (F)	G	6.47	6.4±1.1 d	NR	NR	Kim et al. (2019)
	Rat (M)	G	6.47	54.6±2.5	NR	NR	Kim et al. (2019)
	Mice (F)	G	2.16	25.7(23-29) d	NR	192(165-220)	Tatum-Gibbs et al. (2011)
	Mice (M)	G	2.16	34.4(29-41) d	NR	328(0-1060)	Tatum-Gibbs et al. (2011)
	Mice (F)	G	21.6	68.8(42-120) d	NR	192(165-220)	Tatum-Gibbs et al. (2011)
	Mice (M)	G	21.6	228(70-796) d	NR	328(0-1060)	Tatum-Gibbs et al. (2011)
	Mice (F)	i.v.	0.31	NR	5.1±2.3	150±40	Fujii et al. (2015)
	Mice (M)	i.v.	0.31	NR	3.9±1.9	220±60	Fujii et al. (2015)
	Mice (F)	i.v.	3.13	NR	2.4±1.0	NR	Fujii et al. (2015)
	Mice (M)	G	3.13	NR	4.0±1.7	NR	Fujii et al. (2015)
PFDA	Rat (F)	i.v.	48.6	58.6±5.8 d	5.3±0.2	441±55	Ohmori et al. (2003)
	Rat (M)	i.v.	48.6	39.9±8.6 d	5.2±1.3	348±15	Ohmori et al. (2003)
	Rat (F)	i.v.	1.95	50.0±2.3 d	0.8±0.1	58.4±4.5	Kim et al. (2019)
	Rat (M)	i.v.	1.95	109.4±18.7 d	0.8±0.1	118.2±9.3	Kim et al. (2019)
	Rat (F)	G	1.95	74.6±10.2 d	NR	NR	Kim et al. (2019)
	Rat (M)	G	1.95	80.0±5.5 d	NR	NR	Kim et al. (2019)
	Mice (F)	i.v.	0.31	NR	2.8±1.2	200±50	Fujii et al. (2015)

PFAS	Species (sex)	Route[j]	Dose (µmol/kg)	Half-life	Clearance$_{tot}$ (mL/kg per day)	Volume of distribution (mL/kg)	Reference
	Mice (M)	i.v.	0.31	NR	2.2±0.9	250±60	Fujii et al. (2015)
	Mice (F)	G	3.13	NR	2.2±1.1	NR	Fujii et al. (2015)
	Mice (M)	G	3.13	NR	3.9±1.8	NR	Fujii et al. (2015)
PFUnDA	Mice (F)	i.v.	0.31	NR	3.4±1.5	280±80	Fujii et al. (2015)
	Mice (M)	i.v.	0.31	NR	2.8±1.0	33±60	Fujii et al. (2015)
	Mice (F)	G	3.13	NR	3.1C1.7	NR	Fujii et al. (2015)
	Mice (M)	G	3.13	NR	5.7±2.6	NR	Fujii et al. (2015)
PFDoDA	Mice (F)	i.v.	0.31	NR	4.8±2.4	350±100	Fujii et al. (2015)
	Mice (M)	i.v.	0.31	NR	4.4±1.6	570±210	Fujii et al. (2015)
	Mice (F)	G	3.13	NR	5.2±3.2	NR	Fujii et al. (2015)
	Mice (M)	G	3.13	NR	9.4±4.1	NR	Fujii et al. (2015)
PFTrDA	Mice (F)	i.v.	0.31	NR	7.2±3.2	430±140	Fujii et al. (2015)
	Mice (M)	i.v.	0.31	NR	6.8±2.5	580±200	Fujii et al. (2015)
	Mice (F)	G	3.13	NR	17.1±12.0	NR	Fujii et al. (2015)
	Mice (M)	G	3.13	NR	34.2±16.6	NR	Fujii et al. (2015)
PFTeDA	Mice (F)	i.v.	0.31	NR	10.4±6.0	430±130	Fujii et al. (2015)
	Mice (M)	i.v.	0.31	NR	10.4±4.6	550±180	Fujii et al. (2015)
	Mice (F)	G	3.13	NR	48.7±381	NR	Fujii et al. (2015)
	Mice (M)	G	3.13	NR	106.3±46.6	NR	Fujii et al. (2015)
PFBS	Rat (F)	i.v.	33.3	0.6 h	7464	288	Chengelis et al. (2009)
	Rat (M)	i.v.	33.3	2.1 h	946	118	Chengelis et al. (2009)
	Rat (F)	i.v.	100	4.0±0.2 h	56280±4800[a]	351±34	Olsen et al. (2009)
	Rat (M)	i.v.	100	4.5±2.2 h	11424±3264[b]	330±32	Olsen et al. (2009)
	Rat (F)	G	100	7.4±0.8 h	NR	391±105	Olsen et al. (2009)
	Rat (M)	G	100	4.7±0.4 h	NR	676±55	Olsen et al. (2009)
	Rat (F)	i.v.	11.8	0.36±0.03 h[k]	6048±432	123±12[l]	Huang et al. (2019a)
	Rat (M)	i.v.	11.8	2.26±0.3 h[k]	8028±48	113±16[l]	Huang et al. (2019a)
	Rat (F)	G	11.8	1.5±0.1 h[k]	3648±480	328±57[l]	Huang et al (2019a)
	Rat (M)	G	11.8	4.4±18.1 h[k]	624±60	164±677[l]	Huang et al (2019a)
	Rat (F)	G	59.1	1.2±0.1 h[k]	4392±936	326±95[l]	Huang et al (2019a)
	Rat (M)	G	59.1	2.7±0.8 h[k]	902±74	148±52[l]	Huang et al (2019a)
	Rat (F)	G	295.7	1.1±0.1 h[k]	6216±792	415±83[l]	Huang et al (2019a)
	Rat (M)	G	295.7	2.86±0.4 h[k]	1812±139	311±55[l]	Huang et al (2019a)
	Monkey (F)	i.v.	33.3	11.3±2.5 h 83.2±41.9 h[g]	8832±2880[h]	255±17	Olsen et al. (2009)
	Monkey (M)	i.v.	33.3	13.2±2.9 h 95.2±27.1 h[g]	12264±3384	254±31	Olsen et al. (2009)
	Monkey (F)	i.v.	33.3	8.1±2.0 h	552±240	248±45	Chengelis et al. (2009)
	Monkey (M)	i.v.	33.3	15±9.4 h	298±158	209±29	Chengelis et al. (2009)
PFHxS	Rat (F)	i.v.	22.8	1.83±0.26 d	119±47	278±66	Sundström et al. (2012)
	Rat (M)	i.v.	22.8	6.83 d	40.32	NR	Sundström et al. (2012)
	Rat (F)	G	22.8	0.83±0.53 d	NR	NR	Sundström et al. (2012)
	Rat (M)	G	22.8	NR	NR	NR	Sundström et al. (2012)
	Rat (F)	i.v.	22.8	1.64±0.08 d	53.35±4.38	126±14	Sundström et al. (2012)
	Rat (M)	i.v.	22.8	29.1±0.6 d	6.75±0.06	275±5	Sundström et al. (2012)
	Rat (F)	i.v.	10	0.88±0.07 d[i]	227.9±6.7[i]	289.3±23.8[i]	Kim et al. (2016)
	Rat (M)	i.v.	10	20.7±4.0 d[i]	9.0±0.05[i]	268.9±52.1[i]	Kim et al. (2016)
	Rat (F)	G	10	1.72±0.11 d[i]	124.8±3.4[i]	255.9±18.2[i]	Kim et al. (2016)
	Rat (M)	G	10	26.9±0.4 d[i]	7.2±0.06[i]	277.6±3.9[i]	Kim et al. (2016)
	Rat (F)	i.v.	10	1.61±0.06 d	56.2±6.2	130.4±5.5	Kim et al. (2018)
	Rat (F)	i.v.	25	2.03±0.09 d	52.6±8.6	153.9±20.2	Kim et al. (2018)
	Rat (M)	i.v.	25	34.1±0.88 d	6.4±0.06	314.8±23.2	Kim et al. (2018)
	Rat (F)	G	10	1.69±0.06 d	65.3±8.2	158.6±7.8	Kim et al. (2018)
	Rat (M)	G	25	34.1±4.85 d	6.6±1.2	326.7±20.0	Kim et al. (2018)
	Rat (F)	i.v	9.1	0.7±0.08 d[k]	65.5±3.1	66.3±7.6[l]	Huang et al. (2019a)
	Rat (M)	i.v.	9.1	13.0±1.5 d[k]	4.7±0.5	123±11[l]	Huang et al. (2019)
	Rat (F)	G	9.1	2.33±0.07 d[k]	46.1±2.2	155±9[l]	Huang et al. (2019)
	Rat (M)	G	9.1	17.6±1.8 d[k]	4.8±0.4	123±11[l]	Huang et al. (2019a)
	Rat (F)	G	36.5	2.19±0.06 d[k]	59.0±3.6	186±14[l]	Huang et al. (2019a)
	Rat (M)	G	36.5	16.5±1.1 d[k]	5.7±0.3	137±9[l]	Huang et al. (2019a)

PFAS	Species (sex)	Route[j]	Dose (µmol/kg)	Half-life	Clearance$_{tot}$ (mL/kg per day)	Volume of distribution (mL/kg)	Reference
	Rat (F)	G	73	1.98±0.05 d[k]	96.2±5.8	264±20[l]	Huang et al. (2019a)
	Rat (M)	G	73	14.8±1.2 d[k]	9.0±0.6	192±17[l]	Huang et al. (2019a)
	Mice (F)	G	2.3	24.8 d	2.7	96	Sundström et al. (2012)
	Mice (M)	G	2.3	30.5 d	2.9	129	Sundström et al. (2012)
	Mice (F)	G	45.7	26.8 d	3.8	147	Sundström et al. (2012)
	Mice (M)	G	45.7	28.0 d	4.8	195	Sundström et al. (2012)
	Monkey (F)	i.v.	22.8	87±27 d	1.9±0.4	213±28	Sundström et al. (2012)
	Monkey (M)	i.v.	22.8	141±30 d	1.3±0.1	287±52	Sundström et al. (2012)

Unless otherwise specified, values are means ± SD or means and range
Unless otherwise stated, single dose exposure is given
y = years; d = days
NR = Not Reported
(a): based on an average weight of 200 g
(b): based on an average weight of 250 g
(c): based on an average weight of 25 g
(d): based on an average weight of 35 g
(e): based on an average weight of 3 kg
(f): based on an average weight of 7 kg
(g): calculated from γ phase of elimination profile
(h): expressed as ml/d (weight of the animals not reported)
(i): values reported as mean ± SEM
(j): Unless otherwise stated, single dose exposure is given
(k): elimination half life based on k_{10} calculation
(l): volume of distribution for the central compartment

C.3 Mixtures

Benskin et al. (2009) conducted an isomer-specific disposition study following a single oral dose of a PFAS mixture administered by gavage to male Sprague Dawley rats. The dose consisted of 400 µg/kg bw PFOS, 500 µg/kg bw PFOA, 390 µg/kg bw PFNA (200 µg/kg bw n-PFNA and 190 µg/kg bw iso-PFNA), and 30 µg/kg bw PFHxS isomers which were present as impurities. The PFNA isomer profiles in the dose and in blood suggested both preferential uptake and elimination of iso-PFNA. The half-lives for iso-PFNA and n-PFNA, were 20.7 days and 40.6 days respectively. On day 3 post-dosing, maximum concentrations were found in liver (2.7 and 2.3 µg/g for iso-PFNA and n-PFNA, respectively). Approximately 33% of administered PFNA was excreted in urine throughout the experiment (38 days), with the remainder in feces. The portion of iso-PFNA in urine and feces was 63 and 57% of the dose, respectively. The same team repeated the experiment selecting a subchronic exposure instead of a single dose study (De Silva et al., 2009). Male and female Sprague Dawleys rats were dietary exposed to the same mixture of isomers for 12 weeks, followed by a 12-week depuration period. On day 38 of the exposure period (steady state), the greatest site of accumulation was the liver. Levels of n-PFNA and iso-PFNA in female liver tissue corresponded to 1.3 and 0.37 µg/g, respectively, whereas in males the corresponding levels were 9.9 and 6.3 µg/g, respectively. Half-lives of 47 and 31 days for n-PFNA and iso-PFNA in male rats and 2.1 and 0.8 days in females were reported. PFHxS data suggest preferential absorption of the linear isomer. Branched isomers were eliminated quickly from all tissues, such that only n-PFHxS was detectable on day 38. In blood, the half-life of linear PFHxS was 15.9 days whereas for branched isomers it varied from 3.5 days to 6.9 days. Liver half-life of n-PFHxS was over 3 times longer than that estimated for blood. Elimination of branched isomers occurred primarily *via* urine.

In a study carried out by Numata et al. (2014), the fate of 7 PFCAs and PFSAs, was investigated in male and female pigs fed during three weeks a diet contaminated with a mixture of these PFASs. Absorption, tissue distribution and excretion were measured and the half-lives were estimated. The concentration of PFBS, PFHxS, PFHxA, PFHpS, PFHpA, PFOS and PFOA in feed was 132 ± 11, 91.3 ± 8.0, 47.8 ± 4.4, 3.99 ± 0.50, 10.2 ± 1.7, 137 ± 13, 22.4 ± 2.6 µg/kg. Only the data for PFHxA suggest a steady state after 3 weeks of exposure. For PFBS, the estimation of the time to 95% completion of the steady state

was 217 days, whereas it was between 1 and 10 years for the rest of the compounds. At the end of the experiment, more than 80% of the total mass of ingested PFBS, PFHxS, PFHpS, PFOS, PFOA was found in blood and other tissues. For PFHpA and PFHxA, this percentage was approximately 60% and 20%, respectively. For all investigated substances except PFOS, blood plasma was the largest reservoir of unexcreted PFAS and less than 7% of unexcreted PFAS was present in the liver (for PFOS approximately 35% of the ingested dose was found in the liver). Fecal excretion occurred at a limited extent for all investigated PFASs (less than 8% of the ingested dose). Elimination in urine was < 5% of the dose for PFHxS, PFHpS, PFOS and PFOA, ranging from 10 to 20% for PFHpA and PFBS and higher than 60% for PFHxA. No significant differences were observed between males and females. The half-life was estimated to be 4, 43 and 74 days for PFHxA, PFBS and PFHpA, respectively. It was approximately 0.6, 1.1, 1.8 and 2 years for PFOA, PFHpS, PFOS and PFHxS, respectively.

Guruge et al. (2016) investigated the toxicokinetics of a mixture of 10 PFASs (PFOS, PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA and PFDoDA) in adult female micro-minipigs weighing 9-14 kg. After a single gelatin capsule filled with a mixture, corresponding to a dose of 3 mg/kg bw of each of the 10 tested substances, was given, they found that absorption of PFBA, PFPeA and PFHxA was rapid, with concentrations in the blood reaching the maximum in less than 12 h. The maximum levels of the rest of the PFASs in the blood were reached at 24–48 h after the exposure. PFPeA had the shorterstblood half-life followed by PFHxA, being 1.6 and 2.7 days, respectively. In contrast, PFOS elimination from the blood was the slowest, with a half-life of 86.6 days. The half-lives of PFBA, PFHpA, PFOA, PFNA, PFDA, PFUnDA and PFDoDA were 13.9, 34.7, 63.0, 49.5, 40.8, 38.5 and 31.5 days, respectively. Three weeks post-dosing, PFDoDA, PFUnDA, PFOS, PFDA and PFNA were mainly present in the liver, whereas for PFBA, PFHpA and PFOA, the concentration was higher in the blood than in the liver. At the end of the experiment, PFPeA and PFHxA were undetected in blood and liver whereas the PFNA body burden was the highest among the tested PFASs.

C.4 Other PFASs

Some other PFASs can be precursors and degrade or biotransform to PFSAs and PFCAs. The environmental degradation is reported in the environmental fate section, whereas the biotransformation in experimental animals or in biological *in vitro* models is described hereinafter. Most of the studies have been performed on 8:2 fluorotelomer alcohol (8:2 FTOH), whereas there have been limited investigations on non-FTOH metabolism.

The toxicokinetics of 8:2 fluorotelomer alcohol was reviewed by ECHA (ECHA, 2013) and is summarised in Table C.4. In rats **8:2 FTOH** is rapidly absorbed (absorption rate estimated between 27 and 57%, depending on the dose), and the parent compound and metabolites are quickly distributed to blood and tissues (Hagen et al., 1981; Martin et al., 2005; Fasano et al., 2006). At 7 days following the administration of radiolabelled 8:2 FTOH, 4-7% of the orally administered dose was present in the tissues as parent compound and related metabolites and the levels in the majority of these tissues were greater than in whole blood, with the highest levels observed in fat, liver, thyroid and adrenals (Fasano et al., 2006). Elimination was mainly via faeces (>70%) and biliary excretion was between 20 and 45 % of the administered dose, depending on the dose. Less than 4% of the administered dose was excreted in urine, and females eliminated more than males (Fasano et al., 2006). Metabolites identified in plasma, urine and feces were in principle glucuronide and glutathione conjugates of the parent compound, oxidised and reduced intermediates and PFOA, PFNA, PFHpA and PFHxA (Table C.4). 8:2 FTOH and most metabolites were rapidly or completely cleared from the tissues (8:2 FTOH $t_{1/2}$ = approximately 5 h), with the exception of PFOA (particularly in males). The half-life based on total radioactivity (Fasano et al., 2006) was approximately 9 and 7 days in males and females respectively. Whereas single dose studies showed no differences between sexes in metabolic profiles, the repeated dose study carried out by Fasano et al. (2009) resulted in levels of PFCAs (PFNA, PFOA and PFHpA) consistently higher in male livers than in female livers (Table C.4).

Dagnino et al. (2016) administered by oral gavage a single dose of 8:2 FTOH at 5 or 50 mg/kg bw to male Sprague Dawley rats and blood, urine, and feces samples were collected at 8, 24, 48, 72, 96, and 120 h after dosing. In the 50 mg/kg bw group, the highest concentration of metabolites measured in serum was for PFOA (1,995 ng/mL), whereas PFNA was found at a concentration of 25.66 ng/mL. In urine, PFOA (303.6 ng/mL) was the main transformation product and PFNA concentration was 0.84 ng/mL. Other PFCAs were not examined (Table C.4).

Recently, Huang et al. (2019b) investigated the toxicokinetics of 8:2 FTOH in male and female Sprague Dawley rats given a single dose via gavage or i.v. of 8:2 FTOH. The parent substance and its two metabolites (PFOA and 7:3-fluorotelomer acid [7:3-FTA]) were determined in plasma, liver, kidney, and brain. There was rapid absorption and distribution of 8:2-FTOH after gavage administration and the plasma elimination half-life ranged from 1.1 to 1.7 h. Bioavailability of 8:2-FTOH ranged from 22 to 41% for both sexes with no dose-dependent trends. 8:2-FTOH metabolites, PFOA and 7:3-FTA were detected in plasma following administration of the parent FTOH. The plasma half-life of PFOA was longer in males than in females (198–353 h and 4.47–6.9 h, respectively). The plasma half life of 7:3-FTA was around 2–3 days in both sexes. 8:2-FTOH and 7:3-FTA were detected in all tissues; PFOA was found in the liver and kidney (Table C.4), but not in the brain. Detectable concentrations of metabolites persisted longer than the parent FTOH. Sex differences were observed in the tissue distribution and elimination of PFOA, but not 8:2-FTOH and 7:3-FTA.

Henderson and Smith (2007) examined the metabolism and disposition of 8:2 FTOH in timed-pregnant mice exposed to a single gavage dose (30 mg /kg bw) (Table C.4). During gestation (GD9 to GD18), maternal serum and liver concentrations of PFOA decreased from 789 ± 41 to 668 ± 23 ng/mL and from 673 ± 23 to 587 ± 55 ng/g, respectively. PFOA was transferred to the developing fetuses as early as 24-h post-treatment with concentrations increasing from 45 ± 9 ng/g (GD10) to 140 ± 32 ng/g (GD18), while PFNA was quantifiable only at GD18 (31 ± 4 ng/g). Post-partum, maternal serum PFOA concentrations decreased from 451 ± 21 ng/mL on postnatal day (PND) 1 to 52 ± 19 ng/mL on PND15 and PFNA concentrations, although five-fold less, exhibited a similar trend. Immediately after birth, pups were cross-fostered with dams that had been treated during gestation with 8:2 FTOH or vehicle in order to investigate exposure through lactation. At both PND3 and PND15, PFOA and PNDA were detected in serum and liver from neonates exposed pre- and/or postnatally, indicating that maternal exposure to 8:2 FTOH results in both *in utero* and lactational exposure to PFOA and PNDA.

In vitro data (Table C.4) suggest that hepatocytes from rats, mice and humans have the ability to biotransform 8:2 FTOH into several PFCAs (Nabb et al., 2007). Human hepatocytes produced about 20- and 12-fold less PFOA than mouse and rat hepatocytes, respectively.

PAPs biotransformation proceeds via the hydrolysis of the phosphate linkage, yielding the corresponding FTOH, which is then available for oxidation. Both mono-AP and di-PAP congener uptake, biotransformation and elimination were investigated in rats (see Table C.4).

Male Sprague Dawley rats were administered a single dose of 200 mg/kg by oral gavage of mono-phosphate (**8:2 monoPAPS**), or the corresponding di-phosphate (**8:2 diPAPS**), with blood taken over 15 days post-dosing (D'Eon and Mabury, 2007). Both compounds were synthesised by the authors and were 97% pure and contained < 0.01% PFOA. Upon completion of the time-course study, the animals were redosed using an identical dosing procedure, with sacrifice and necropsy 24 h after the second dosing. Increased levels of PFOA, along with both 8:2 PAPs congeners, were observed in the blood of the dosed animals. In the 8:2 monoPAPS-dosed animals, 8:2 monoPAPs and PFOA blood concentrations peaked at 7900 ± 1200 ng/g and 34 ± 4 ng/g, respectively. In the 8:2 diPAPS-dosed animals, 8:2 diPAPS concentration peaked at 32 ± 6 ng/g, and 8:2 monoPAPs and PFOA peaked at 900 ± 200 ng/g and 3.8 ± 0.3 ng/g, respectively. As indicated in Table C.4, in addition to PFOA, PFHpA was also observed in the animals dosed with monoPAPs. Consistent with other fluorinated contaminants, the tissue distributions showed increased levels of both PFOA and the 8:2 PAPs congeners in the liver relative to the other tissues measured.

A subsequent study by the same authors carried out in rats administered by gavage or by iv injection a single dose of various congeners of mono-PAPs or di-PAPs (see Table C.4), resulted in biotransformation yields of 1% for 6:2 diPAP (to PFHxA), 9% for 8:2 diPAP (to PFOA), and 8% for 10:2 diPAP (to PFDA). Half-lives of the investigated mono- and di-PAP congeners were estimated to be in the 1.6-4.8 d range. (D'Eon and Mabury, 2011).

Dagnino et al. (2016) investigated the metabolism of 8:2 diPAP in male Sprague Dawley rats (see above experimental details). PFOA and PFNA highest concentrations in serum of animals dosed at 50 mg/kg dosed animals were 36.1 ng/mL and 1.5 ng/mL, respectively. In urine, PFOA and PFNA concentrations were approximately 6 and 0.6 ng/mL, respectively. Other PFCAs were not examined (Table C.4).

Ross et al. (2012) investigated the isomer specific fate of perfluorooctane sulfonamide (**FOSA**) in male Sprague–Dawley rats exposed to commercial FOSA (purity not indicated) via food for 77 days (83.0 ng/kg bw per day), followed by 27 days of depuration. Elimination half-lives of the two major branched FOSA isomers (2.5 ± 1.0 days and 3.7 ± 1.2 days) were quicker than for linear FOSA (5.9 ± 4.6 days), resulting in a depletion of branched FOSA isomers in blood and tissues relative to the total dosed FOSA. A significant enrichment of 5m-PFOS and a significant depletion of 1m-PFOS were observed in serum, relative to authentic electrochemical PFOS. The results confirm that *in vivo* exposure to commercially relevant PFOS-precursors can result in a distinct PFOS isomer profile.

Female Sprague Dawley rats were administered **EtFOSE** (purity > 99%) by gavage for 3 weeks, at 5 mg/kg bw per day and several putative metabolites were analysed in liver and serum (Xie et al., 2009). Levels of **EtFOSE** and **FOSE** in serum and liver were in the low µg/kg range, whereas EtFOSA was not detected in either matrix. In contrast, levels of **EtFOSAA** were in the low mg/kg range in the liver and serum. FOSA hepatic levels were also in the low mg/kg range, but were approximately one order of magnitude lower in serum. The major metabolite detected in both liver and serum was PFOS (Table C.4), with levels that were at least 10 times higher compared to the five perfluorooctanesulfonamides. The liver-to-serum ratios ranged from 1.3 for EtFOSAA to 10.1 for FOSA and decreased in the order FOSA>FOSE>PFOS>EtFOSAA. The formation of EtFOSAA, FOSA and PFOS was also observed in rat liver slices incubated with EtFOSE (Xu et al., 2004; data not presented in Table C.4).

9766 Table C.4 Summary of perfluorinated biotransformation products observed in metabolic studies of fluorotelomer compounds (FTOHs), polyfluoroalkyl
9767 phosphoric acid esters (PAPs) and other PFAS precursors. Only end-metabolites are mentioned

Systems	Precursors	Metabolites								References
		PFBA	PFPA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFOS	
Male rats (gavage, single dose)	8:2 FTOH	NE	NE	NE	NE	Plasma	ND	NS		Hagen et al., 1981
Male rats (single ip injection)	8:2 FTOH	NE	NE	NE	NE	Plasma, Liver Kidney	Plasma, Liver Kidney	NE		Martin et al., 2005
Rats (gavage, single dose)	^{14}C-8:2 FTOH	ND	ND	Plasma Urine Feces	Plasma	Plasma Urine Feces	Plasma	ND		Fasano et al., 2006
Rats (gavage, repeated doses)	8:2 FTOH + ^{14}C-8:2 FTOH	ND	Plasma, Tissues[a]	Plasma, Tissues[b], Urine	Plasma, Tissues[c], Urine	Plasma, Tissues[c], Urine	Plasma, Tissues[c], Urine	ND		Fasano et al., 2009
Male and female rats (inhalation)	8:2 FTOH	NE	ND	Plasma	Plasma	Plasma	Plasma	NE		Himmelstein et al., 2012
Male rats (gavage, single dose)	8:2 FTOH	NE	NE	NE	NE	Serum Urine Feces	Serum Urine Feces	NE		Dagnino et al., 2016
Male and female rats (single i.v. dose)	8:2 FTOH	NE	NE	NE	NE	Serum	NE	NE		Huang et al., 2019b
Male and female rats (gavage, single dose)	8:2 FTOH	NE	NE	NE	NE	Serum, liver, kidney				Huang et al., 2019b

Systems	Precursors	Metabolites								References
		PFBA	PFPA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFOS	
Male rats (gavage, single dose or two doses)	8:2 monoPAPs 8:2 diPAPs	NE	NE	ND	Blood[d]	Blood Liver Kidney	ND	NE		D'Eon and Mabury, 2007
Male rats (single iv injection or gavage, single dose)	Mixtures of 4:2, 6:2, 8:2, 10:2 monoPAPs or diPAPs	Blood Urine	Blood[e] Urine	Blood Urine	Blood Urine	Blood Urine[f]	Blood	Blood		D'Eon and Mabury, 2011
Male rats (gavage, single dose)	8:2 diPAP	NE	NE	NE	NE	Serum Urine Feces	Serum Urine Feces	NE		Dagnino et al., 2016
Female rats (gavage, repeated doses)	EtFOSE	NE	NE	NE	NE	NE	NE	NE	Liver Serum	Xie et al., 2009
Male rat hepatocytes	8:2 FTOH	NE	NE	NE	NE	Major PFCA[g]	Traces	NE		Martin et al., 2005
Rat hepatocytes [h]	^{14}C-8:2 FTOH	NE	0.15%	0.27%	0.08%	0.24%	0.06%	NE		Nabb et al., 2007
Rat microsomes [i]	^{14}C-8:2 FTOH	NE	< LOQ	0.06%	0.15%	1.33%	0.02%	NE		Nabb et al., 2007
Male mice (dietary exposure)	8:2 FTOH	NE	NE	NE	NE	Liver	Liver	NE		Kudo et al., 2005

Systems	Precursors	Metabolites								References
		PFBA	PFPA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFOS	
Pregnant mice (gavage, single dose at GD8)	8:2 FTOH	NE	NE	NE	NE	Liver, serum (dams) ; Liver, serum (pups) ; Placenta	Liver, serum (dams) ; Liver, serum (pups) ; Placenta	NE		Henderson & Smith, 2007
Mouse hepatocytes [h]	^{14}C-8:2 FTOH	NE	0.21%	0.21%	0.20%	0.47%	0.08%	NE		Nabb et al., 2007
Mouse microsomes [i]	^{14}C-8:2 FTOH	NE	< LOQ	0.04%	0.05%	0.48%	< LOQ	NE		Nabb et al., 2007
Human hepatocytes [h]	^{14}C-8:2 FTOH	NE	0.08%	0.04%	0.03%	0.02%	0.02%	NE		Nabb et al., 2007
Human microsomes [i]	^{14}C-8:2 FTOH	NE	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	NE		Nabb et al., 2007

a present in liver, kidney and thyroid
b present in liver > kidney > thyroid > adipose tissue ≈ skin
c present in liver > kidney > thyroid > adipose tissue ≈ bone marrow ≈ skin ≈ thymus
d detected in animals exposed to 8:2 monoPAPS
e not detected in blood from animals exposed via oral gavage
f not detected in urine from animals injected intravenously
g After 4 h incubation with 18µM 8:2 FTOH, 78% of the parent material had been biotransformed, PFOA representing 1.4% and PFNA <0.2% of the formed products
h Values are reported as percent of incubated dose following 120 min incubations.
i Values are reported as percent of incubated dose following 30 min incubations
ND: Not detected
NE: Not evaluated
NS: Not specified

9782 Luebker et al. (2002) determined the relative effectiveness of EtFOSA and EtFOSE to inhibit 11-(5-
9783 dimethylaminonapthalenesulphonyl)-undecanoic acid (DAUDA) binding to L-FABP, compared to PFOS
9784 and PFOA. They found that PFOS exhibited the highest level of inhibition of DAUDA-L-FABP binding in
9785 the competitive binding assays, followed by EtFOSA, and, with equal IC50s, N-EtFOSE and PFOA.
9786

Appendix D – Effects following acute exposure

Due to the limited number of published data, studies were considered even if PFASs were not applied via water or food or by gavage (oral exposure). Reports on acute exposure effects were identified for PFHxA, PFDA, EtFOSE (as listed in Table D1) and 8:2 FTOH. Studies on effects following acute exposure to PFOS and PFOA, which have been published between 2008 and 2016, are documented in the previous opinion (EFSA CONTAM Panel, 2018). An additional study on PFOS has been published.

For PFHxA, Loveless et al. (2009) reported that the LD_{50} ranged between 1750 and 5000mg/kg bw in female rats.

For PFDA, Harris et al. (1989b) determined an oral LD_{50} of 120 mg/kg bw in female C57BL/6N mice and of 129 mg/kg bw for female C57BL/6J mice.

Adinehzadeh et al. (1999) studied the impact of PFDA (Sigma-Aldrich, commercial source,) at single i.p. doses of 2-50 mg/kg bw in male F344 rats (6 rats/group). At doses of < 20 mg/kg bw, PFDA was less toxic than above, as indicated by measurements of daily food intake. At 50 mg/kg bw, serum concentrations of tumor necrosis factor (TNF)-alpha were elevated about 8-fold. Hepatic fatty acyl-CoA oxidase activity showed a dose-dependent increase from 5-25 mg/kg bw. At 15 or 50 mg/kg bw there were increases in hepatic phosphatidylcholine and phosphatidylethanolamine concentrations. Both doses, however, did not alter liver ATP content.

Cheng and Klaassen (2008a) investigated the effects of PFDA on Na(+)-taurocholate cotransporting polypeptide (Ntcp) and organic anion transporting polypeptides (Oatp) 1a1, 1a4, and 1b2, major transporters for the uptake of bile acids and other organic compounds into the liver. Male C57BL/6 mice received a single i.p. administration of PFDA at 0.5, 1, 10, 20, 40, or 80 mg/kg bw. The highest dose of PFDA elevated serum bile acid concentrations about 3-fold. mRNA and protein expression of all four transporters were lowered after PFDA exposure. The subsequent use of PPARα-/- mice indicated that the down-regulation of the transporters appears to involve PPARα.

Cheng and Klaassen (2008b) treated male C57BL/6 mice with a single i.p. administration of PFDA at 0.5, 1, 10, 20, 40, or 80 mg/kg bw. Two days after treatment the expression of Cyp2B10, Cyp3A11, and Cyp4A14 was increased significantly. By using CAR-, PXR-, PPARα-, or FXR-knockout mice, it was determined that PPARα and CAR contribute to the induction of Cyps by PFDA.

Maher et al. (2008) treated male C57BL/6 mice with single i.p administrations of PFDA at 0, 0.25, 0.50, 1, 10, 20, 40, or 80 mg/kg bw. PFDA elevated the liver/body weight ratios and mRNA of the PPARα-target gene Cyp4a14 at all doses tested. The transporter proteins Mrp3 and Mrp4 were induced by 10 and 20 mg/kg bw, respectively. Single application of 80 mg/kg bw of PFDA elevated transcript levels of hepatic Mrp3 (4-fold) and Mrp4 (31-fold) and also serum levels of conjugated bilirubin and bile acids, indicating that PFDA interferes with the transporters for hepatic efflux of bilirubin and bile acids to serum.

Luo et al. (2017) treated wild-type and PPARα-null 129/Sv mice with a single i.p. dose of PFDA at 80 mg/kg bw. Five days after treatment, metabolomic analyses of blood and liver tissue samples revealed elevated direct and indirect bilirubin levels, increases in liver enzymes ALP, ALT and AST in the serum, and weak hepatocellular injury and inflammation in wild-type mice, associated with adaptive regulations of bile acid synthesis and transport. While both wild-type and PPARα-null mice exhibited elevated liver/body weight ratios, there was no disruption of bile acid homeostasis, hepatocellular injury or inflammation in the knock-out animals.

Berthiaume and Wallace (2002) treated male Sprague–Dawley rats with a single i.p. injection of 100 mg/kg bw of EtFOSE. PFOA and PFOS at 100 mg/kg bw served as positive control. Animals were sacrificed on the third day post treatment. Peroxisome proliferation was determined by lauroyl CoA oxidase activity, reduction of serum cholesterol concentration, and relative liver weights were recorded as well. The degree of mitochondrial biogenesis was estimated by measurements of cytochrome oxidase

activity, cytochrome content and mitochondrial DNA copy number. In contrast to PFOS and PFOA, EtFOSE exhibited no potency as peroxisome proliferator and exerted no effects.

Chang et al (2017) treated cynomolgus monkeys with a single dose of 9 mg PFOS/kg bw by gavage and reported on an insignificant reduction of serum cholesterol in the post treatment phase. No further alterations could be observed.

Finlay et al. (2008, cited from CLH, 2012) treated male and female SD rats with 8:2 FTOH by single gavage with 2000 and 500 mg/kg bw, respectively. The authors reported on no mortality, no signs of toxicity, no body weight loss and no gross lesions at necropsy.

9841 Table D.1. Acute Studies

Substance (Purity)	Species/ dose route / doses	Observed effects	Highest dose with no effect (mg/kg bw)	Lowest dose with effect (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
PFHxA						
PFHxA (sodium salt; 100% purity)	SD rats (f) No/sex/group: not given single application: 0, 175, 550, 1750, 5000 mg/kg bw; route not specified.				>1750 <5000	Loveless et al., 2009
PFDA						
PFDA (96% purity)	C57BL/6N mice (f) No/sex/group: 10 Single gavage: 0, 20, 40, 80, 160, or 320 mg/kg bw				120	Harris et al., 1989b
PFDA (96% purity)	C57BL/6J mice (f) No/sex/group: 19-20 Single gavage: 0, 20, 40, 80, or 160 mg/kg bw				129	Harris et al., 1989b
PFDA (purity not specified)	F344 rats (m) No/sex/group: ≤6	Fatty-acyl-CoA oxidase activity		5	N/A	Adinehzadeh et al., 1999

Substance (Purity)	Species/ dose route / doses	Observed effects	Highest dose with no effect (mg/kg bw)	Lowest dose with effect (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
	single i.p. administration: 0, 5, 15, 25, 50 mg/kg bw					
PFDA (free acid, 98% purity)	C57BL/6 mice (m) No/sex/group: 4-5 single i.p. administration: 0, 0.5, 1.0, 10, 20, 40, or 80 mg/kg bw	Decr mRNA of Oatp1a1, Oatp1a4, Oatp1b2	10 20	40 0.5 40	N/A	Cheng and Klaassen, 2008a
PFDA (98% purity)	C57BL/6 mice (m) No/sex/group: 5 single i.p. administration: 0, 0.5, 1.0, 10, 20, 40, or 80 mg/kg bw	Incr mRNA of: Cyp4A14 Cyp2B10	1 40	10 80	N/A	Cheng and Klaassen, 2008b
PFDA (> 99% purity)	C57BL/6 mice (m) No/sex/group: 5 single i.p. administration: 0, 0.25, 0.5, 1, 10, 20, 40, or 80 mg/kg bw	Incr rel liver weight Incr mRNA of Cyp4a14 Mrp3 Mrp4	 1 10	0.25 0.25 10 20	N/A	Maher et al., 2008

Substance (Purity)	Species/ dose route / doses	Observed effects	Highest dose with no effect (mg/kg bw)	Lowest dose with effect (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
PFDA (98% purity)	129/Sv wt mice No/sex/group: 5 single i.p. administration: 80 mg/kg bw	Incr ALT Incr AST Incr ALP Hepatic inflammation		80 80 80 80	N/A	Luo et al., 2016
PFDA (98% purity)	129/Sv wt mice (sex?) No/sex/group: 5 single i.p. administration: 0 or 80 mg/kg bw	Incr ALP, ALT Incr total bile acids in serum Incr indirect and direct serum bilirubin		80 80 80		Luo et al., 2017
EtFOSE						
EtFOSE (purity not specified)	SD rats (m) No/sex/group: 3 single i.p. administration: 0 or 100 mg/kg bw	Peroxisomal ß-oxidation	100		N/A	Berthiaume and Wallace, 2002
PFOS						

Substance (Purity)	Species/ dose route / doses	Observed effects	Highest dose with no effect (mg/kg bw)	Lowest dose with effect (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
PFOS (Potassium salt from 3M, approx. 88.9% puritiy, containing PFHxS at 3.2%, PFHpS at 1.2%, PFPeS at 1.1%, PFBS at 0.97%, PFPS at 0.74%)	Cynomolgus monkey (m/f) No/sex/group: 6 Single gavage of 9 mg/kg bw Observation period for 316 days post gavage	Non-significant reduction of serum cholesterol post treatment (m)				Chang et al., 2017
PFOA, > 98% pure	Sprague Dawley rats, 10-11 weeks old at start. 0, 0.625, 1.25, 2.5, 5, 10 mg/kg bw per day, N=10 per sex per group 28 days	Reduced epidydimal weight Reduced epidydimal sperm count	5 5	10 10		NTP 2019a

PFPeS - perfluoropropanesulfonate
PFPS - perfluoropropanesulfonate

Appendix E – Effects following repeated exposure tables

9844

9845 **Table E.1. Repeated dose toxicity studies for PFCAs**

9846

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
Perfluorobutanoic acid (PFBA)						
PFBA (purity not specified)	SV/129 wt mice (m) No/sex/group: 10 Duration: 28 days Gavage: 0, 35, 175, or 350 mg/kg bw per day	Incr rel liver weight Incr hepatic replicative DNA synthesis Incr mRNA of Cyp4A10/ACO Hepatocellular necrosis	 35	35 35 35 175	At 35 mg/kg bw per day Serum: ~80 µg/mL Liver: ~27 µg/g	Foreman et al., 2009
PFBA (ammonium salt, 3M, purity not specified)	SD rats (m/f) No/sex/group: 10 Duration: 28 days Gavage: 0, 6, 30 or 150 mg/kg bw per day	Incr abs liver weight (m) Decr serum cholersterol (m) Decr serum total T4 (m) Decr free T4 in serum (m) Incr mRNA of Acox, Cyp4A1 (m)	6 6 6	30 30 6[a] 6[a] 30	At 6 mg/kg bw per day in males[f] Serum: 24.7 +/- 17.6 µg/mL Liver: 7.5 +/- 4.5 µg/g At 30 mg/kg bw per day in males[f] Serum: 38.04 +/- 23.2 µg/mL Liver: 17.4 +/- 8.2 µg/g	Butenhoff et al., 2012

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
PFBA (ammonium salt, 3M, purity not specified)	SD rats (m/f) No/sex/group: 10 Duration: 90 days Gavage: 0, 1.2, 6, or 30 mg/kg bw per day	Incr abs liver weight (m) Incr mRNA of Cyp4A1 (m) Decr serum total T4 (m)	6 6 6	30 30 30	At 6 mg/kg bw per day in males[f] Serum: 13.6 +/- 9.1 µg/mL Liver: 3.1 +/- 2 µg/g At 30 mg/kg bw per day in males[f] Serum: 52.2 +/- 25 µg/mL Liver: 16.1 +/- 9.1 µg/g	Butenhoff et al., 2012
Perfluorohexanoic acid (PFHxA)						
PFHxA (Sigma-Aldrich)	ddY mice (m/f) No/sex/group: 3-5 Duration: 5 days i.p.: 50, 100, 150 mg/kg bw per day	Incr abs & rel liver weight (f)	50	100		Kudo et al., 2006
PFHxA (98.5% purity)	SD rats (m) No/sex/group: 10-15 Duration: 28 days	Lethality, reduced body weight Decr mean corpuscular hemoglobin Incr abs and rel liver weight	150 150 50	450/300 450/300 150		WIL Research Laboratories, 2005

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
	Gavage: 0, 50, 150, 450/300* mg/kg bw/day (*reduced on day 4 from 450 to 300mg/kg bw/day due to lethality in 5/15 males)	Decr serum cholesterol	50	150		
PFHxA (98.5% purity)	SD rats (m, f) no/sex/group: 10 Duration: 90 days Gavage: 0, 10, 50, or 200 mg/kg bw per day	Incr rel liver weight, (m) Incr rel kidney weight, (m) Decr serum cholesterol, (m) Incr serum ALT and ALP, (m)	50 10 50	200 10 50 200		Chengelis et al., 2009
PFHxA (sodium salt, 100% purity)	Crl:CD(SD) rats (m/f) No/sex/group: 10 Duration: 92 days Gavage: 0, 20, 100, or 500 mg/kg bw per day	Decr body weight (m) Incr perox ß-oxidation (m/f) Incr rel & abs liver weight (m/f) Incr ALT (m) Incr rel kidney weight (m/f) Incr urin volume (m/f) Degeneration/atrophy in nasal cavity (m,f)	100 20/100 m/f: 100/100 100 100 20	500 100/500 m/f: 500/500 20 500 500 100		Loveless et al., 2009

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
PFHxA (sodium salt, 100% purity)	Crl:CD(SD) rats (m) No/sex/group: 10 Duration: ~110 days Gavage: 0, 20, 100, or 500 mg/kg bw per day	Decr body weight (m, day 105)	20	100		Loveless et al., 2009
PFHxA (>99% purity)	SD rats (m, f) No/sex/group: 10 Duration: 28 days Gavage: 0, 62.6, 125, 250, 500, or 1,000 mg/kg per day	Incr rel liver weight (m) Incr abs liver weight (m) Incr rel+abs liver weight (f) Incr. acyl-CoA oxidase activity (m) Decr hematocrit, hemoglobin, erys (m) Decr blood cholesterol (m) Decr T3 and free+total T4 (m) Incr ALT and AST (m+f) and ALP (m) Degeneration and hyperplasia of olfactory epithelium (m+f)	125 250 250 125 250 125	250 500 500 250 62.6 62.6 62.6 500 250	*Plasma conc. (ng/ml)* at 62.6 mg/kg bw/day: 378 ± 178 (m) 129 ± 16 (f) at 250 mg/kg bwper day: 1297± 265 (m) *Liver conc (ng/g)* at 250mg/kg bw/day 655 ± 148 (m)	NTP, 2019a
Perfluoroheptanoic acid (PFHpA)						
PFHpA (analytical grade)	Wistar rats (m/f) No/sex/group: 4	Incr hep peroxisomal ß-oxidation (m) Incr hep peroxisomal ß-oxidation (f)	30[b] 160[b]	160[b]		Kudo et al., 2000

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
	Duration: 5 days i.p.: 0-160 mg/kg bw per day					
PFHpA (Sigma-Aldrich)	ddY mice (m/f) No/sex/group: 3-5 Duration: 5 days i.p.: 0, 20, 50, 100 mg/kg bw per day	Incr abs & rel liver weight (m) Incr abs & rel liver weight (f) Incr hep peroxisomal ß-oxidation (m) Incr hep peroxisomal ß-oxidation (f)	20 50 20	50 100 20 50		Kudo et al., 2006
PFHpA (Sigma, purity not specified)	C57BL/6 mice (sex) No/sex/group: 4 Duration: 3 days i.p. 0 or 20 mg/kg bw per day	Incr rel liver weight	20			Abe et al., 2017
Perfluoroctanoic acid PFOA						
PFOA (> 96% puritiy)	Balb/c mice (m) No/sex/group: not reported Duration: 7 days	Incr ALT Necrosis and vacuolation of hepatocytes Incr abs liver weight Incr triglycerides in liver	 1	1 1 5		Hui et al., 2017

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
	Via distilled water: 0, 1 or 5mg/kg bw per day	Decr body weight Decr free fatty acids in serum Decr triglycerides in serum	 1	1 1 5 1		
PFOA (96% purity)	Balb/c mice (m) No/sex/group: 3-10 Duration: 28 days Orally: 0 or 1.25mg/kg bw per day	Incr rel liver weight incr fasting blood glucose levels Decr glycogen and glucose content in the liver Incr blood glucagon		1.25 1.25 1.25 1.25		Zheng et al., 2017
PFOA	SD rats (m) No/sex/group: 7 Duration: 14 days Gavage: 0, 1, 5, 25 mg/kg bw per day	Incr abs & rel liver weight Incr activity of superoxide dismutase and glutathione peroxidase in the liver Incr MDA content in liver	1 1	5 1 5		Wang et al., 2017c
PFOA (commercial source, puritiy not specified)	C57BL/6 mice (sex) No/sex/group: 4 Duration: 3 days i.p. 0 or 20 mg/kg bw per day	Incr rel liver weight		20		Abe et al 2017

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
PFOA (98% purity)	Kunming mice (m) No/sex/group: 8 Duration: 21 days Gavage: 0, 1 or 5 mg/kg bw per day	Incr abs & rel liver weight Incr ALT and AST Decr serum triglycerides Incr hepatic triglycerides Decr hepatic FGF21 protein	1 1 1 1 1	5 5 5 5 5		Wu et al., 2018
PFOA (ammonium salt, >98% purity)	C57BlL/6 mice (m) No/sex/group: 5 Duration: 2, 8 or 16 weeks Gavage: 1mg/kg bw/day	Decr body weight (week 8+16) Incr liver weight (week 8) Incr rel liver weight (week 2-16) Incr replication of hepatocytes (week 2+8) Incr hepatic peroxisomal ß-oxidation activity (week 2-16)		1 1 1 1 1 1		Li et al., 2019
PFOA (>98% purity)	SD rats (m, f) No/sex/group: 10 Duration: 28 days Gavage: males: 0, 0.625, 1.25, 2.5, 5, or 10 mg/kg bw per day; females: 0, 6.25, 12.5,	Incr rel+abs liver weight (m) Incr acyl-CoA-oxidase activity (m) Incr rel kidney weight (m) Incr rel +abs liver weight (f) Incr rel kidney weight (f) Incr rel thyroid weight (m) Decr serum cholesterin&triglyceride (m)	 25 25 0.625	0.625 0.625 0.625 50 50 1.25 0.625	*Plasma conc. (ug/ml)* at 0.625 mg/kg bw per day: 50.7 ± 2.2 (m) at 5 mg/kg bw per day: 110.7 ± 3.8 (m) at 6.25 mg/kg bw per day:	NTP, 2019a

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
	25, 50, or 100 mg/kg bw per day	Incr ALT, ALP, albumin/globulin ratio (m) Decr T3, free+total T4 (m) Decr hematocrit (f) Incr TSH, ALP (f) Incr serum cholesterin+triglycerides (f) Degeneration and inflammation of olfactory epithelium (m)	25	0.625 0.625 6.25 6.25 50 0.625	491 $\pm$ 72.1 (f) *Liver conc (ug/g)* at 0.625mg/kg bw per day 54.6 $\pm$ 2.2 (m)	
Perfluorononanoic acid (PFNA)						
PFNA (analytical grade)	Wistar rats (m/f) No/sex/group: 4 Duration: 5 days i.p.: 0, 2.5, 5, 10,15,20 mg/kg bw per day	Incr hep peroxisomal ß-oxidation (m) Incr hep peroxisomal ß-oxidation (f)	5[b]	2.5[b] 10[b]	*Liver conc (ug/g)* 20 mg/kg bw: 358 +/-19 (m) 102 +/-11 (f)	Kudo et al., 2000
PFNA (commercial source; purity not specified)	ddY mice(m/f) No/sex/group: 3-5 Duration: 5 days	Incr abs & rel liver weight (f) Incr abs liver weight (m) Incr rel liver weight (m) Incr perox ß-oxidation (m/f)	2.5	2.5 5 2.5 2.5		Kudo et al., 2006

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
	i.p.: 0, 2.5, 5, 10, 20 mg/kg bw per day					
PFNA (97% purity)	SD rats (m) No/sex/group: 6 Duration: 14 days Gavage: 0, 0.2, 1 or 5 mg/kg bw per day	Incr abs & rel liver weight Decr total serum cholesterol Incr mRNA of SREBP-1c, ACOT1/2 Incr hepatic levels of of Il1ß,Il10,TNFa Incr serum levels of ALT, AST, ALP, LDH	0.2 0.2 1	1 0.2 1 0.2 5	*Liver conc. (ug/g)* 0.2 mg/kg bw/day: 12.2 5 mg/kg bw/day: 135	Fang et al., 2012a
PFNA (97% purity)	SD rats (m) No/sex/group: 6 Duration: 14 days Gavage: 0, 0.2, 1 or 5 mg/kg bw per day	Incr serum glucose Decr serum HDL Incr liver glycogen Incr liver MDA Incr mRNA of G6PC/GLUT2	0.2 1 1 1	1 0.2 5 5 5		Fang et al., 2012b
PFNA (97% purity)	Balb/c mice (m) No/sex/group: 8 Duration: 14 days Gavage: 0, 0.2, 1 or 5 mg/kg bw per day	Incr rel liver weight Incr total hepatic cholesterol/triglycer. Incr mRNA of Cyp4A1/ACOX1 Incr serum levels of AST, ALT	 1	0.2 0.2 0.2 5		Wang et al., 2015

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
PFNA (97% purity)	SD rats (m)[c] No/sex/group: 10 Duration: 7 days Gavage: 0, 0.2, 1, 5 mg/kg bw per day	Incr hepatic cholesterol Incr activity of glucose-6-P-dehydrogenase Incr serum ALT	0.2 0.2 0.2	1 1 1		Fang et al., 2015
PFNA (purity not specified)	Wistar rat (m) No/sex/group: 10 Duration: 14 days Gavage: 0, 0.0125, 0.25, or 5mg/kg bw per day	Incr plasma corticosterone Decr hepatic OATP4C1 protein		0.0125[b] 0.0125[b]	*Serum levels (ug/ml):* at 0.0125 mg/kg bw per day: 0.396 at 0.25 mg/kg bw per day: 30 at 5 mg/kg bw per day: 602	Hadrup et al., 2016
PFNA (Sigma purity not specified)	SV129 mice (m) No/sex/group: 4 Duration: 7 days Gavage: 0, 1 or 3 mg/kg bw per day	Incr abs & rel liver weight		1		Rosen et al., 2017

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
PFNA (97% purity)	SV129 mice (m) No/sex/group: 4 Duration: 7 days Gavage: 0 or 10mg/kg bw per day	Incr abs & rel liver weight Incr hepatic lipid and triglyceride content		10 10		Das et al., 2017
PFNA (Sigma, purity not specified)	C57BL/6 mice (sex) No/sex/group: 4 Duration: 3 days i.p. 0 or 20mg/kg bw per day	Incr rel liver weight		20		Abe et al., 2017
PFNA (>98% purity)	SD rats (m, f) No/sex/group: 10 Duration: 28 days Gavage: males: 0, 0.625, 1.25, 2.5, 5, or 10 mg/kg bw per day; females: 0, 1.56, 3.12, 6.25, 12.5, or 25 mg/kg bw per day	Decr body weight (m) Decr body weight (f) Incr rel + abs liver weight (m) Incr acyl-CoA oxiase activitiy (m) Incr rel kidney weight (m) Incr rel + abs liver weight (f) Incr rel kidney weight (f) Decr serum cholesterol+triglyceride (m) Decr free + total T4 (m)	0.625 0.156	1.25 3.12 0.625 0.625 0.625 1.56 1.56 0.625 0.625	*Plasma conc. (ug/ml)* at 0.625 mg/kg bw per day: 56.7 $\pm$ 1.9 (m) at 1.56 mg/kg bw per day: 26.4 $\pm$ 1.1 (f) *Liver conc (u g/g)* at 0.625 mg/kg bw per day 145.5 $\pm$ 2.7 (m)	NTP, 2019a

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
		Incr serum bile salts (m) Incr urea, albumin/globulin ratio (m) Incr total+direct bilirubin, ALP/ALT/AST (m) Incr albumin/globulin ratio (f) Incr urea and serum bile salts (f) Decr total+free T4 (f)	 0.625 0.625 1.56	0.625 1.25 1.25 1.56 3.12 3.12		
Perfluorodecanoic acid (PFDA)						
PFDA (Aldrich)	Wistar rats (m) No/sex/group: 4 Duration: 1 week Diet: 0, 0.00125, 0.0025, 0.005 or 0.01%; equivalent to 0, 1.5, 3, 6, or 12 mg/kg bw per day [e)]	Incr rel liver weight Incr abs liver weight Incr peroxisomal ß-oxidation Incr acyltransferase activity Incr intrahepatic triacylglycerol	 1.5 1.5	1.5 3 3 1.5 1.5		Kawashima et al., 1995
PFDA (analytical grade)	Wistar rats (m/f) No/sex/group: 4 Duration: 5 days.	Incr hep peroxisomal ß-oxidation (m) Incr hep peroxisomal ß-oxidation (f)	2.5[d] 2.5[d]	5[d] 5[d]	*Liver conc (ug/g)*	Kudo et al., 2000

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
	i.p.: 0, 2.5, 5, 10, 15, 20 mg/kg bw per day				20 mg/kg bw: 453 +/-19 (m) 412 +/-33 (f)	
PFDA (Sigma, purity not specified)	C57BL/6 mice (sex not reported) No/sex/group: 4 Duration: 3 days i.p. 0 or 20 mg/kg bw per day	Incr rel liver weight		20		Abe et al., 2017
PFDA (97.8% purity)	SD rats (f) No/sex/group: 8 Gavage at 0, 0.125, 0.25, 0.5, 1, or 2 mg/kg bw per day Duration: 28 days	Incr rel liver weight Incr abs liver and rel kidney weight Incr abs kidney weight	 0.125 0.25	0.125 0.25 0.5		Frawley et al., 2018
PFDA (97.8% purity)	B6C3F1 mice (f) No/sex/group: 8 Gavage at 0, 0.31, 0.625, 1.125, 2.5, or 5 mg/ kg bw per day	Incr abs & rel liver weight Incr rel spleen weight	0.31 0.625	0.625 1.125		Frawley et al., 2018

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
	Duration: 28 days					
PFDA (>97% purity)	SD rats (m, f) No/sex/group: 10 Duration: 28 days Gavage: 0, 0.156, 0.312, 0.625, 1.25, or 2.5 mg/kg bw per day	Incr abs+rel liver weight (m, f) Incr acyl-CoA-oxidase activity (m) Decr abs weight of adrenal gland (m) Incr abs+rel weight of thyroide (f) Incr albumin/globulin ratio (m, f) Decr cholesterol in blood (m) Incr AST (m/f) Incr ALT (m/f) Incr ALP (m, f)	 0.156 -/0.625 0.156/0.625 0.156	0.156 0.156 0.156 0.312 0.156 0.156 0.156/1.25 0.312/1.25 0.312	*Plasma conc. (ug/ml)* at 0.156 mg/kg bw/day: 8.5 ± .6 (m) 11.2 ± 0.4 (f) *Liver conc (ug/g)* at 0.156 mg/kg bw/day 44.7 ± 1.5 (m)	NTP, 2019a
Perfluoroundecanoic acid (PFUnDA)						
PFUnDA (98.5% purity)	Crl:CD (SD) rats (m/f) No/sex/group: 12 Duration: 42 days Gavage: 0, 0.1, 0.3, 1 mg/kg bw per day	Incr abs liver weight (m, f) Incr rel liver weight (m/f) Decr abs & rel spleen weight (m) Incr serum ALP, AST (m) Incr serum BUN (m/f)	0.3 0.1/0.3 0.3 0.3 0.3	1 0.3/1 1 1 1		Takahashi et al., 2014

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
		Decr serum albumin (m)	0.3	1		
Perfluorododecanoic acid (PFDoDA)						
PFDoDA (>99% purity)	SD Rats (m) No/sex/group: 6 Duration: 14 days Gavage: 0, 1, 5, or 10 mg/kg bw per day	Incr abs & rel liver weight Incr serum triglyceride Incr hepatic triglyceride Incr hepatic SOD activity Incr hepatic mRNA of PPARα/g, ACOX, CypA4 Incr hepatic content of cholesterol	1 5 5 5	5 10 10 1 1 10		Zhang et al., 2008
PFDoDA (95% purity)	SD Rats (m) No/sex/group: 10 Duration: 110 days Gavage: 0, 0.02, 0.05, 0.2, or 0.5 mg/kg bw per day	Incr serum glucose Incr serum albumin Incr hepatic mRNA of PPARα, Cyp4A1, ACOX, cd36	 0.05	0.02 0.02 0.2		Ding et al., 2009
PFDoDA (95% purity)	SD Rats (m) No/sex/group: 6 Duration: 110 days	Incr protein level of pyruvate carboxylase in kidney Incr protein level of isovaleryl coenzyme A dehydrogenase,	0.05	0.05 0.2		Zhang et al., 2011

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
	Gavage: 0, 0.05, 0.2, or 0.5 mg/kg bw per day	malate dehydrogenase 1 and dihydrolipoamide S-acetyltransferase in kidney				
PFDoDA (Sigma, purity not specified)	SD rats (m) No/sex/group: 6 Duration: 110 days Gavage: 0, 0.2, or 0.5mg/kg bw per day	Incr hepatic cholesterol Incr hepatic triglycerides Altered hepatic levels of signal transduction proteins (e.g. glycogen synthase kinase, insulin receptor substrate)		0.2 0.2 0.2		Zhang et al., 2013d
PFDoDA (97% purity)	SD Rats (m/f) No/sex/group: 7 Duration: 42 days Gavage: 0, 0.1, 0.5, or 2.5 mg/kg bw per day	Incr rel liver weight (m/f) Decr weight of spleen/heart (f) Decr in reticulocytes (m) Incr serum ALP (m) Decr serum total cholesterol (m) Liver hypertrophy (m) Hepatic necrosis (f) Pancreas: decr zymogen granules (m)	0.1 0.1 0.5 0.1 0.5 0.5 0.5	0.5 0.5 2.5 0.5 0.1 2.5 2.5 2.5		Kato et al., 2015b

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
		Decr serum glucose (m)	0.5	2.5		
PFDoDA (>95% purity)	SD Rats (m) No/sex/group: 4-10 Duration: 110 days Gavage: 0, 0.05, 0.2, or 0.5 mg/kg bw per day	Incr hepatic SOD activity Incr TBARS in liver Decr hepatic GPX activity Incr mRNA of PPARα/Cyp4A1 Incr mRNA of mitochondrial acyl-CoA-thioesterase 1 and hydroxyacyl-CoA-dehydrogenase	0.2 0.2 0.2 0.05	0.5 0.5 0.5 0.2 0.05		Liu et al., 2016
Perfluorotetradecanoic acid (PFTeDA)						
PFTeDA (96.5% purity)	Crl:CD (SD) rats (m) No/sex/group: 12 Duration: 42 days Gavage: 0, 1, 3, or 10 mg/kg bw per day	Decr body weight Decr hindlimb strength Incr serum ALP and BUN Incr abs & rel liver weight Centrolob. liver hypertrophy & steatosis Decr abs & rel pituitary gland weight	3 1 3 1 1 1	10 3 10 3 3 3		Hirata-Koizumi et al., 2015

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
		Decr abs weight of semin. vesicles Hypertrophy of thyroid follicular cells	0 1	1 3		
Perfluorohexadecanoic acid (PFHxDA)						
PFHxDA (95.3% purity)	Crl:CD (SD) rats (m) No/sex/group: 12 Duration: 42 days Gavage: 0, 4, 20, or 100 mg/kg bw per day	Decr body weight Incr abs & rel liver weight Centrolob. liver hypertrophy & steatosis Incr rel thyroid weight	20 20 20 20	100 100 100 100		Hirata-Koizumi et al., 2015
Perfluorooctadecanoic acid (PFODA)						
PFODA (98.9% purity)	Crl:CD (SD) rats (m) No/sex/group: 12 Duration: 42 days Gavage: 0, 40, 200, or 1000 mg/kg bw per day	Decr body weight/food consumption Decr red blood cells/hemoglobin/hematocrit Decr serum gamma-GTP Incr abs & rel liver weight Liver hypertrophy Incr serum ALP, ALT	200 40 40 40 40 200	1000 200 200 200 200 1000		Hirata-Koizumi et al., 2012

(a): Please note that the effect was not evident at the 90 day time point of investigation
(b): Adequate statistical evaluation is missing
(c) rats were treated with streptozotocin to induce diabetes
(d) Derived from figure 2D of Kudo et al (2000). No statistical evaluation is given and significance of data is unclear.

(e) applying default values, provided by EFSA
(f) More data on serum and tissue concentrations are given in Butenhoff et al. (2012). The table is confined to serum/tissue concentration at doses with sensitive endpoints in males of the highest dose-group.

9854 **Table E.2. Repeated dose toxicity studies for PFSAs**

9855

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
Perfluorobutane sulfonic acid (PFBS)						
PFBS, potassium salt (98.2% purity)	Sprague Dawley rats (m/f) No/sex/group: 10 Duration: 28 days Gavage: 0, 100, 300, or 900 mg/kg bw per day	Decr serum phosphorus and potassium (m) Incr rel and absolute liver weight (m)	100 300	300 900	N/A	NICNAS, 2005
PFBS, potassium salt (98.2% purity)	Crl:CD(SD)IGS BR VAF/PlusTM rats (m/f) No/sex/group: 10 Duration: 90 days Gavage: 0, 60, 200, or 600 mg/kg bw per day	Decr abs &rel spleen weight(m) Decr red blood cells (m) Decr hematocrit (m) Decr hemoglobin (m) Incr serum chloride (m) Decr serum albumin and total protein (f)	 200 60 60 200 200	60 600 200 200 600 600		Lieder et al., 2009a

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
PFBS, potassium salt (97.9% purity)	Crl:CD(SD)IGS BR VAF/PlusTM rats (m) No/sex/group: 30 Duration: 10 weeks Gavage: 0, 30, 100, 300 or 1000 mg/kg bw per day	Incr abs & rel liver weight	100	300		Lieder et al., 2009b
PFBS (>97% purity)	Sprague Dawley rats (m, f) No/sex/group: 10 Duration: 28 days Gavage: 0, 62.6, 125, 250, 500, or 1,000 mg/kg bw per day	Incr rel liver weigt (m,f) Incr abs liver weight (m/f) Incr acyl-CoA-oxidase activity (m) Incr rel kidney weight (f) Decr haematocrit, RBC, cholesterol, T3, total+free T4 (m,f) Incr albumin/globulin ratio (m,f)	-/62.6 125 62.6	62.6/125 125/250 250 62.6 62.6 125	*Plasma conc. (ug/ml)* at 62.6mg/kg bw/day: 2.2 ± 4.8 (m) 0.2 ± 0.05 (f) *Liver conc (ug/g)* at 62.6mg/kg bw/day 1.3 ± 0.2 (m)	NTP, 2019b
Perfluorohexane sulfonic acid (PFHxS)						
PFHxS (potassium salt; 99.98% purity)	Sprague Dawley rats (m) No/sex/group: 10	Incr rel liver weight	1	3	Concentration in µg/g at	Butenhoff et al., 2009

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
	Duration: 42 days Gavage: 0, 0.3, 1, 3, or 10 mg/kg bw per day	Decr prothrombin time Decr serum cholesterol Decr hemoglobin Decr hematokrit Decr RBC Thyroid hypertrophy/hyperplasia	 0.3 1 1 1	0.3 0.3 1 3 3 3	0.3 mg/kg bw per day in liver 43.8 +/- 8.1 in serum 44.2 +/- 12.7 3 mg/kg bw per day in liver 339 +/- 128 in serum 128 +/- 10 10 mg/kg bw per day in liver 593 +/- 81.4 in serum 201.5 +/- 20	
PFHxS (3M, salt, purity not specified*)	SV129 mice (m) No/sex/group: 4 Duration: 7 days Gavage: 0, 3 or 10 mg/kg bw per day	Incr rel liver weight Incr abs liver weight	 3	3 10		Rosen et al., 2017
PFHxS (potassium salt, 97% purity)	SV129 mice (m) No/sex/group: 4 Duration: 7 days Gavage: 0 or 10 mg/kg bw per day	Incr abs and rel liver weight Incr hepatic lipid and triglyceride content		10 10		Das et al., 2017
PFHxS (potassium salt; 98.9% purity)	Crl:CD1 (IRC) mice (m) No/sex/group: 30	Incr abs & rel liver weight	0.3	1	*Liver conc. (µg/g):*	Chang et al., 2018

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
	Duration: 42 days Gavage: 0, 0.3, 1, 3 mg/kg bw per day	Centrilobular hypertrophy Incr ALP Decr Serum cholesterol Necrotic hepatocytes and lipid vesicles in hepatocytes	 1 1 1	0.3 3 3 3	0.3 mg/kg bw/day: 25.9 ± 3.5 1 mg/kg bw/day: 98.5 ± 22.7 3 mg/kg bw/day: 281.1 ± 45.4	
PFHxS (potassium salt; >98% purity)	Sprague Dawley rats (m, f) No/sex/group: 10 Duration: 28 days Gavage: 0, 0.625, 1.25, 2.5, 5, or 10 mg/kg bw per day (males) 0, 3.12, 6.25, 12.5, 25, or 50 mg/kg bw per day (females)	Incr rel and abs liver weight (m/f) Decr T3 and cholesterol (m) Decr total T4 (m/f) Decr free T4 (m/f) Incr acyl-CoA-oxidase activity (m)	0.625/- -/3.12 -/6.25 2.5	1.25/3.12 0.625 0.625/6.25 0.625/12.5 5	*Plasma conc. (ug/ml)* 0.625mg/kg bw/day: 66.8 ± 3.5 (m) 3.12mg/kg bw/day: 37.0 ± 1.7 (f) *Liver conc (ug/g)* 0.625mg/kg bw/day 39.9 ± 1.3 (m)	
Perfluorooctane sulfonic acid (PFOS)						
PFOS (commercial source, Sigma)	Swiss albino rats (m) No/sex/group: 6 Duration: 30 days Gavage every second day: 0, 0.6, 1.25 or 2.5mg/kg bw	DNA fragmentation in liver (Comet Assay), indication of apoptosis/necrosis?		~0.6		Eke et al., 2017

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
PFOS (Potassium salt from 3M, approx. 88.9% puritiy, containing PFHxS at 3.2%, PFHpS at 1.2%, PFPeS at 1.1%, PFBS at 0.97%, PFPS at 0.74%)	Cynomolgus monkey (m/f) No/sex/group: 4-6 Gavage: day 43: 14 mg/kg (m, f) day 288: 14.8 (m) and 17.2 mg/kg (f) day 358: 11 mg/kg (m, f) End of observation: day 422	Insiginificant reduction of serum cholesterol post treatments (m)			*Serum conc (ug/ml):* 11mg/kg bw/day at day 365: 160.8 +/- 14.2 (m) 165.0 +/-6.7 (f)	Chang et al., 2017
PFOS (commercial source, Sigma)	C57BL/6 J mice (m) No/sex/group: 5 Duration: 28 days Diet: 0 or 0.2mg/kg bw per day	Incr epididymal adipose tissue Incr rel liver weight Incr hepatic triglycerides Incr hepatocellular lipid storage Incr blood glucose		0.2 0.2 0.2 0.2 0.2		Huck et al., 2018
PFOS (> 98% puritiy)	Mice (m) No/sex/group: 4 Duration: 21 days Gavage: 0 or 10 mg/kg bw per day	Incr AST, ALT, LDH Incr rel liver weight Incr content of hepatic MDA and H_2O_2 Incr hepatic levels of TNFa and IL6 Incr hepatic caspase 3 activity		10 10 10 10 10		Lv et al., 2018

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
PFOS (potassium salt, 98% purity)	SD rats (m) No/sex/group: 6 Duration: 28 days Gavage: 0, 1 or 10 mg/kg bw per day	Incr ALT Incr AST Incr TNFa in serum Incr hepatic MDA content Decr hepatic catalase activit Decr hepatic content of GSH and GSH/GSSG Incr cleavage of caspase 3 in liver	1	1 10 1 1 1 1 1		Han et al., 2018a
PFOS (potassium salt, 98% purity)	SD rats (m) No/sex/group: 6 Duration: 28 days Gavage: 0, 1 or 10 mg/kg bw pday	Incr ALT Incr AST Incr TNFa in serum Incr IL6 in serum Incr PCNA positive nuclei	1	1 10 1 1 1		Han et al., 2018b
PFOS (commercial source, no further details indicated purity and type of salt not specified)	C47Bl6/J mice (m) No/sex/group: 5 Duration: 6 weeks	Incr rel liver weigt Incr hepatic triglyceride conc. Incr blood glucose		0.089 0.089 0.089		Huck et al., 2018

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
	Diet: 0.089mg/kg bw/day	Incr. serum triglyceride & cholesterol		0.089		
PFOS (type of salt not specific, 98% purity)	CD mice (f) No/sex/group: 4 Duration: 7 weeks Diet: 0, 0.3, or 3mg/kg bw per day	Incr abs & rel liver weight Incr liver triglyceride Decr serum triglyceride Altered pyruvate tolerance test Altered gut microbiome	0.3 0.3	3 0.3 3 0.3 0.3	*Plasma conc (ug/g)* at 0.3mg/kg bw/day: 33.8 ± 4.4 at 3 mg/kg bw/day: 109.5 ± 19.4 *Liver conc (ug/g)* at 0.3mg/kg bw/day: 32.9 ± 13.5 at 3 mg/kg bw/day: 503.8 ± 326	Lai et al., 2018
PFOS (98% purity, salt not specified)	ICR mice (m) No/sex/group: 10 Duration: 21 days Treatment: 10mg/kg bw per day	Incr. ALT & AST Incr total cholesterol & triglycerides in serum Incr TNFa & IL6 in serum		10 10 10		Su et al., 2019
PFOS (>96% purity)	SD rats (m, f) No/sex/group: 10 Duration: 28 days	Incr rel and abs liver weight (m/f) Incr acyl-CoA-oxidase activity (m) Decr blood cholesterol (m)	1.25	0.312 2.5 0.312	*Plasma conc. (ug/ml)* at 0.312mg/kg bw per day: 23.7 ± 1.1 (m) 30.5 ± 0.9 (f)	NTP, 2019b

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
	Gavage: 0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg bw per day	Decr total and free T4 (m, f)		0.312	*Liver conc (ug/g)* at 0.312 mg/kg bw per day 87.2 $\pm$ 3.04 (m)	

9856 *Purity assumed to be 99.98%

9857

9858 **Table E.3. Repeated dose toxicity studies for further PFASs**

9859

Substance (Purity)	Species/Experimental design and doses	Observed effect	Highest dose with no effects (mg/kg bw per day)	Lowest dose with effect (mg/kg bw per day)	Serum/tissue level of compound	Reference
N:2 fluorotelomer alcohols (n:2 FTOHs)						
8:2 FTOH (commercial source, purity not specified)	Wistar rat (m) No/sex/group: 4 Duration: 14 days Diet: 0, 0.2, 0.4, or 0.8% corresponding to 0, 240, 480, or 960 mg/kg bw per day[a]	Incr rel liver weight Decr of 16:0 fatty acid in liver		240 240		Iwase et al., 2006
8:2 FTOH (99.2% purity)	Crl:CD(SD)IGS BR rat (m/f) No/sex/group: 10 Duration: 90 days Gavage: 0, 1, 5, 25, or 125 mg/kg bw per day	Focal hepatic necrosis (m) Incr rel liver weight (m/f) Incr abs liver weight (f) Decr RBC, m Incr serum cholesterol, (f) Incr hepatic perox ß-oxidation (f)	5 5 5 5 25 5	25 25 25 25 125 25		Ladics et al., 2008

		Incr urinary fluorides (m) Incr urinary fluorides (f)	1 5	5 25		
8:2 FTOH (>97% purity)	C57Bl/6 mice (m) No/sex/group: 6 Duration: 28 days Gavage: 0, 10, 30, or 100 mg/kg bw per day	Incr abs& rel liver weight Incr abs thymus weight Serum SOD activity Serum GSH content		10 10 10 10		Wang et al., 2018
N-ethyl perfluorooctanesulfonamidoethanol (EtFOSE)						
EtFOSE (>99% purity)	SD rat (f) No/sex/group: 9 Duration: 5 days/week for 3 weeks Gavage: 0 or 5 mg/kg bw/day	Decr body weight gain Incr rel liver weight Incr rel spleen weight Incr catalase activity (uterus) Decr total GPX activity (uterus) Incr CuZn-SOD activity (uterus) Incr CuZn-SOD and MnZn-SOD activity (liver)		5 5 5 5 5 5 5	Serum concentr. (ng/mL) 177+/-86	Xie et al., 2009

(a): Applying default values, provided by EFSA.

Appendix F – Developmental and Reproductive toxicity tables

9861

9862 Table F.1 Studies on mammary gland development in mice with PFOA exposure in utero, postnatally or during puberty. Results concern decreased scores
9863 unless otherwise indicated

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
Prenatal and lactational exposure								
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[1] 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)	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

	continuously from P0 GD 7							
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	
Pubertal exposure								
Balb/c	From PND 21 for 28 days, 5 days/week[2]	0, 0.7, 3.6, 7.1	0.7[2]	3.6[2]	<10, 29500, 109000, NR	29500	109000	Yang et al. 2009[1,3]
C57Bl/6		0, 0.7, 3.6, 7.1	For ↓ 3.6[2]	For ↓ 7.1[2] For ↑ 0.7[2]	<10, 26000, 68200, 96600		For ↓ 96600 For ↑ 26000	
Balb/c	From PND 21 for 28 days, 5 days/week[2]	0, 1.8		1.8[2]	Serum at termination: <10, 51100		51100	Zhao et al. 2012[1]
C57Bl/6		0, 5.4		5.4[2]	Serum at termination: <10, 93400		93400	

[1]Concentration in serum extracted from figure;

[2]Values adjusted for dosing 5 days per week;

[3]Serum levels reported in Zhao (2012);

NR: not reported.

9868 Table F.2. Perfluoroalkyl carboxylic acids (PFCAs): reproductive and developmental toxicity

Substance/ (Purity)	Species/Experimental design and doses	Observed effects	Highest dose with no effect (mg/kg bw per day)		Lowest dose with effects (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
Perfluorobutanoic acid (PFBA)								
PFBA (98% pure)	CD-1 mice 0, 35, 175, 350 mg/kg per day Exposure GD 1 – GD 17 30 mice per dose group, in three blocks	Increased liver weight Increased liver weight (PND 1) Delayed eye opening Delayed vaginal opening Delayed preputial separation	35	 35 n.d. 35 175	175	 175 35 175 350	Serum in µg/mL, liver in µg/g. Serum in non-pregnant female: <LOD (0.01), 1.96, 2.41, 2.67. Serum in pregnant dam: <LOD (0.01), 3.78, 4.44, 2.49 Liver in non-pregnant female: 0.038, 0.51, 0.86, 0.89. Liver in pregnant female: <LOD (0.01), 1.41, 1.60, 0.96. Serum pups PND 1: ND, 0.56, 0.61, 0.37.	Das et al. 2008

Substance/ (Purity)	Species/Experimental design and doses	Observed effects	Highest dose with no effect (mg/kg bw per day)		Lowest dose with effects (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
							Serum pups PND 10: <LOD (0.01), 0.11, 0.14, 0.12	
Perfluorohexanoic acid (PFHxA)								
PFHxA, 93.4% pure (ammonium salt)	CD-1 mice Exposure GD 6 – GD 18 20 mice per dose group Phase 1: 0, 100, 350, 500 mg/kg per day, gavage. Phase 2: 0, 7, 35, 175 mg/kg per day, gavage.	Maternal mortality Increased % pups found dead day 1-4 Increase in stillborn pups and pups dying on PND1	100 35	 100 	350 175	 350 	Concentrations in serum and liver from dams at weaning and in F1 at day 41 after birth were generally below the LOQ (LOQ 0.02 µg/mL)	Iwai and Hoberman 2014
As above	As Phase 2 above	Reassessment of Phase 1 in Iwai and Hoberman 2014, combining Phase 1 and Phase 2 controls in statistical analysis.	175					Iwai et al., 2019
PFHxA, 100% pure (sodium salt)	Crl:CD(SD) rats	Reduced body weight gain (females) during	100		500		Not reported	Loveless et al. 2009

	One generation reproduction study: 0, 20, 100, 500 mg/kg bw per day, gavage. Females and males: 70 days prior to cohabitation until weaning, approximately 126 day totally for females and 110 days totally for males	first week of exposure and during lactation Reduced mean F1 pup weight		100		500		
PFHxA, 100% pure (sodium salt)	Crl:CD(SD) rats Developmental toxicity study: 0, 20, 100, 500 mg/kg bw per day, gavage. 22 female rats/group. Exposure from GD 6 – GD 20 according to OECD Guideline 414, sacrifice on GD 21.	Reduced maternal body weight 10% decrease of fetal weight	100	100	500	500	Not reported	Loveless et al. 2009
Perfluorononanoic acid (PFNA)								
PFNA 97% pure, (linear isomer according to supplier) free acid	CD-1 mice 0, 1, 3, 5, 10 mg/kg bw per day Exposure GD 1 – GD 17 n=11-27 animal per group, depedning on outcome (details not specified) Experiment performed in three blocks	Dams at 10 mg/kg failed to carry pregnancy (no follow up in this dose group) Increase in absolute and relative liver weight	5 n.d.		10 1		Read from graphs at 1, 3, 5 mg/kg bw per day: Serum, non-pregnant: 30, 45, 210 µg/mL Serum, dam at term: 20, 25, 75 µg/mL	Das et al. 2015

		Dose dependent increase in relative liver weight in pups Neonatal mortality within the first 10 days Postnatal reduction of body weight gain Delay in eye opening, preputial separation, and vaginal opening		n.d. 3 n.d. 1		1 5 1 3	Serum dam after weaning: 10, 25, 85 µg/mL Liver, non-pregnant: 170, 320, 470 µg/g Liver, dam at term: 100, 270, 320 µg/g Dam, post weaning: 35, 125, 210 µg/g Fetal liver at term: 10, 35, 70 µg/g Serum levels in pups at PND 1: 25, 50 and 75 µg/mL Liver from pups at PND 1 and PND 10: 60, 150, 200 µg/g, thereafter decreasing, but still elevated at PND 40 and 70	
PFNA source, purity and salt not specified	Sprague-Dawley rats 5 mg/kg bw per day, gavage. Exposure GD 1-20	Delayed weight gain	n.d.	n.d.	5	5	Not reported	Rogers et al. 2014

	10-12 Offspring animals per group	Lower birth weight in females Increased blood pressure in males and females at 10 weeks of age Reduced nephron endowment (nephrons per kidney) in males at week 22		n.d. n.d.		5 5		
PFNA 97% pure	Parkes mice, 0, 2, 5 mg/kg bw per day, by oral feeding needle, from GD 12 to parturition. 10 dams per group, 2 male offspring per dam investigated on PND 3	Decreased testicular testosterone PND3		2		5	Not reported	Singh and Singh, 2019a
Perfluorodecanoic acid (PFDA)								
PFDA, 96% pure salt not specified	C57BL/6N Mice, gavage, GD10-13 at 0, 0.25, 0.5, 1, 2, 4, 8, 16 and 32 mg/kg bw per day. GD 5-15 at 0.03, 0.1, 0.3, 1, 3, 6.4, 12.8 mg/kg bw per day.	Increased liver weight Reduced bw Increased liver weight Reduced body weight	0.25 8 0.3 3	0.25 0.1	0.5 16 1.0 6.4	0.5 0.3	Not reported	Harris and Birnbaum 1989a
Perfluoroundecanoic acid (PFUnDA)								
PFUnDA, 98.5% pure, salt not specified	Crl:CD Sprague-Dawley rats, 0, 0.1, 0.3, 1 mg/kg per day by gavage.	Lowered body weights in male		0.3		1	Not reported	Takahashi et al. 2014

	Males: 12 per group for 42 days beginning 14 days before mating Females (12 per group): 14 days prior to mating, through gestation until 4 days of lactation. Recovery groups: 5 of 12 males were allowed a 14 days recovery period. Females in recovery group were treated like males in this group	and female pups at PNDs 0 and 4						
Perfluorododecanoic acid (PFDoDA)								
PFDoDA (97% pure, salt not specified	Crl:CD Sprague-Dawley rats. 0, 0.1, 0.5, 2.5 mg/kg per day by gavage. Males: 12 per group for 42 days beginning 14 days before mating Females: 14 days prior to mating, through gestation until 6 days of nursing	*Reproductive endpoints only* Decreased spermatid and spermatozoa count at 2.5 mg/kg per day		0.5		2.5	Not reported	Kato et al.2015b
		Continuous diestrus	0.5		2.5			
		Death in late pregnancy (7 out of 12 animals)	0.5		2.5			
		Failure to deliver live pups (4 out of 12 animals)	0.5		2.5			

Perfluorotetradecanoic acid (PFTeDA)								
PFTeDA, (salt not specified), 96.5% pure	Crl:CD(SD) rats, males and females, from 14 days before mating (12 per group) were dosed for 42 days (males), or until PND5 (females), Dosage 0, 1, 3 and 10 mg/kg bw per day, gavage.	Females: centrilobular hepatocyte hypertrophy. Decreased body weight.	3	3	10	10	Not reported	Hirata-Koizumi et al. 2015
Perfluorohexadecanoic acid (PFHxDA)								
PFHxDA (salt not specified), 95.3% pure	Crl:CD(SD) rats males and females, from 14 days before mating (12 per group) were dosed for 42 days (males), or until PND5 (females). Dosage 0, 4, 20 and 100 mg/kg bw per day, gavage.	Centrilobular hepatocellular hypertrophy of hepatocytes Reproductive/ developmental parameters not affected	20 100	100	100		Not reported	Hirata-Koizumi et al. 2015
Perfluorooctadecanoic acid (PFODA)								
PFODA, 98.9% pure	Crl:CD(SD) rats, males were dosed for 42 days, females until PND 5, dosage 0, 40, 200 and 1000 mg/kg per day, gavage	Hepatic changes (centrilobular hepatocellular hypertrophy) Reduced numbers of implantation, total number of born pups and number of live pups on PND 0	200	200	1000	1000	Not reported	Hirata-Koizumi et al. 2012

		and 4, decreased birth weight and postnatal weight gain						

n.d: not determined.

9870 Table F.3. PFCA reproductive toxicity studies with exposure in pubertal or adult animals.

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
Perfluorohexanoic acid acid (PFHxA)						
PFHxA (>99% purity)	Sprague Dawley rats Gavage 0, 62.6, 125, 250, 500, or 1,000 mg/kg per day	Cauda epidydimis sperm count Serum testosterone Oestrus cyclicity	1000 1000 1000		*Plasma conc. (ng/ml)* at 62.6 mg/kg bw per day: 378 ± 178 (m) 129 ± 16 (f) at 250 mg/kg bw per day: 1297± 265 (m) *Liver conc (ng/g)* at 250 mg/kg bw/day 655 ± 148 (m)	NTP 2019a
Perfluorononanoic acid (PFNA)						
PFNA (>98% purity)	Sprague Dawley rats Gavage Male: 0, 0.625, 1.25, 2.5 mg/kg bw per day Female: 0, 1.56, 3.12, 6.25 mg/kg bw per day	Decreased epidydimal weight with histopatological findings. Reduced testis weight with histopatological changes Decrease serum testosterone	 0.625 0.625	0.625 1.25 1.25	*Plasma conc. (ug/ml)* at 0.625 mg/kg bw per day: 56.7 ± 1.9 (m) at 1.56 mg/kg bw per day: 26.4 ± 1.1 (f) *Liver conc (u g/g)* at 0.625 mg/kg bw per day 145.5 ± 2.7 (m)	NTP 2019a

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
PFNA 97% pure	Male Parkes mice, 0, 0.2 and 0.5 mg/kg bw per day by oral feeding needle. 90 days exposure (PND 25 to PND 114) n=14 per group 7 animals were selected for fertility test, the remaining 7 for assessment of toxicological paramters	Reduced male fertility (reduced sperm number, viability and motility) Decreased cholesterol Decreased testosterone Reduced litter size Reduced expression of steroidogenic enzymes in testes Reduced PCNA (proliferation marker) and increased caspase3 (apoptosis marker) expression in testis Decreased SOD and catalase activity in testes	0.2 0.2 0.2 0.2 0.2 0.2 n.d.	0.5 0.5 0.5 0.5 0.5 0.5 0.2	Not reported	Singh and Singh 2019b
PFNA 97% pure	Male Parkes mice, 0, 2, 5 mg/kg bw per day, by oral feeding needle for 14 days, from PND 25 to PND 38. 10 mice per dose group. 5 mice per dose group for some outcomes.	Reduced body weight gain Reduced serum and testicular testosterone Degenerative changes in seminiferous tubules	2 n.d. n.d	5 2 2	Not reported	Sing and Singh, 2019c.

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
PFNA 97% pure	Male Parkes mice, 0, 2, 5 mg/kg bw per day, by oral feeding needle for 14 days, from PND 25 to PND 38. 10 mice per dose group treated, but generally 5 mice per dose group for outcomes.	Increased liver weight and hepatocellular hypertrophy. Altered proportions of 4C and 2C cells in testis	n.d. n.d.	2 2	Not reported	Singh and Singh, 2019d
Perfluorodecanoic acid (PFDA)						
PFDA (>97% purity)	Sprague Dawley rats Gavage 0, 0.156, 0.312, 0.625, 1.25 and 2.5 mg/kg bw per day	Reduced epidydimal weight and cauda epidydimis sperm count Reduced testis weight Reduced testosterone	0.625 1.25 1.25	1.25 2.5 2.5	*Plasma conc. (ug/ml)* at 0.156mg/kg bw/day: 8.5 ± .6 (m) 11.2 ± 0.4 (f) *Liver conc (ug/g)* at 0.156mg/kg bw/day 44.7 ± 1.5 (m)	NTP, 2019a
Perfluorododecanoic acid (PFDoDA)						
PFDoDA (95% pure, salt not specified)	Female Sprague-Dawley rats, weaned (PND 21; 8 per group) 0, 0.5, 1.5, 3 mg/kg bw per day orally 28 days (PND 24 – PND 52)	Body weight decreased Absolute and relative weight of uterus and ovary Age, weight at vaginal opening Estrus cyclicity Increased cholesterol	1.5 3 3 3 1.5	3 3	Not given	Shi et al. 2009a

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
		Decreased estradiol	1.5	3		
PFDoDA (95% pure, salt not specified)	Male Sprague-Dawley rats, weaned (PND21; 6 per group) 0, 0.02, 0.05, 0.2, 0.5 mg/kg bw per day orally 110 days exposure	Body weight decreased Absolute and relative weight of testis, prostate, seminal vesicle, vas deferens Cholesterol Decreased testosterone	0.2 0.5 0.5 0.2	0.5 0.5	Not given	Shi et al. 2009b
PFDoDA (purity and salt not specified)	Male Sprague-Dawley rats 21 days old (8 per group) 0, 5, 10 mg/kg per day gavage 14 days exposure (sacrifice PND 35)	Body weight decrease Decreased testis weight Decreased testosterone, LH, FSH Leydig and Sertoli cell number	5 5	10 10 5	Not given	Chen et al. 2019
Perfluorotetradecanoic acid (PFTeDA)						
PFTeDA, (salt not specified), 96.5% pure	Crl:CD(SD) rats, 12 males per group dosed for 42 days. Dosage 0, 1, 3 and 10 mg/kg bw per day, gavage.	Decreased weight of seminal vesicles		1	Not reported	Hirata-Koizumi et al. 2015

n.d: not determined

9873 Table F.4. Perfluoroalkane sulfonic acids (PFSAs): reproductive and developmental toxicity

9874

Substance/ (Purity)	Species/Experimental design and doses	Observed effects	Highest dose with no effect (mg/kg bw per day)		Lowest dose with effect (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
Perfluorobutane sulfonic acid (PFBS)								
PFBS (97.9% pure, K^+-salt)	Sprague Dawley rats 2-generation reproduction study according to OECD guideline 416 Exposure: 0, 30, 100, 300, 1000 mg/kg bw per day, gavage. Parental (F0) animals (males and females, n=29-30 per sex per group) dosed from 70 days prior to mating, females were continued through gestation and lactation. F1 offspring (n=29-30) was dosed from weaning (lactational day 22) onwards. F2 generation was exposed through placenta and	Increased liver weight Increased hepatocellular hypertrophy (P and F1 adult males) Increased incidence of mild microscopic findings in kidney No reproductive and developmental toxicity findings	100 100 100	 1000	300 300 300		Not determined	Lieder et al., 2009b

Substance/ (Purity)	Species/Experimental design and doses	Observed effects	Highest dose with no effect (mg/kg bw per day)		Lowest dose with effect (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
	lactation. Experiment was terminated at lactational day 22 of F2 generation animals							
PFBS (97% pure, K^+-salt)	ICR mice Dosing: 0, 50, 200, and 500 mg/kg bw per day from GD 1 -20, orally. Only female offspring follow-up reported. 30 dams per dose group, randomly allocated to three experimental sub-groups per dose: *group 1:* perinatal survival and growth, pubertal onset, and ovarian and uterine development (10 dams, 50 female offspring per dose), *group 2:* hypothalamic–pituitary–gonadal hormone and hypothalamic–pituitary–thyroid hormone levels (10 dams, 30 PND 1	Decreased body weight from PND 1 to adulthood Delayed eye opening Delayed vaginal opening Impaired ovarian and uterine development Delayed estrus cyclicity and reduced E2/ increased LH Decreased T3/T4, increased TSH	50	50 50 50 50 50 50	200	200 200 200 200 200 200	Mean (10 dams) serum concentration (ng/mL) on GD 20, 12h after last dosing, at 0, 50, 200 and 500 mg/kg bw per day: 1.73, 74, 332, 721.	Feng et al., 2017

Substance/ (Purity)	Species/Experimental design and doses	Observed effects	Highest dose with no effect (mg/kg bw per day)		Lowest dose with effect (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
	female offspring, 10 female PND 30 offspring, and 10 PND 60 female offspring), *group 3:* levels of serum PFBS (10 dams)	Decreased T3/T4, increased TSH at GD 20						
Perfluorohexane sulfonic acid (PFHxS)								
PFHxS (99.98% pure, K^+-salt)	Sprague-Dawley rats, OECD guideline 422-based design Exposure: 0, 0.3, 1, 3, 10 mg/kg bw per day by oral gavage to F0 (n=15+3; main study + serum sampling) starting 14 days prior to mating until PND 22 or presumed gestation day 25 for rats without litter	No reproductive or developmental effect No treatment related effects in dams	10	10			Elaborated serum and liver values in dams and fetuses. Serum levels at study day 14 and GD 21 in dams were similar. For GD 21 females in serum and liver: 0 mg/kg per day: <LOQ of 0.1 µg/mL and 0.1 µg/g. 0.3 mg/kg per day: 3.32 µg/mL and 0.79 µg/g	Butenhoff et al., 2009

							1 mg/kg per day: 10.65 µg/mL and 2.61 µg/g 3 mg/kg per day: 32.75 µg/mL and 7.80 µg/g 10 mg/kg per day: 59.80 µg/mL and 16.53 µg/g	
PFHxS, K^+-salt, purity > 98%	Wistar rats. Oral gavage from GD 7 until PND 22 (except for day of delivery). Range finding study (8 time mated rats per group): 0, 25, 46 mg/kg bw per day. Main study (16 -20 time mated rats per group): 0, 0.05, 5, 25 mg/kg bw per day.	Increased liver weight. - Males - Females Pronounced reduction of T4 levels, detectable at different time points. Dams: Offspring: Mildly decreased body weight. Male pups Female pups	0.05	5 0.05 0.05 5 0.05	5	25 5 5 25 5	Serum levels in dam at PND 22 in the range finding study. 139 and 174 µg/mL in 25 and 45 mg/kg bw per day groups, respectively	Ramhøj et al. 2018
K^+PFHxS (98.9% pure)	Crl:CD1 (IRC) mice	Reduced litter size (without impact on born pup to implant ratio)		0.3		1	Serum and liver was measured at study day 14, GD 18 (toxicokinetic study	Chang et al., 2018

	Study design according to OECD guideline 422, modified). Exposure: gavage at 0, 0.3, 1, 3 mg/kg bw per day (n=30+12 per treatment; main study + toxicokinetic arm). Main experiment: For F_0 males treatment from 14 days prior to cohabitation for to at least 42 days total (one day post-last dosing). Treatment of F_0 females started 14 days prior to cohabitation with continuation through mating, gestation, and lactation. F_0 dams were sacrificed on lactation day 22 (one day after last dosing). F1 offspring, first exposure in utero and via lactation. After weaning (PND 22), F1 direct dosing for 14 days at maternal dose. Toxicokinetic experiment: 12 animals per sex and dose. Subset 1 (5/sex/dose group) daily	*F0 animals:* Increased mean and relative liver weight. F1 animals: Increased relative liver weight ♀ and ♂ Increased thyroid weight ♂	0.3 1 1		1 3 3		arm) and lactational day 22 (main experiment) in dams and in F1 at GD 18 (pooled serum foetuses), PND 4 (pooled liver litter) and PND 21 and 36 (males and females). Serum and liver in dams, and serum from pooled fetus on GD18: 0 mg/kg per day: <0.001 µg/mL, <0.005 µg/g and <0.001 µg/mL. 0.3 mg/kg per day: 16.8 µg/mL, 5.3 µg/g, and 20.8 µg/mL 1 mg/kg per day: 51.5 µg/mL, 15.1 µg/g and 62.3 µg/mL 3 mg/kg per day: 111.3 µg/mL, 88.4	

	oral gavage for 14 days prior to sacrifice. Subset 2 (7/sex/dose group) dosing for 14 days prior to cohabitation. Serum and liver samples were collected at study day 14 for both sexes, at study day 28 for males and GD 18 (for females). On GD 18, pooled fetal blood and fetal liver samples were collected						µg/g and 137.7 µg/mL.	

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9877 Table F.5. 8:2 FTOH and EtFOSE reproductive and developmental toxicity

Substance/ (Purity)	Species/Experimental design and doses	Observed effects	Highest dose with no effect (mg/kg bw per day)		Lowest dose with effect (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
8:2 FTOH (99.2% pure)	Crl:CD (SD)IGS BR rats (time mated) 0, 50, 200, 500 mg/kg bw per day, oral gavage Exposure GD 6- GD 20 22 rats per dose group	Mortality Decreased body weight and body weight gain (Slight) reduction in food consumption Slight increases in delayed skull ossification Increased skeletal variations (due to delayed ossification)	200 200 100	 100 200	500 500 200	 200 500		Mylchreest et al. 2005
8:2 FTOH (purity not specified)	CD1 mice Experiment 1: 30 mg/kg per day on GD 8 (n=15 control, n=26 treatment group). Follow up by serial sacrifice at GD 9,	Pre- and postnatal exposure to PFOA and PFNA following following maternal treatment at GD 8 with 8:2 FTOH					No 8:2 FTOH detectable after 24 h of treatment of dams. Only quantifiable compounds PFOA and PFNA.	Henderson & Smith 2007

	GD 10, GD 13, GD 15, and GD 18 Experiment 2 (cross fostering): 30 mg/kg per dayon GD 8 (n=34 control, n= 36 treatment group). From PND 0 cross-fostering of half of the animals Follow-up on PND 1, 5, 15						8:2 FTOH not detectable in fetal or neonatal tissue. Placental PFOA 49 ± 13 ng/g. *Fetal period:* Maternal serum: Decrease 789 to 668 ng/mL (GD 9 – 18) Maternal liver: Decrease 673 to 587 ng/g (GD 9 - 18). Foetuses (whole body burden): Increase PFOA 49 ng/g to 140 ng/g (GD 9 -18), PFNA only quantifiable at GD 18 at 31 ng/g *Postpartum period:* Maternal serum: Decrease PFOA 451 – 52 ng/mL (PND 1 – PND 15) Neonates (treated mothers, whole	

							body burden): 200 to 149 ng/g (PND 1 – PND 3) Neonates (untreated mothers, cross fostering, whole body burden): 57 to 58 ng/g (PND 3 – PND 15) Levels of PFOA were higher than levels of PFNA in serum and liver of dams, foetuses and neonates Cross fostering experiments provide evidence for lactational exposure	
EtFOSE (98.2% pure	Female Crl:CD BR rats Dose range finder study: Dosage: 0, 1, 5, 10, 20, 25, 35 mg/kg bw per day, 8 pregnant animals/group, oral administration from GD 6 – 17. Necroscopy on GD20.	*Results from dose range finding study* Emaciation Reduced maternal body weight Reduced fetal body weight	 25 5 10		 35 10 20		No information given	Case et al. 2001

	Developmental toxicity (teratology) study: Dosage 0, 1, 5, 10, 20 mg/kg bw per day, 25 pregnant animals/group, oral administration GD 6 – 17. Necroscopy at GD 20	*Results from developmental toxicology study* Reduced body weight gain during pregnancy Reduction of fetal weight	5	5	10 10			
	Rabbits Dose range finder study: Dosage: 0, 1, 5, 10, 25, 50, 75 mg/kg bw per day, 5 mated rabbits, oral administration GD 6 – 20. Sacrifice at GD 29. Developmental toxicity (teratology) study: Dosage 0, 0.1, 1, 2.5, 3.75 mg/kg bw per day, 22 pregnant does/group, oral administration GD 7 – 20. Sacrifice at GD 29.	*Results from dose range finding study* Weight loss Severe toxicity and death Abortion *Results from developmental toxicology study* Abortion	1 10 1 1	1	5 25 2.5 2.5	5	No information given	Case et al. 2001

		Permanent reduced body weight gain Transient reduction of body weight (GD 7 – 13)	0.1		1			

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9880 **Table F.6** : PFOS reproductive and developmental toxicity studies. Studies are in chronological order with studies in rats listed before studies in mice.

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	Offspring	Mother	offspring		
PFOS (91% K^+-Salt) *"Our analysis indicated that approximately 71% of the chemical was straight-chain, and the remaining 29% was branched. Additional analysis indicated that the chemical obtained from Fluka appeared to be identical to that produced by 3M."*	Sprague-Dawley rats Exposure: 0, 1, 2, 3, 5, 10 mg/kg per day per gavage GD2 – GD21	Decreased postnatal survival Decreased growth in surviving pups Delay in eye opening Decreased thyroxin No consistent change in liver weight or relative liver weight in surviving pups PND 0-35 Maternal effects not assessed		1 ($BMDL_{05}$ 0.58) 1 1 1		2 2 2 2	**Read** from figures. Serum in pups at pnd 1: Control: 0 1 mg/kg: 36 µg/mL 2 mg/kg: 71 µg/mL 3 mg/kg: 85 µg/mL 5 mg/kg: 108 µg/mL Slightly lower at pnd 5 Liver in pups at pnd 1: Control: 0 1 mg/kg: 45 µg/g 2 mg/kg: 68 µg/g 3 mg/kg: 100 µg/g 5 mg/kg: 160 µg/g	Lau et al. 2003
PFOS (86.9%, K^+-Salt. Lesser homologs (C4–C7) at 8.4%; impurities (by	Sprague Dawley rats, 20 dams per group. Exposure: 0, 0.1, 0.4, 1.6, and 3.2 mg/kg bw per day by gavage for 6	F0 males and females: reduced bw gain.	0.4 1.6		1.6 3.2		Serum levels (µg/mL) from cross foster study at end of lactation.	Luebker et al. 2005a

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	Offspring	Mother	offspring		
quantitative 19F Nuclear Magnetic Resonance) at 1.9%; metals (calcium, magnesium, sodium, nickel, and iron) at 1.5%; inorganic fluoride at 0.6%; perfluorooctanoic acid at 0.3%; nonofluoropentanoic acid at 0.3%; heptafluorobutyric acid at 0.1%.	weeks prior to mating, during mating, and through gestation and lactation, across two generations for females (only 0, 0.1, 0.4 mg/kg bw per day continued to F2). Cross fostering (0 and 1.6 mg/kg bw per day) as follow up study	In F0 shorter gestation, lower n implantation sites, increase in stillborn pups or early neonatal death of litter. F1 reduced survival and bw gain. Delayed eye opening F1 Pre- and postnatal exposure additive to pup toxicity	0.4 0.1		1.6 0.4		Control litter nursed by control dams:<0.05. Control litter nursed by treated dam: 22.4 Treated litter nursed by control dam: 53.9 Treated litter nursed by treated dam: 89.7 Control dams nursing control litter: <0.05 Treated dams nursing control litter: 83.0 Control dams nursing treated litter: 2.02 Treated dams nursing treated litter: 89.0	
PFOS (86.9%, K^+-Salt. Impurities as in Luebker 2005a	Sprague Dawley rats, 35 dams per group. Exposure: 0, 0.4, 0.8, 1.0, 1.2, 1.6, 2,0 mg/kg bw per day by gavage from 6 weeks prior to mating to day four of lactation	Decrease in gestation length Decrease in postnatal survival day 5	$BMDL_{05}$ 0.31	$BMDL_{05}$ 0.89 $BMDL_{05}$ 0.39			Measured in dams GD 1, 7, 15 and 21. Constant GD 1-15, drop at GD 21 Serum levels GD 1-15 (µg/mL): 0.1 mg/kg: 7.8-8.9	Luebker et al 2005b

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	Offspring	Mother	offspring		
		Mean pup weight at birth Increase in serum T4 on lactation day 5			 0.4	 0.4	0.4 mg/kg: 40.7- 41.4 1.6 mg/kg: 154-160 3.2 mg/kg: 275-318	
PFOS (purity 98 %, salt not specified)	Sprague-Dawley rats Exposure: 0, 0.1, 0.6, 2 mg/kg per day per gavage GD2 – GD21 Dams: 10 per group (not studied) Follow up offspring: Neonatal survival PND 4 (all), survivors until PND 21: 6 per group	Increased mortality of offspring at 2 mg/kg per day Reduced body weight offspring Increased heart to body weight ratio at PND 21 Cardiac mitochondrial injury		0.6 0.6 0.6 0.6		2 2 2 2	Serum PND21, dose dependent increase: 4.26 µg/ml at 2 mg/kg per day Heart, dose dependent increase: 9.59 µg/g at 2 mg/kg per day	Xia et al., 2011
PFOS, purity not specified	Sprague-Dawley rats	At GD 20: Decreased body weight	5	 5	20	 20	Not reported	Zhao et al., 2014

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	Offspring	Mother	offspring		
	Exposure: 0, 5, 20, mg/kg per day by oral gavage, GD 11-19 n=4 per group	Decreased testis weight and liver HDL- cholesterol						
		Decreased body weight		n.d.		5		
		Decreased testis weight		5		20		
		Change anogenital distance		5		20		
		Increased apoptosis rate in testicular cells		5		20		
		Decreased number of Leydig cells		5		20		
		Decreased testis testosterone		5		20		
		Decreased testis progesterone		5		20		

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	Offspring	Mother	offspring		
PFOS (98% K^+-Salt)	ICR mice 15 dams/goup (5 each selected for specific endpoints Exposure: 0, 1, 10, 20 mg/kg per day , GD 1 – 17/18	Reduced weight gain Increased liver weight Liver hypertrophy Decreased neonatal survival Developmental/ teratological alterations Sternal defects	10 1 10	 1 1 n.d	20 10 20	 10 10 1	Not reported	Yahia et al., 2008
PFOS, 91%, K^+-Salt	B6C3F1 mice Exposure: GD 1-17, 0, 0.1, 1.0, 5.0 mg/kg bw per day by gavage.	Decrease in NK cell activity at 8 weeks Decrease in IgM production assessed by PFC assay at 8 weeks (spleen)		0.1 (males) 1 (females) 1 (males) 5 (females)		1 (males) 5 (females) 5 (males)	Not reported	Keil et al., 2008
PFOS (purity 98%, K+-salt)	ICR mice	Sharp increase in cleft palate		13		20	Serum level on GD17 (µg/mL) reported for	Era et al., 2009

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	Offspring	Mother	offspring		
	Exposure 0, 9, 13, 20, 30 mg/kg bw per day by gavage GD1-17 (3 dams/group). 20 mg/kg bw per day GD 1-17 and 50 mg/kg per day GD11 – 15. 5 – 8 dams per group 67 – 103 fetuses: (examined animals, total number higher) 20 mg/kg per day GD 1-15/18 for histology.	between 13 (7.3%) and 20 (78.35) mg/kg per day.		50 % effective dose expected 17.7 mg/kg per day			dams at 30 mg/kg per day and other values estimated from figure: 9 mg/kg bw per day: 58 in dam, 62 in fetus. 13 mg/kg bw per day: 105 in dam and fetus. 20 mg/kg bw per day: 135 in dam and fetus. 30 mg/kg bw per day: 162.3 in dams and 130 in fetus. 50 % effective fetal serum concentration for cleft palate: 121 µg/mL	
PFOS (K^+-salt, >91% pure)	129S1/Svlm WT and PPARαa KO. Exposure: Gavage, (WT) 0, 4.5, 6.5, 8.5, 10.5 mg/kgbw per day, (KO) 0, 8.5 10.5 mg/kg per day GD 15-18. Number of dams varied from 8 (WT. 4.4 mg) to 20 (WT control).	Postnatal death Delayed eye opening (Litter loss not affected)		4.5		4.5 6.5	Measured at PND15 in adult females with and without pups and in pups.	Abbott et al., 2009

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	Offspring	Mother	offspring		
PFOS (98% K^+-Salt)	C57BL/6J-$Apc^{+/+}$ female mated with C67BL6J-$^{Min}/^+$ males (n=20/21) Exposure: 0, 0.1, 3 mg/kg per day experimental block 1 per drinking water 0, 0.01, 0.1, 3 mg/kg per day experimental block 2 per drinking water GD 1-17 $Apc^{Min/+}$ genotype mice were terminated at 11 weeks of age for tumorigenesis. Wildtype mice were kept until week 20 for obesogenic effects.	No obesogenic effects, no intestinal tumorigenesis Comparative study approach revealed that mild toxicity effects seen for PFOA did not occur in response to PFOS		3			0.1 mg/kg PFOS: 2.2/2.7 µg/mL in GD 18 Dams, 0.48/0.54 µg/mL dams after weaning and 0.38/0.3 µg/mL pups after weaning 3.0 mg/kg PFOS: 36.6/44.6 µg/mL in GD 18 dams;17.2/22.2 µg/mL Dams after weaning and n.d in pups after weaning	Ngo et al., 2014
PFOS (purity 98%, salt not specified)	CD1 mice Exposure: 0, 0.3, 3 mg/kg per day GD1 – PND21 by	Increased liver weight	3	0.3 (male)		3 (male)	Serum levels in dams at	Wan et al., 2014

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	Offspring	Mother	offspring		
	gavage. Then no dosing in offspring until sacrifice at PND 63. Dams: 6 per group (sacrifice after weaning (PND 21)) Offspring: animals per treatment equally distributed in a low and a high fat feeding group. Termination on PND 63	Relative liver weight increase Increased HOMA-IR Elevated fasting glucose Additional effects related to high fat diet, but only results from normal diet included here	0.3	0.3 3 3 (PND 21)	3 0.3	3 0.3 (PND 63)	0, 0.3 and 3 mg/kg per day: 0.25, 15.3 and 131.7 µg/mL. Liver levels in dams at 0, 0.3 and 3 mg/kg per day: 0.15, 49.1 and 338.9 µg/g. Serum levels in pups PND21 at 0.3 mg/kg per day: 12.7 µg/mL in males and 11.4 µg/mL in females Serum levels at 3 mg/kg per day: 98.7 µg/mL in males and 87.2 µg/mL in females Liver levels in pups PND21 at 0.3 mg/kg per day: 20.1 µg/g in males and 18.0 µg/g in females Liver levels at	

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	Offspring	Mother	offspring		
							3 mg/kg per day: 243 µg/g in males and 178 µg/g in females	
PFOS (source, purity and salt not specified)	Sprague-Dawley rats Exposure: 18.75 mg/kg per day GD 2-6 by gavage 10-12 Offspring animals per group	delayed weight gain Reduced birth weight both sexes Increase blood pressure in male offspring from PND7 to PND52 Increase blood pressure in female offspring from PND37 to PND65	n.d. n.d. n.d. n.d.		18.75 18.75 18.75 18.75			Rogers et al., 2014
PFOS (purity not specified, K^+-salt)	CD1 mice Exposure: Gavage, 0, 0.5, 2, 8 mg/kg per day, GD11 – GD16 10 dams per group	Body weight decrease Dose dependent decrease of placental weight and capacity	2	n.d	8	0.5	Not reported	Lee et al., 2015

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	Offspring	Mother	offspring		
		Dose dependent increase of number of resorptions and dead foetuses		n.d.		0.5		
		Decrease in the numbers of glycogen trophoblast cells in the junctional zone and the number of sinusoidal trophoblast giant cells in the labyrinth zone		n.d.		0.5		
		Decrease of mPL-II, mPLP-Cα and mPLP-K expression levels and serum concentrations		n.d.		0.5		

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	Offspring	Mother	offspring		
PFOS, purity not given	Kunming mice. Exposure: 0, 0.5, 5 mg/bw per day, intragastric, from GD1 to parturition, 5 dams per group	Increased liver weight and triglycerides Changes in liver lipid homeostasis		0.5 0.5		5 5	Not given	Liang et al., 2019

9881 n.d: not determined.

9882 **Table F.7** PFOA reproductive and developmental toxicity studies with pre- and perinatal exposure.

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
PFOA ammonium salt, 98% pure	CD1 mice, GD1-birth, 0, 1, 3, 5, 10, 20, 40 mg/kg per day	Liver weight resorption of litters, reduced percentage of live foetuses, reduced weight of foetuses, reduced postnatal survival and growth deficits (ossification)	n.d.	 1	1	 3	Maternal serum levels (ng/mL) at term (estimated from figure). 0: n.d. 1: 20 000 3: 40 000 5: 70 000 10: 110 000 20: 170 000 40: 260 000	Lau et al., 2006
PFOA ammonium salt, 97,99% pure	129S1/SvlmJ wild-type and PPARα knockout mice, 0.1, 0.3, 0.6, 1, 3, 5, 10, 20 mg/kg per day, GD 1-17. Results for WT	Relative liver weight Relative liver weight Litter loss Postnatal survival	0.6	 n.d. 0.3 0.3	1	 0.1 0.6 0.3	P0 with no pups, PND22 serum at 0, 0.1, 0.3, 0.6 and 1 mg/kg per day:131, 4400, 10400, 17400 and 26300 ng/mL P0 with pups, PND22 serum at 0, 0.1, 0.3, 0.6 and 1 mg/kg per day: 33.2, 1600, 2840, 5170, 9290 ng/mL	Abbott et al., 2007

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
							Pups at PND22 serum at 0, 0.1, 0.3, 0.6 and 1 mg/kg per day: 17.3, 798, 2150, 3810, 9860 ng/mL	
PFOA ammonium salt, 98% pure	CD1 mice GD1-17, GD8-17, GD12-17: 0 and 5 ng/kg bw/day	Reduced bw Decreased weaning-induced mammary involution (P0) Mammary gland development retardation irrespective of the timing of exposure			5	5 5		White et al., 2007
PFOA ammonium salt, 98% pure	CD1 mice Full gestational study (GD 1-17): 0 (n=48), 3 (n=28), 5 (n=36) mg/kg bw (pregnant dams) cross-fostering during lactational exposure leading to 7 groups.	Mammary gland development retardation irrespective of the timing of exposure		n.d.		3 5	Serum levels (ng/mL). Exposure GD 8-17, dams on lactation day 1,3,5,10: Control dams nursing control pups: 11, 6, 4, 0. Control dams nursing treated	White et al., 20091

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
	GD 8-17: 0 (n=56), 5 (n=56) ng/kg bw (pregnant dams). Cross-fostering during lactational exposure. Early life effects restricted gestational (GD 7,10,13,15-17) exposure study at 5 mg/kg per day					5	pups: 26, 1300, 6510, 5000. Treated dam nursing control pups: 47900, 39300, 34000, 16400. Treated dam nursing treated pups: 42200, 43000, 38800, 24400. Pups on PND 1, 3, 5, 10 (exposure GD 8-17) and PND 22, 42, 63 (exposure GD 1-17). Control pup nursed by control dam: 23, 16, 10, 8, nd, nd, nd. Treated pup nursed by control dam: 66200, 43900, 33100, 20500, 8300, 646, nd. Control pups nursed by treated dam:	

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
							1560, 10500, 15000, 15700, 12100, 894, nd. Treated pups nursed by treated dam: 70000, 54400, 52200, 31300, 21900, 4050, 500.	
PFOA, ammonium salt > 98% pure	CD-1 mice Exp. 1: 0 ,1, 3, or 5 mg/kg; n=5, 5, 8, 7 dams Exp. 2: 0, 0.01, 0.1, 0.3, 1 or 5 mg/kg; n=14 dams except for 5 mg/kg n=10 Exposure GD 1 -17	Transient body weight gain at mid age observation group of the pups (21-33 weeks) Increase of insulin and leptin 0.01 – 0.1 mg/kg.		n.d. n.d.		0.01 - 0.3 0.01		Hines et al., 2009
PFOA, purity 90%, salt unknown	ICR mice, 15 – 19 dams/group, 1, 5, 10 mg/kg per day, exposure GD 1-17/18	Dose dependent liver weight gain, Significant change of 12 out of 20 metabolic parameters with phosphorus and urea levels being most sensitive	1 n.d.	1	5 1	5		Yahia et al., 2010

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
		Decreased neonatal survival						
PFOA, ammonium salt > 98% pure	CD1 mice Full gestational study (GD 1-17): 0, 0.3, 1, 3 mg/kg per day, dams (n=13) Late gestational study (GD 10-17): 0, 0.01, 0.1, 1.0 mg/kg per day, dams (n=7–13) Offspring: 7-9 animals per litter	Full gestational exposure: Transient (until PND7) increase in liver weight Decreased mammary gland developmental score Late gestational exposure: decreased mammary gland developmental score number of terminal endbuds		n.d n.d. n.d. 0.01		0.3 0.3 0.01 0.1	Full gest. exposure, female serum at 0.3 mg/kg per day: 4980 (PND7) – 16 ng/mL (PND84) Liver at 0.3 mg/kg per day: 2078 (PND7) – 43 ng/g (PND84) Late gest. exposure: Control 22.6 (PND1) – 4.1 ng/mL (PND21) Female serum at 0.01 mg/kg per day: 284.5 (PND1) – 16.5 ng/mL (PND21)	Macon et al., 2011

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
							Serum at 0.1 mg/kg per day: 2303.5 ng/mL (PND1) – 131.7 ng/mL (PND21) More concentration data in supplementary	
PFOA, ammonium salt, purity not stated	CD-1, 10 dams per group, 2, 10, 25 mg/kg bw/day, GD 11-16. Sacrificed on GD 16	No of resorptions and dead fetuses Decrease of placental weight Decrease in trophoblast cells in the placenta Decrease of mPL-II, mPLP-Cα and mPLP-K expression levels and serum concentrations	10		2 2 25 2			Suh et al, 2011
PFOA, ammonium salt 98 % pure	Pregnant CD-1 mice receiving 0, 1, 5 mg/kg	Prenatal loss (P0)	1		5		P0 dams at weaning (PND 22) Control: 4.0 ng/mL	White et al., 2011

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
	bw per day PFOA by oral gavage from GD1-17. Pregnant CD-1 mice receiving 0 and 1 mg/kg bw per day PFOA by gavage from GD1-17 and additional drinking water containing 5 µg/L (0.00045 mg/kg bw per day) of PFOA from GD7 until termination of the experiment for P0, F1 and F2 generations Dams: n=5 -12 Litter size neonates F1: 12-13 pups Litter size neonates F2: 10 pups	Decreased weaning-induced mammary involution (P0) Postnatal survival (F1)		1	0.00045 (5µg/L in water)	5	Control + 5 µg/L in water: 74.8 ng/mL 1 mg/kg bw per day: 6658 ng/mL 1 mg/kg bw per day + 5 µg/L in water: 4772 ng/mL 5 mg/kg/ bw per day: 26980 ng/mL	
		F1 developmental indices mammary gland without PFOA in drinking water (PND 22, 42, 63)		n.d.		1	F1 pups PND 22 Control 0.6 ng/mL Control + 5 µg/L in water: 21.3 ng/mL 1 mg/kg bw per day: 2444 ng/mL 1 mg/kg bw per day + 5 µg/L in water: 2744 ng/mL 5 mg/kg bw per day: 10045 ng/mL	
		F1 developmental indices mammary gland with PFOA in drinking water (PND 22, 42, 63)		n.d.		0.00045 5µg/L in water	F1 pups PND 42 Control:1.4 ng/mL Control + 5 µg/L in water: 48.9 ng/mL 1 mg/kg bw per day: 610 ng/mL	

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
							1 mg/kg bw per day + 5 µg/L in water: 558 ng/mL 5 mg/kg bw per day: 1581 ng/mL	
		F1 maternal indices mammary gland without PFOA in drinking water (PND 10)	n.d.		1		F1 pups PND 63 Control 3.1 ng/mL Control + 5 µg/L in water: 66.2 ng/mL 1 mg/kg bw per day: 211 ng/mL 1 mg/kg bw per day + 5 µg/L in water: 187 ng/mL 5 mg/kg bw per day: 760 ng/mL	
		F1 maternal indices mammary gland with PFOA in drinking water (PND 10)	n.d.		0.00045 5µg/L in water		F1 dams at weaning (PND 22) Control 2.0 ng/mL Control + 5 µg/L in water: 86.9 ng/mL 1 mg/kg bw per day: 9.3 ng/mL 1 mg/kg bw per day + 5 µg/L in water: 173 ng/mL	

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
							5 mg/kg bw per day: 18.7 ng/mL	
		F2 developmental indices mammary gland without PFOA in drinking water (PND 63)		n.d.		1	F2 pups PND 22 Control 0.4 ng/mL Control + 5 µg/L in water: 26.6 ng/mL 1 mg/kg bw per day: 4.6 ng/mL 1 mg/kg bw per day + 5 µg/L in water: 28.5 ng/mL 5 mg/kg bw per day: 7.8 ng/mL	
		F1 developmental indices mammary gland with PFOA in drinking water (PND 42)		n.d.		0.00045 5µg/L in water	F2 pups PND 42 Control 0.7 ng/mL Control + 5 µg/L in water: 57.4 ng/mL 1 mg/kg bw per day: 0.4 ng/mL 1 mg/kg bw per day + 5 µg/L in water: 72.8 ng/mL 5 mg/kg bw per day: 0.4 ng/mL	
							F1 pups PND 63	

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
							Control: 1.1 ng/mL Control + 5 µg/L in water: 68.5 ng/mL 1 mg/kg bw per day: 1.1 ng/mL 1 mg/kg bw per day + 5 µg/L in water: 69.2 ng/mL 5 mg/kg bw per day: 1.2 ng/mL	
PFOA ammonium salt, 98% pure	CD-1 mice, 5 dams/group, 5 mg/kg bw per day, exposure GD 1 - 17	Decreased neonatal survival		n.d.		5		Abbott et al., 2012
PFOA, purity not stated	Sv/129 Mice WT, mPPARα KO and expressing hPPARα GD 1-17 3 mg/kg bw per day. Results for WT (5-6 dams per group)	Decrease postnatal survival Decrease mammary development		 3		3	19000 ng/mL	Albrecht et al, 2013
PFOA ammonium salt, 98% pure	C57BL/6J-Apc+/+ female mated with C67BL6J-Min/+ males 10 – 24 dams/group 0.1 and 3 mg/kg bw per day (study 1) and 0.01	Decreased neonatal survival (not detectable for PFOS)		0.1 n.d.		3	Serum levels in pups at 0, 0.01/0.1/3 mg/kg bw per day: <0.05, 12-26, 213 – 216/n.d. ng/mL	Ngo et al., 2014

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
	and 0.1 mg/kg bw per day (study 2) Exposure: GD 1 – 14-18	Small increase of liver weigt at 0.01 mg/kg per day, but not at 0.1 mg/kg per day				0.01 (not detectable at 0.1)		
PFOA ammonium salt, 98% pure	CD-1 mice, 12, 12, 14, 13, 12, and 6 pregnant dams resulting in 29, 29, 37, 26, 31, and 21 female offspring, exposure 0.01, 0.1, 0.3, 1, 5 mg/kg bw per day, GD 1 -17 129/Sv WT mice, 7, 7, 5, 3, and 5 pregnant dams resulting in 10, 10, 8, 6, and 8 female offspring, exposure 0, 0.1, 0.3, 0.6, 1 mg/kg bw per day, GD 1-17 129/Sv PPARα ko mice, 5, 9, 8, 7, and 9 pregnant dams resulting in 6, 10, 10,9, and 9 female offspring, exposure 0.1, 0.3, 1, 3 mg/kg bw per day , GD1-17	CD1 mice: Several non-neoplastic alterations in livers 129/Sv mice: bile duct hyperplasia in 129/Sv PPARα knockout, but not wildtype mice liver lesions can occur independent of PPARα		1 1		5 5		Filgo et al., 2014

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
	Investigation at 18 months							
PFOA, ammonium salt 98% pure	CD-1 mice, 163 dams equally distributed to treatment groups, 6-7 females and 3-4 males per litter after birth C57Bl/6 mice, 41 dams divided to 5 groups, litter sizes > 5 were maintained Exposure 0, 0.01, 0.1, 0.3 1.0 mg/kg bw per day, GD1-17	CD-1 mice: Decrease of mammary gland development score C57Bl/6 mice: Decrease of mammary gland development score		0.01 (PND21) n.d. (PND 35, PND 56) 0.1 (all time points)		0.1 (PND21) 0.01 (PND 35, PND 56) 0.3 (all time points)	CD-1: Female pups, PND 21 serum at 0, 0.01, 0.1, 0.3 and 1 mg/kg bw per day: <5, 74.8, 457.3, 904.8, 3119 ng/mL. C57Bl/6: Female pups, PND 21 serum at 0, 0.01, 0.1, 0.3 and 1 mg/kg bw per day: <10, 26.1, 247.1, 891.3, 2141.7 Concentrations in CD-1 at PND 35 and 56 and in B6 at PND 61 available	Tucker et al., 2015
PFOA Na+ Salt >99 %	Female C57BL/6J mice mated with male FVB mice. Supplemented through feed: 0, 0.017, 0.056,	Decreased litter size Decreased Body weight	BMDL 0.299	BMDL5 (week 25) 0.85	1			Van Esterik et al., 2016

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
	0.17, 0.56, 1.7, 5.6 and 17 mg/kg bw per day. Corresponding exposure: 0, 3, 10, 30, 100, 300, 1000 and 3000 µg/kg bw per day. Feeding to females was started 2 weeks prior to mating and maintained through mating, gestation and lactation (6 F0 females) Follow up of 9 offspring animals per sex into juvenile and adult stages Switch to high fat diet at 21 weeks	Decreased perirenal fat pads weight Decreased cholesterol (female) Triglycerides (female offspring)		BMDL5 0.65 BMDL5 0.40 BMDL5 0.006 (BMDU/BMDL=100)				
PFOA salt not specified, 96% pure	C57BL/6/Bkl female mice Exposure 0, 0.3 mg/kg per day, during gestation, n=6 dams per group	periostal areas and medullary areas of the femur were increased at 17 months of age, the bone mineral density of the femur unaffected. Tibial bone mass was decreased	n.d.		0.3		Bone levels: 13 months: control 0.73 ng/g, PFOA 3 ng/g 17 months: control 0.64 ng/g, PFOA 3.7 ng/g	Koskela et al., 2016

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
		both at 13 and 17 months. Biomechanical properties unaffected						
PFOA, 99.2% pure	Kunming mice Gavage, 0, 1, 5, 10, 20, 40 mg/kg bw per day, GD 1 to 17, 10 dams per group. Terminated on GD 18.	Decreased bw gain Increased relative liver weight Decreased relative uterus weight Decreased embryo weight Decreased embryo survival	10 n.d. 1 1 5		5 1 5 5 10			Li et al., 2018b
PFOA > 98% pure	Kunming mice, Gavage, 1, 2.5, 5 mg/kg bw per day from GD 1 to 17, 10 dams per group. Follow up on male offspring PND 21 and 70	Decreased postnatal survival Decreased bw PND 21 Dereased testosterone Decreased number of Leydig cells		2.5 1 n.d. 1		5 2.5 1 2.5	Mice serum 0.17 µg/mL. At which dose or age of mice was not explained, and method not gven	Song et al., 2018
PFOA, 99.2% pure	Kunming mice Gavage 0, 1, 2.5, 5, 10 mg/kg bw per day, GD 1 to 17, 10 dams per group.	Decreased postnatal survival Decreased weight gain		2.5 1		5 2.5		Li et al., 2019

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)		Significant effect level (mg/kg bw per day)		Serum/tissue levels of compound	Reference
			Mother	offspring	Mother	offspring		
	Follow up on female offspring on PND21	Increased liver weight		n.d.		1		

n.d.: not determined from the study. [1]Concentrations in serum quantified based on figure.

9884 **Table F.8** PFOA developmental toxicity studies with exposure in pubertal or adult animals

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
PFOA ammonium salt, 98% pure	BALB/c and C57BL/6 mice (comparative approach); n=20 0, 1, 5, 10 mg/kg bw per day, 5 days per week Exposure: PND 21 for 28 days (pubertal period)	Increase of relative liver weight in both strains Delayed vaginal opening Balb/c mice Delayed vaginal opening C57BL/6 mice Dose dependent decrease uterine wet weight in Balb/c mice Increase of uterine wet weight for C57/BL6 mice Reduction of ductal length, number of terminal endbuds and number of	n.d. n.d. 1 n.d. n.d. 1	1 1 5 1 1 5	BALB/c at 0, 1, 5 ng/kg bw/day: <10, 29500, 109000 ng/mL. C57BL/6 at 0, 1, 5, 10 ng/kg bw/day: <10, 26000, 68200, 96600 ng/mL	Yang et al. 2009

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
		terminal ducts in Balb/c mice. Increase of mammary gland parameters in C57/BL6 mice	n.d.	1		
PFOA ammonium salt, 98% pure	129/Sv wildtype, PPARα knockout mice, humanized PPARα mice, adult 0, 1, 5 mg/kg per day, n=8-10, 42 days	sperm abnormalities, decreased testosterone level	n.d.	1		Li et al., 2011
PFOA ammonium salt, 98% pure	Exposure: PND 21 for 28 days (pubertal period). 5-10 mice per group. Dosing 5 days a week Balb/c: 0 and 2.5 mg/kg bw per day C57Bl/6: 0 and 7.5 mg/kg bw per day	Reduction in mammary gland development (reduced ductal length, number of terminal end buds, stimulated terminal ducts)		2.5 7.5	Balb/c <10, 51100 C57Bl/6 <10, 93400	Zhao et al., 20121
PFOA ammonium salt, 98% pure	CD1 mice PND18, three day uterotrophic assay 0, 0.01, 0.1, 1 mg/kg bw per day (n=8)	1.46-fold increase of uterine wet weight. Further supported by histopathological examination	n.d.	0.01		Dixon et al., 2012

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
PFOA ammonium salt, 98% pure	BALB/c mice, adult 0, 0.31, 1.25, 5, 20 mg/kg bw per day, n=16, 28 days	Sperm count reduced, sperm motility, sperm progression increased, percentage of teratosperm increased Testosterone and progesterone levels drecreased Mild phenotype in seminiferous tubules	1.25 0.31 0.31	5 1.25 1.25		Zhang et al., 2014
PFOA, > 98% pure	Sprague Dawley rats, 10-11 weeks old at start. 0, 0.625, 1.25, 2.5, 5, 10 mg/kg bw per day, N=10 per sex per group 28 days	Reduced epididymal weight Reduced epididymal sperm count	5 5	10 10	Plasma conc. (ug/ml) at 0.625 mg/kg bw per day: 50.7 + 2.2 (m) at 5 mg/kg bw per day: 110.7 + 3.8 (m) at 6.25 mg/kg bw per day: 491 + 72.1 (f) Liver conc (ug/g) at 0.625mg/kg bw	NTP 2019a

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
					per day 54.6 + 2.2 (m)	

n.d.: not determined from the study; [1]Concentration in serum extracted from figure in Zhao et al., 2012.

Appendix G – Developmental Neurotoxicity tables

9886

9887 Table G.1. Developmental neurotoxicity studies

Substance (Purity)	**Species/Experimental design and doses**	**Observed effects**	**Highest dose with no effect (mg/kg bw per day**	**Lowest dose with effect (mg/kg bw per day)**	**Serum/tissue concentration**	**Reference**
Perfluorodecanoic acid (PFDA)						
PFDA; purity 98%	mouse, NMRI developmental exposure, single exposure at PND10 1.4, 21 µmol/kg bw (0.72; 10.8 mg/kg bw per day) oral (n=4 to 7 per group)	Decreased locomotor activity	N/A	10.8 at 2 months; 0.72 at 4 months	N/A	Johansson et al., 2008
Perfluorohexane sulfonic acid (PFHxS)						
PFHxS; purity >98%	mouse, NMRI developmental exposure, single exposure at PND10 1.4; 14; 21 µmol/kg bw (0.61; 6.1; 9.2 mg/kg bw per day) oral (n=12 per group)	Decreased locomotor activity at 2 months	N/A	0.61	N/A	Viberg et al., 2013
PFHxS; purity >98%	mouse, NMRI developmental exposure, single exposure at PND10	Increased expression of CaMKII and Tau in	N/A	6.1	N/A	Lee and Viberg, 2013

Substance (Purity)	**Species/Experimental design and doses**	**Observed effects**	**Highest dose with no effect (mg/kg bw per day**	**Lowest dose with effect (mg/kg bw per day)**	**Serum/tissue concentration**	**Reference**
	14, 21 µmol/kg bw (6.1; 9.2 mg/kg bw) oral (n=not reported)	hippocampus at PND11; increased Tau level in cortex at 4 months				

PND=postnatal day. Oral defines exposure via ingestion of drinking water, food, or via gavage; N/A: Not applicable.

Appendix H – Immunotoxicity

Table H.1 Immunotoxicity studies PFOS from 2018 opinion (EFSA CONTAM Panel, 2018)

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
PFOS, 98%	C57BL/6 mice (4–8 males per group), administered via diet for 10 days, at 0.001 or 0.02% (2 or 40 mg/kg bw per day) (0.02% equals a total of 6 mg/animal over 10 days)	Several immune parameters up or down, but also reduced body weight gain	N/A	0.02% (40 mg/kg bw per day)	340 µg/mL	Qazi et al. (2009a,b)
PFOS, 98%	C57BL/6 mice (4 males), restricted food diet, 10 days, 0.001, 0.002, 0.02 (1.6, 3.1 or 23.5 mg/kg bw per day)	Reduced B-cell numbers	0.001% (1.6 mg/kg bw per day)	0.02% (23.5 mg/kg bw per day)	50.8 µg/mL at 0.001% 340 µg/mL at 0.02%	Qazi et al. (2012)
PFOS, 98%	Balb/c mice (8 per group, males and females), gavage, 5, 20 mg/kg bw per day, 14 days	Thymus and spleen histopathology, but in addition to liver effects and PPARα changes	5	20	4.89 µg/mL at 5 mg/kg bw per day 25.2 µg/mL at 20 mg/kg bw per day	Wang et al. (2011a)
PFOS, 85%	Balb/c mice (5 females per group), gavage, 20 mg/kg bw per day, 7 days	Values various immune parameters reduced, but in addition to body weights	N/A	20	NR	Vetvicka and Vetvickova (2013)

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
PFOS, 98%	C57BL/6 mice (12 males per group), gavage, 1, 5, 10 mg/kg bw per day, 7 days	Inconsistent; some ex vivo apoptotic parameters in splenocytes and thymocytes up and others go down	1	5	24.37 µg/mL at 1 mg/kg bw per day 87.56 µg/mL at 5 mg/kg bw per day	Zhang et al. (2013d)
PFOS, 98%	C57BL/6 mice (12 males per group), gavage, 5, 20, 40 mg/kg bw per day, for 7 days	Reduced NK activity, antibody response, lymphocyte proliferation	N/A	5	97.25 µg/mL	Zheng et al. (2009, 2011)
PFOS, 85%	BALB/c mice (5 females per group), gavage, 20 mg/kg bw per day, 21 days	Reduced antibody response and NK activity, but accompanied by body weight effects	N/A	30	NR	Vetvicka and Vetvickova (2013)
PFOS	B6C3F1 mice (5 males per group), food, 0.25 mg/kg bw per day for 28 days	Effects on body weight and liver No immune effects	0.25	N/A	11.6 µg/mL	Qazi et al. (2010)
PFOS, 98%	B6C3F1 mice (5 females per group), gavage, 0.0331, 0.0993, 9.3 mg/kg bw per day, 28 days	TNFα and IL-6 up and down, but no dose-response	N/A	0.0331	NR	Mollenhauer et al. (2011)
PFOS, 98%	B6C3F1 mice (5–10 females), gavage, 3.31,	Increased ex vivo IL-6	N/A	3.31 µg/kg bw per day (0.00331 mg/kg bw	<1 ng/mL (LOQ)	Fair et al. (2011)

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
	16.6, 33.1, 166 µg/kg bw per day, 28 days			per day)		
PFOS, 98%	Sprague-Dawley rats (15 males or female per group), diet, 0.14, 1.33, 3.21, 6.31 mg/kg bw per day, 28 days	Reduced total IgG1 levels in serum	0.14	1.33	0.95 µg/mL at 0.14 mg/kg bw per day 13.45 µg/mL at 1.33 mg/kg bw per day	Lefebvre et al. (2008)
PFOS, 98%	B6C3F1 mice (5 males or females per group), gavage, 0.166, 1.66, 3.31, 16.6, 33.1, 166 µg/kg per day, 28 days	Reduced specific antibody Response (PFCs)	0.166 µg/kg per day (0.000166 mg/kg bw per day)	1.66 µg/kg bw per day (0.00166 mg/kg bw per day)	17.8n/ ng/mL at 0.000166 mg/kg bw per day 91.5 ng/mL at 0.00166 mg/kg bw per day	Peden-Adams et al. (2008)
PFOS	B6C3F1 mice (30 females per group), gavage, 5, 25 µg/kg per day, 21 days	Reduced survival after challenge with influenza virus	5 µg/kg per day (0.005 mg/kg bw per day)	25 µg/kg per day (0.025 mg/kg bw per day)	189 ng/mL at 0.005 mg/kg bw per day 670 ng/mL at 0.025 mg/kg bw per day	Guruge et al. (2009)

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
PFOS, 98%	C57BL/6 mice (10–12 males per group), gavage, 8.3, 16.7, 83.33, 416.7, 833.3 µg/kg bw per day for 60 days	Antibody responses down.	8.3 µg/kg per day (0.0083 mg/kg bw per day)	83.33 µg/kg bw per day (0.0833 mg/kg bw per day)	0.84 µg/mL at 0.0083 mg/kg bw per day 8.21 µg/mL at 0.0833 mg/kg bw per day	Dong et al. (2009)
PFOS, 98%	C57BL/6 mice (6 males per group), gavage, 8.3, 16.7, 83.33, 416.7, 833.3 µg/kg bw per day for 60 days	IL-4 and IL-10, TNF-a, IL-1b, values up and down. IgM decrease IgG, IgE increase	16.7 µg/kg per day (0.0167 mg/kg per day)	83.33 µg/kg per day (0.0833 mg/kg per day)	10.75 µg/mL at 0.0833 mg/kg per day 2.36 µg/mL at 0.0167 mg/kg per day	Dong et al., 2011
PFOS, 98%	C57BL/6 mice (12 males per group), gavage, 8.3, 16.7, 83.33, 416.7, 833.3 µg/kg bw per day for 60 days	Apoptotic lymphocytes,	0.0167 mg/kg per day	83.33 µg/kg per day (0.0833 mg/kg per day)	0.84 µg/mL at 0.0833 mg/kg per day NR at 0.0167 mg/kg per day	Dong et al., 2012
PFOS, 98%	C57BL/6 mice (4 per group), 6-8 weeks old, exposed to 2 mg PFOS/kg bw per day for a period of 7 days	IL 12 production after Citrobacter rodentium infection	N/A	2 mg/kg bw per day	NR	Suo et al., 2017

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
PFOS (purity NR)	ICR mice (number, sex NR), sensitized to ovalbumin on day 0, and orally exposed to 50-150 mg PFOS/kg bw on days 9, 11, and 13,.	Aggrevated allergic inflammation in an ovalbumin model.	N/A	50 mg/kg, three times	NR	Lee et al., 2018

bw: body weight; IgG: immunoglobulin G; IL: interleukin; NK: natural killer (cell); N/A: not applicable; NR: not reported; PFOS: perfluorooctane sulfonic acid; PPAR: peroxisome proliferator activated receptors; TNF: tumour necrosis factor.

9896 Table H.2 Immunotoxicity studies PFOA from 2018 opinion (EFSA CONTAM Panel, 2018)

9897

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
PFOA, 97%	BALB/c mice (5 females per group), 7 days, gavage, 20 mg/kg bw per day	Several immune parameters reduced, but also reduced body weight gain and increased liver weight	N/A	20	NR	Vetvicka and Vetvickova (2013)
PFOA, 98%	C57BL/6J mice (16 females), 10 days, drinking water, 0, 30 mg/kg bw per day. Followed by nil or exposure for 5 days	Reduced IgMantibody responses but enhanced IgG response. Also effects on body weight gain	N/A	30	NR	DeWitt et al. (2008)
PFOA, 98%	C57BL/6J mice (6 males), Drinking water for 15 days 3.75, 7.5, 15, 30 mg/kg bw per day	Reduced IgM antibody response	3.75	7.5	NR	Dewitt et al. (2009)
PFOA, 98%	C57BlL6N (6 females) drinking water for 15 days 0.94, 1.88, 3.75, 7.5, 30 mg/kg bw per day	Reduced Tcell dependent and T cell independent IgM antibody responses	1.88	3.75	NR	DeWitt et al., (2016)
PFOA, 98%	C57BL/6 mice (4–8 males per group), food for 10 days, 0.001 or 0.02% (2 or 40 mg/kg bw per day) (0.02% equals a total of	Values several immune parameters up or down, but also body weight gain	N/A	0.02% (40 mg/kg bw per day)	152 µg/mL	Qazi et al. (2009a, b)

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
	5.2 mg/animal over 10 days)					
PFOA, 98%	C57BL/6 mice (4 males per group), restricted food diet, 10 days, 0.001, 0.002 or 0.02% (1.6, 3.1 or 23.5 mg/kg bw per day)	Reduced B-cell numbers	0.002% (3.1 mg/kg bw per day)	0.02% (23.5 mg/kg bw per day)	87.6 µg/mL at 0.002% (3.1 mg/kg bw per day) 152 µg/mL At 0.02% (23.5 mg/kg bw per day)	Qazi et al. (2012)
PFOA, 96%	BALB/c mice (5 females per group), gavage, 20 mg/kg bw per day, 21 days	Various immune parameters reduced	N/A	20	NR	Vetvicka and Vetvickova (2013)
PFOA, 100%	ICR mice (5–6 males per group), drinking water, 21 days, 0.49, 2.64, 17.63, 47.21 mg/kg bw per day	Reduced CD8 levels	N/A	0.49	NR	Son et al. (2009)
PFOA, 100%	CD-1 mice (10–20 males), gavage, 0.3, 1, 10, 30 mg/kg bw per day, 29 days	Various immune parameters reduced, but also body weight gain, increased liver weight	1	10	27 µg/mL at 1 mg/kg bw per day 190 µg/mL at 10 mg/kg bw per day	Loveless et al. (2008)

Substance/ (Purity)	Species/Experimental design and doses	Most sensitive endpoints	Highest dose with no effect (mg/kg bw per day)	Significant effect level (mg/kg bw per day)	Serum/tissue levels of compound	Reference
PFOA, 100%	CD(SD) rats, gavage (10–20 males, 0.3, 1, 10, 30 mg/kg bw per day, 29 days	Body weight, haematopoiesis, but no immune effects	No immune effects	N/A	NR	Loveless et al. (2008)
PFOA	BALB/c mice (8–10 females per group, diet, 4 mg/kg diet at day 2 of gestation though to 12 weeks of age	Increased airway hyperresponsiveness	N/A	4 mg/kg diet	4.8 µg/mL	Ryu et al. (2014)

bw: body weight; N/A: not applicable; NR: not reported; PFOA: perfluorooctanoic acid.

Table H.3 Data provided by Peden-Adams on request of EFSA, showing the results from two independent studies, each with both female and male B6C3F1 mice treated with PFOA via gavage (n=5 per dose per sex). Studies were carried out in two subsequent years. The data show that the results were reproducible. Data on serum levels were only provided for the second study. TAD is the total applied dose over the 28-day study.

first experiment

	females						males					
TAD	serum (ng/g)			PFCs/million cells		% of	serum (ng/g)			PFCs/million cells		% of
(mg/kg bw)	n	mean	SD	mean	SD	control	n	mean	SD	mean	SD	control
0	5	ND		2631	814	100%	5			4641	879	100%
0.005	4	ND		1539	563	58%	5			4019	2026	87%
0.05	5	ND		1472	306	56%	5			1853	579	40%
0.1	5	ND		1422	550	54%	5			2225	567	48%
0.5	5	ND		766	267	29%	5			2006	296	43%
1	5	ND		891	548	34%	5			1016	437	22%
5	5	ND		750	166	29%	5			1416	618	31%

second experiment (published data)

	females						males					
TAD	serum (ng/g)			PFCs/million cells		% of	serum (ng/g)			PFCs/million cells		% of
(mg/kg bw)	n	mean	SD	mean	SD	control	n	mean	SD	mean	SD	control
0	5	17	4	3013	694	100%	5	12	5	3519	1848	100%
0.005	5	ND		2706	733	90%	5	18	4	2747	1211	78%
0.05	5	88	11	3250	514	108%	5	92a	22	1328	471	38%
0.1	5	123	19	2509	816	83%	5	131	15	1094	270	31%
0.5	5	666	108	1494	997	50%	5	ND		1413	467	40%
1	5	ND		1019	362	34%	5	ND		1488	323	42%
5	5	NR		791	364	26%	5	NR		1353	472	38%

ND: not done

NR: not reported, outside calibration curve

a: n=4

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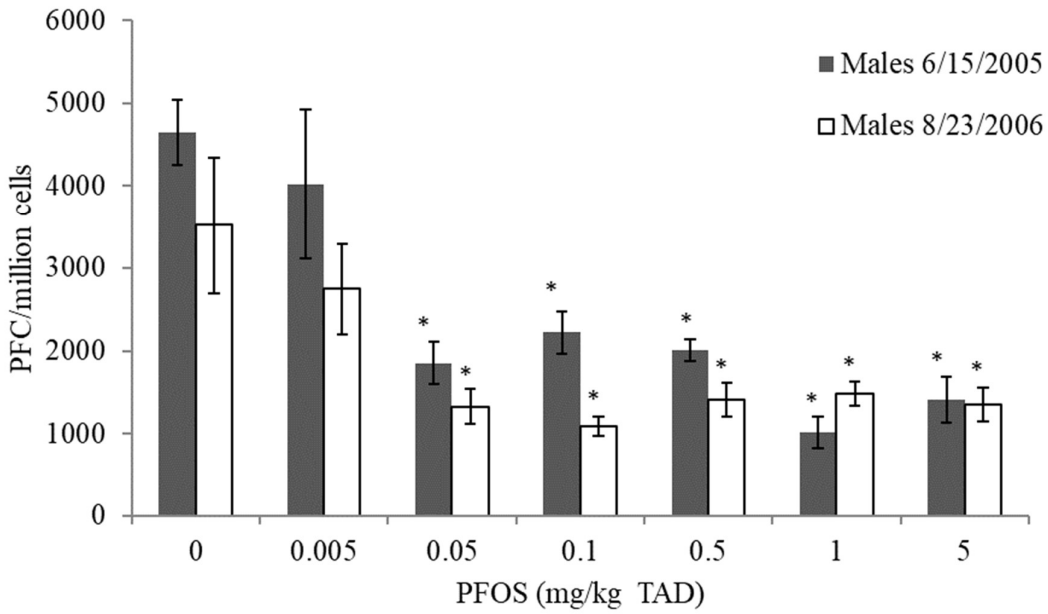

9905

9906 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). *
9907 significantly different from control ($p<0.05$). (Mean and SEM). Two independent experiments.

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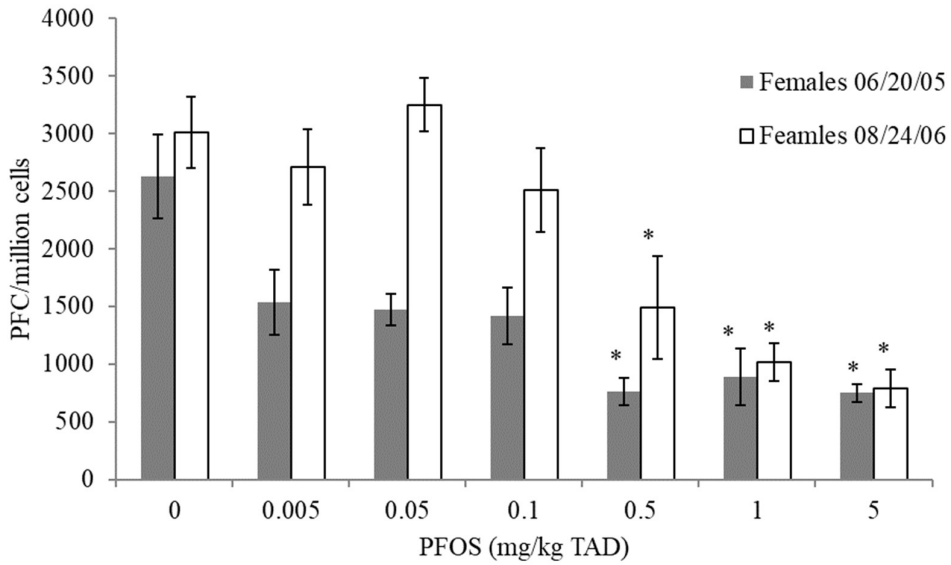

9909

9910 Figure H.2. PFC response in female 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). *
9911 significantly different from control ($p<0.05$). (Mean and SEM). Two independent experiments.

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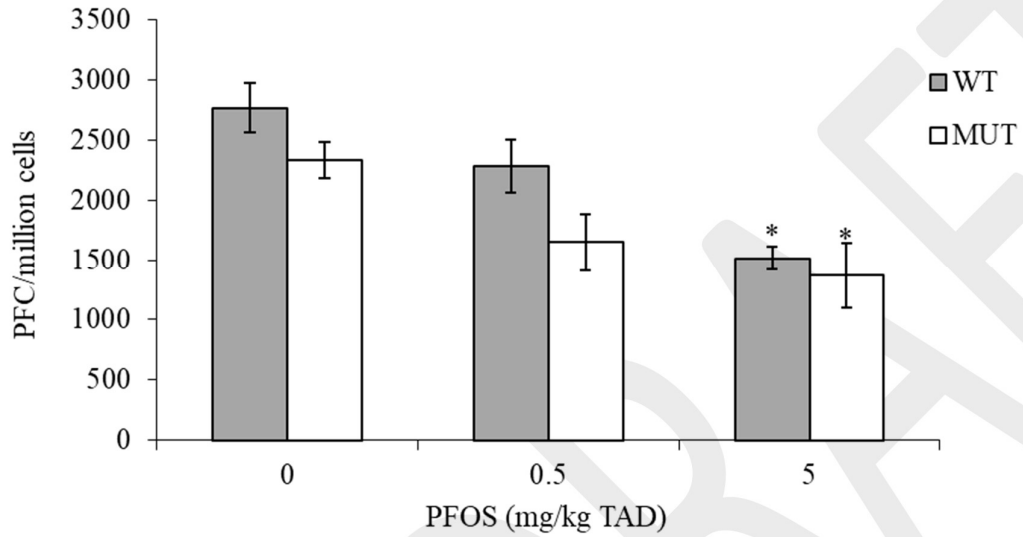

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9915 Figure H.3. PFC response in female C57Bl/6 mice (WT) and PPAR-alpha targeted mutation mouse model (MUT; Taconic), treated with PFOS for 28-d by
9916 gavage, with N=5 (mean, SEM). Samples were blinded to person reading slides. Doses of 0, 0.5 and 5 mg/kg bw (TAD).

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Appendix I – In vitro genotoxicity of PFASs tables

Table I.1 *Invitro* genotoxicity studies

Test system	Cells/animals	Concentration/ Treatment for genotoxicity endpoints	Result	Comment	Reference
In vitro					
Bacterial reverse mutation assay (Ames test)	S. typhimurium TA98,TA100, TA1535, TA1537, TA1538	**PFBA**: 20 µmol/plate (+/-S9)	Negative	Only highest applied non-cytotoxic dose dose shown; two independent experiments	Buhrke et al., 2013
		PFHxA: 20 µmol/plate (+/-S9)	Negative	Only highest applied non-cytotoxic dose dose shown; independent experiments	
		PFHpA: 10 µmol/plate (+/-S9)	Negative	Only highest applied non-cytotoxic dose dose shown; independent experiments	
		PFNA: 5 µmol/plate (+/-S9)	Negative	Only highest applied non-cytotoxic dose dose shown; independent experiments	
		PFDA: 5 µmol/plate (+/-S9)	Negative	Only highest applied non-cytotoxic dose dose shown; independent experiments	
		PFDoDA: 1 µmol/plate (+/-S9)	Negative	Only highest applied non-cytotoxic dose dose shown; independent experiments	

Test system	Cells/animals	Concentration/ Treatment for genotoxicity endpoints	Result	Comment	Reference
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98,TA100, TA1535, TA1537, WP2*uvr*A	**PFHxA**: 0, 333 – 5000 µg/plate (+/-S9)	Negative		Loveless et al., 2019
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98,TA100, E. Coli WP2 uvrA pKM 101	**PFHxA**: 10-750 µg/plate (TA98, TA100, +/-S9)	Negative		NTP, 2019a
		PFHxA: 100-2000 µg/plate E. Coli WP2 uvrA pKM 101, +/-S9)	Negative		
		PFNA: 50-500 µg/plate (TA98, TA100, +/-S9)	Negative		
		PFNA: 500-5000 µg/plate E. Coli WP2 uvrA pKM 101, +/-S9)	Negative		
		PFDA: 100-1000 µg/plate (TA98, TA100, +/-S9)	Negative		
		PFDA: 500-5000 µg/plate E. Coli WP2 uvrA pKM 101, +/-S9)	Negative		

Test system	Cells/animals	Concentration/ Treatment for genotoxicity endpoints	Result	Comment	Reference
		PFBS: 50-5000 µg/plate (TA98, +/-S9)	Equivocal		NTP, 2019b
		PFBS: 50-5000 µg/plate (TA100, +/-S9)	Negative		
		PFBS: 50-1000 µg/plate E. Coli WP2 uvrA pKM 101, +/-S9)	Negative		
Chromosomal aberration assay	Human peripheral blood lymphocytes	**PFHxA**: 0, 2000 – 3860 µg/mL (-S9), 4 h	Negative	No significant increase of structural or numerical chromosome aberrations activated test systems	Loveless et al., 2019
		PFHxA: 0, 250 – 1000, (+S9), 4 h	Negative		
		PFHxA: 0, 250 – 1000, (-S9), 22 h	Negative		
DNA strand breaks and FPG sensitive sites (Comet assay)	HepG2 cells	**PFHxA**: 0, 100-400 µM, 24 h	Negative	No significant increase in ROS production (DCFH-DA), 0.4-2000 µM, 3h; 400 µM for 24h resulted in a LDH release of ≤ 5%	Eriksen et al., 2010
		PFNA: 0, 100- 400 µM, 24 h	**Positive** ≥ 200 µM	No significant increase in ROS production (DCFH-DA), 0.4-2000 µM, 3h; 400 µM for 24h resulted in a LDH release of 66%.	

Test system	Cells/animals	Concentration/ Treatment for genotoxicity endpoints	Result	Comment	Reference
		PFBS: 0, 100- 400 µM, 24 h	Negative	No significant increase in ROS production (DCFH-DA), 0.4-2000 µM, 3h; 400 µM for 24h resulted in a LDH release of ≤ 5%	
DNA strand breaks (Comet assay	HepG2 cells	**PFHxS**: 0, 0.2 – 20 µM, 24 h	**Positive** 1, ≥ 10 µM	Cytotoxicity > 200 µM, 24 h; no clear dose dependency, positive for ROS (DCFDA fluorescence) ≥ 0.2 µM	Wielsoe et al., 2015
		PFNA: 0**,** 0.2 – 20 µM, 24 h	**Positive** 2, 20 µM	Cytotoxicity > 200 µM, 24 h; no clear dose dependency, positive for ROS (DCFDA fluorescence) 0.2, ≥ 20 µM	
		PFDA: 0, 0.2 – 20 µM, 24 h	Negative	Cytotoxicity > 200 µM, 24 h; positive for ROS (DCFDA fluorescence) 0.2, ≥ 20 µM	
		PFUnDA: 0, 0.2 – 20 µM, 24 h	Negative	Cytotoxicity > 200 µM, 24 h; positive for ROS (DCFDA fluorescence) ≥ 2 µM	
		PFDoDA: 0, 0.2 – 20 µM, 24 h	Negative	Cytotoxicity > 20 µM, 24 h; positive for ROS (DCFDA fluorescence) ≥ 2µM	
DNA strand breaks (Comet assay)	Human lymphoplastoid (TK6) cells	**PFNA:** 0, 125, 250 ppm, 2 h	**Positive** 125, 250 µg/ml	The authors state that cells are viable (as measured by trypan blue). However, data are not shown.	Yahia et al., 2014
8-OHdG (LC-MS/MS)	Human lyphopkastoid (TK6) cells	**PFNA:** 0, 125, 250 ppm, 2 h	**Positive** 125, 250 µg/ml	The authors state that cells are viable (as measured by trypan blue). However, data are not shown. 8-OHdG induction was greater than that produced by PFOA.	Yahia et al., 2014

Test system	Cells/animals	Concentration/ Treatment for genotoxicity endpoints	Result	Comment	Reference
Micronuclei (OECD 487)	V79 cells	**PFBA:** 100 µM (-/+S9), 3 h (+21 h)	Negative	IC_{50} (neutral red) > 1000 µM, 72 h	Buhrke et al., 2013
		PFHxA: 100 µM (-/+S9), 3 h (+21 h)	Negative	IC_{50} (neutral red): 344 µM, 72 h	
		PFHpA: 100 µM (-/+S9), 3 h (+21 h)	Negative	IC_{50} (neutral red): 128 µM, 72 h	
		PFNA: 10 µM (-/+S9), 3 h (+21 h)	Negative	IC_{50} (neutral red): 28 µM, 72 h	
		PFDA: 10 µM (-/+S9), 3 h (+21 h)	Negative	IC_{50} (neutral red): 15 µM, 72 h	
		PFDoDA: 1 µM (-/+S9), 3 h (+21 h)	Negative	IC_{50} (neutral red): 7 µM, 72 h	

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Appendix J – Human Observations

J.1 Fertility and Pregnancy Outcomes

Preterm delivery

Papers previously reviewed only for PFOS and PFOA (EFSA CONTAM Panel, 2018)

Maisonet et al. (2012) examined the association between maternal serum concentrations of PFHxS (median: 1.6 ng/mL) with gestational age or preterm delivery in 447 singleton female infants from the ALSPAC cohort. No differences in PFHxS concentrations were observed among preterm cases and non-cases. In a cohort of 638 pregnant women from Denmark, providing blood samples between gestation weeks 8 to 16, Lind et al. (2017) examined differences in maternal serum concentrations (medians) of PFOS (8.1 ng/mL), PFOA (1.7 ng/mL), PFHxS (0.3 ng/mL), PFNA (0.7 ng/mL) and PFDA (0.3 ng/mL) among preterm cases and non-cases. Non-significant differences were observed. Similarly, Hamm et al. (2010) found no association with preterm delivery for PFHxS (mean 2.1 ng/mL) among 252 pregnant women in Canada. Chen et al. (2012) examined, among 429 pregnant women from Taiwan, the association between cord blood concentrations (means) of PFNA (2.4 ng/mL) and PFUnDA (10.3 ng/mL) with preterm delivery. No associations were observed. Bach et al. (2016) examined associations between serum concentrations (medians) of PFHxS (0.5 ng/mL), PFHpS (0.2 ng/mL), PFNA (0.8 ng/mL), PFDA (0.3 ng/mL) and PFUnDA (0.3 ng/mL) in 1,507 primiparous women from Aarhus Denmark with preterm delivery. No associations were observed.

Time to pregnancy

Bach et al. (2015a) observed no association between maternal serum concentrations (medians) of PFHxS (0.5 ng/mL), PFHpS (0.2 ng/mL), PFNA (0.8 ng/mL), PFDA (0.3 ng/mL) and PFUnDA (0.3 ng/mL) with time to pregnancy, among 1,372 women from the Aarhus Birth Cohort (2008–2013). Serum samples were drawn during pregnancy prior to week 20 of gestation and time to the current pregnancy was recorded through questionnaires in early pregnancy.

In another study, 222 Danish women, planning their first time pregnancy (1992–1995), were followed for six menstrual cycles or until conception. No associations between pre-pregnancy serum concentrations (medians) of PFHxS (~1.2 ng/mL) and PFNA (~0.5 ng/mL), PFDA (~0.1 ng/mL), and time to pregnancy (Vestergaard et al., 2012) were observed.

Jørgensen et al. (2014) examined the relationship between serum concentrations (medians) of PFHxS (1.9 ng/mL) and PFNA (0.6 ng/mL) with time to pregnancy in 938 pregnant women from Greenland (48%), Poland (22%) and Ukraine (30%). Time to pregnancy in the current pregnancy was based on self-report and serum samples were drawn in the 2nd or 3rd trimester. PFNA was significantly associated with time to pregnancy of more than 13 months. No association was observed for PFHxS.

Velez et al. (2015) examined associations between maternal serum concentrations of PFHxS (median 1.0 ng/mL) and subfecundity and infertility (defined as time to pregnancy >12 months or infertility treatment for the current pregnancy) among 2,001 women recruited before week 10 of gestation in 10 cities across Canada in 2008–2011. Blood samples were drawn during the first trimester. Maternal PFHxS concentrations were associated with longer time to pregnancy, that was also reflected in increased odds of infertility (OR for infertility per 1-SD increase in PFHxS: 1.3 (95%: 1.1, 1.5)). Results stratified by parity were not reported in this study, and no adjustment was made for parity.

A cohort of 501 US couples who were followed over a 12-month period (Buck Louis et al., 2013) showed a significant association between maternal pre-pregnancy concentrations of PFOSA (mean: 0.1 ng/mL) and longer time to pregnancy.

As explained in the opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018), studies relying on samples drawn in the current pregnancy as proxy for pre-pregnancy exposure are prone to reverse causation. The one study relying on pre-pregnancy samples (Vestergaard et al., 2012) did not observe any association with time to pregnancy for PFHxS and PFNA. Longer time to pregnancy for PFOSA in the study by Buck Louis et al. (2013) needs to be confirmed in an independent setting before any reliable conclusions can be drawn. Based on the small number of studies reviewed above there is little to suggest that PFNA, PFHXs or PFOSA are associated with reduced fertility in terms of time to pregnancy.

Miscarriage

Using a case–control design nested within a prospective cohort of 2,874 women from Odense Denmark, Jensen et al. (2015) examined the association between serum concentrations (medians) of PFHxS (~0.3 ng/mL), PFNA (~0.7 ng/mL) and PFDA (~0.3 ng/mL) and the risk of miscarriage. Of the women recruited into the cohort, 88 suffered miscarriage and 56 of them had stored serum samples drawn prior to week 12 of gestation. These 56 cases were compared with 336 randomly selected controls from the cohort who also had serum drawn prior to week 12 of gestation. In addition, 51 miscarriage cases were compared to 204 controls that were matched on parity and gestational day of serum sampling. In serum samples drawn before week 12 of gestation (prior to miscarriage), higher serum concentrations were observed for miscarriage cases compared to non-cases for PFNA (1.16 vs 0.68. ng/mL) and PFDA (0.33 vs 0.26 ng/mL). In terms of risk, both compounds showed a dose-related increase in risk with an odds ratio of around 38 (95% CI: 10, 145) and 3.7 (95% CI: 1.6, 8.6) when comparing the highest to the lowest tertile of exposure to PFNA and PFDA, respectively.

Buck Louis et al. (2016) examined, in a cohort of 501 US couples, the association between pre-pregnancy serum concentrations of several PFASs and pregnancy loss. Concentrations of (mean or %<LOD) of Et-PFOSA-AcOH (97%<LOD), Me-PFOSA-AcOH (0.3 ng/mL), PFOSA (92%<LOD), PFDA (0.4 ng/mL) and PFNA (1.2 ng/mL) were quantified. Of these 501 women, 344 became pregnant and 98 of them suffered pregnancy loss. However, information on 24 out of the 344 pregnancies was missing due to loss to follow-up. Concentrations of Me-PFOSA-AcOH showed, by linear trend test, an inverse (protective) association with pregnancy loss. For other compounds no association with pregnancy loss was observed.

J.2 Developmental effects

PFHxS and PFNA

Neurodevelopment

Liew et al. (2014) studied the association between plasma PFAS levels in pregnant women and the risk of cerebral palsy (CP) in their children in a case-control study (156 CP cases and 550 controls) from the Danish National Birth Cohort (N=1400) recruited between 1996 and 2002. Cases were collected from a National CP register with validated diagnoses. A number of potential confounders were taken into account. Median PFHxS and PFNA levels were 0.9 and 0.4 ng/mL and not associated with risk of CP.

Liew et al. (2015) also performed a case-control study of attention deficit hyperactivity disorder (ADHD) and autism in the same cohort. Cases of ADHD (N=220) and autism (N=220) were randomly selected among cases identified in the cohort by linking with national disease registries, and 515 random controls were selected from the cohort, frequency-matched for sex. A number of potential confounders were adjusted for. Median PFHxS and PFNA levels in maternal plasma were 0.9 ng/mL and 0.4 ng/mL. Relative risks in the fourth quartile of PFHxS and PFNA were 0.6 (95% CI 0.4 – 0.7) and 1.6 (95% CI 1.2 – 2.1) for ADHD, respectively and 1.3 (95% CI 0.8 – 1.1) and 1.0 (95% CI 0.6 – 1.6) for autism, respectively.

Braun et al. (2014) examined associations between PFHxS and PFNA in pregnant mothers (N=175) in Ohio (the HOME study), and autistic behavior (a Social Responsiveness Scale, SRS, based on questionnaires to mothers) in their children at 4-5 years of age. The median levels were 1.6 ng/mL for PFHxS and 0.9 ng/mL for PFNA in the women (mostly at 16 weeks), recruited in 2003 – 2006. A number of potential confounders were adjusted for. There were no statistically significant associations between PFHxS or PFNA and results from the SRS scale.

The HOME cohort was also used to study associations between maternal serum PFHxS/PFNA (levels as above) and executive function (flexibility, goal planning, and information processing), as estimated from a parent-reported questionnaire (named BRIEF) in about 200 children at 5 and 8 years of age (Vuong et al., 2016). There were no consistent significant associations between maternal PFHxS or PFNA and executive functions in children.

In a Taiwanese cohort of 430 pregnant women, serum PFHxS and PFNA levels were measured in the third trimester (year 2000 – 2001) and then 120 children were followed up at 5 and 8 years of age for assessing IQ (Wang et al., 2015b). The median maternal serum PFHxS was 0.7 ng/mL and for PFNA the median was 1.4 ng/mL. After adjustment for a number of potential confounders, no statistically significant associations were found between maternal PFHxS/PFNA and children's IQ at 5 or 8 years of age.

Two Taiwanese birth cohorts were used to examine the association between PFNA in cord blood and ADHD-related symptoms (parental questionnaires) in 282 children at 7 years of age (Lien et al., 2016). The response rate for follow-up was low. The mean PFNA levels were 4.4 ng/mL. A number of potential confounders were adjusted for. Several scores for hyperactivity and inattention were positively associated with PFNA.

Oulhote et al. (2016) studied associations between maternal serum PFHxS and PFNA at the end of pregnancy and child behavior (parent-reported scales) in 539 Faroese children (born 1997 – 2000) at 7 years of age. In addition, associations between children's behavior and their PFHxS/PFNA levels (at age 5 and 7) were examined. No associations between behavioral problems and maternal (medians PFHxS 4.5 ng/mL, PFNA 0.6 ng/mL) or children's levels at age 7 (PFHxS 0.5, PFNA 1.1 ng/mL) were found. There was, however, a significant association between serum PFNA levels (but not PFHxS) in the children at age 5 and unfavourable behavioural changes (see Section 3.3.4.2).

Dutch children were examined by Quaak et al. (2016) for associations between the sum of PFASs (including PFHxS and PFNA) in cord blood and parental-assessed scales for ADHD and "externalizing behavior" (attention problems and aggressive behaviour) in 59 children. A number of potential confounders were taken into account. No adverse associations were found.

Growth in infancy and childhood, overweight, metabolic risk factors, and timing of puberty

Halldorsson et al. (2012) performed a longitudinal study of the association between PFNA in serum in 665 pregnant women, recruited in 1988-1989 from the Aarhus cohort, Denmark, and risk of overweight (BMI, waist circumference, insulin, leptin, and adiponectin) in their offspring at 20 years of age. The median PFNA level was 0.3 ng/mL. After adjustment for PFOA no significant associations with these outcomes were reported.

Maisonet et al. (2012) studied the association between prenatal PFHxS (serum levels in pregnant mothers; median 1.6 ng/mL) and birth weight and weight at 20 months in 448 girls from Avon, UK (ALSPAC study). The girls were selected for another study (of menarche, see Christensen et al. (2011) below). A number of potential confounders were adjusted for. Findings on birth weight are reported in Section 3.3.4.1.1. No association was found with maternal PFHxS.

A Faroese mother-child cohort (recruited 2007 – 2009) was used by Karlsen et al. (2016) to study the associations between maternal PFHxS and PFNA levels and overweight at 18 months and 5 years of

age. Median PFHxS and PFNA levels 2 weeks post-partum were 0.2 and 0.7 ng/mL. A number of potential confounders were adjusted for. There were no significant associations between these maternal PFAS levels and risk of overweight.

Associations between maternal plasma PFHxS/PFNA in early pregnancy and adiposity in children were examined by Mora et al. (2016) in a US birth cohort. BMI, waist circumference, and skinfold thickness were measured at about 3 and 8 years of age (1006, and 876 children, participation rate 61 and 53% of children with prenatal PFHxS/PFNA levels). At 8 years, also fat mass was estimated (using DXA). A number of potential confounders were adjusted for. Serum albumin and estimated pre-pregnancy eGFR were also taken into account. Median maternal PFHxS and PFNA levels were 2.4 and 0.6 ng/mL. No consistent associations were found between maternal PFHxS/PFNA and adiposity at about 3 years of age or adiposity in boys at about 8 years. In girls there were significant associations between PFHxS/PFNA and measures of adiposity at 8 years. Positive associations with PFHxS/PFNA remained after further adjustment for eGFR and albumin, but were attenuated when levels of several PFASs (PFOS, PFOA, PFHxS, PFNA) were all included in the models.

Fleisch et al. (2016) studied associations between prenatal (maternal plasma at about 10 weeks of gestation in 1999- 2002) PFHxS and PFNA and glucose homeostasis (glucose, insulin, HOMA-IR), leptin and adiponectin in about 500 children from Boston at the median age of eight years. A number of potential confounders were adjusted for. GM maternal PFHxS and PFNA were 2.5 and 0.7 ng/mL. Associations between prenatal PFHxS and PFNA and insulin resistance, leptin and adiponectin were null. As mentioned in section 3.3.4.6.2, associations examined cross-sectionally using PFASs in children's plasma showed an inverse association between PFNA and HOMA-IR.

Associations between prenatal PFHxS and PFNA exposure (serum levels in mothers at week 16) and adiposity at the age of 8 in 204 US children from the HOME study, were examined by Braun et al. (2016). GM for PFHxS and PFNA were 1.5 and 1.0 ng/mL. A number of potential confounders were adjusted for. There were no associations between PFHxS/PFNA and adiposity.

Using a nested case control design Christensen et al (2011) examined the association between maternal concentrations of PFHxS (median 1.6 ng/mL), PFNA (median 0.6 ng/mL), and FOSA (median 0.2 ng/mL) with age of menarche in the British ALSPAC cohort. They selected 218 early menarche cases, which reported age at menarche before 11.5 years (median 11.1 years) and 230 random controls (mean age at menarche 12.6 years). Age at menarche was assessed through self-reported questionnaires administered with 2-year intervals between the age of 8 to 13. Serum PFAS concentrations were quantified in archived serum samples taken during pregnancy (1991-1992). No significant association was observed with early age of menarche with odds ratios centered around 1.

In the same cohort, Hartman et al. (2017) studied the association between prenatal PFHxS and PFNA (serum levels in pregnant mothers; median 1.6 ng/mL and 0.5 ng/mL) and body fat (measured by DXA) at age 9 years in 359 girls from Avon, UK (ALSPAC study). The girls were selected for a study of early menarche. A number of potential confounders were adjusted for. No associations were found between body fat and maternal PFHxS or PFNA.

In a Spanish mother-child cohort (INMA) Manzano-Salgado (2017b) examined associations between maternal PFHxS and PFNA and various outcomes at age 6 months, and/or 4 and 7 years (BMI, waist circumference, blood pressure, blood lipids, and a cardiovascular risk score based on these factors) in their children (N=1086 – 1230). Maternal GM levels of PFHxS and PFNA were 0.6 and 0.7 ng/mL. No significant associations were found with BMI, waist circumference or blood pressure, or total cholesterol. The metabolic score at age 4 years was positively associated with maternal PFNA.

Other PFASs

Some of the above-mentioned studies also examined associations between the same outcomes as mentioned above and prenatal exposure to other PFASs than PFHxS and PFNA. This was the case for

PFDA (Liew et al., 2014, 2015; Vuong et al., 2016; Oulhoute et al., 2016; Wang et al., 2015, Karlsen et al., 2016, Fleisch et al., 2016, Berg et al., 2017), PFUnDA (Wang et al., 2015; Lien et al., 2016; Berg et al., 2017), PFDoDA (Wang et al., 2015), and PFHpS (Berg et al., 2017). None of these studies showed significant associations between prenatal exposure to PFDA, PFUnDA, PFDoDA or PFHpS and adverse outcomes.

J.3 Neurotoxic Outcomes

Associations between PFASs and Attention Deficit Hyperactivity Disorder (ADHD) and learning problems in children were investigated by Stein and Savitz (2011). This was a cross-sectional study in the C8 cohort exposed to PFOA from contaminated drinking water. Apart from levels of PFOS and PFOA, also PFNA and PFHxS were measured in serum in 2005-2006 in 10,000- 11,000 children aged 5-18 years. The median PFNA and PFHxS levels were 1.5 ng/mL and 5.2 ng/mL. Classification of ADHD was based on interviews (diagnosis made in the health sector, or medication against ADHD). A number of potential confounders were adjusted for. There was no association between PFNA and ADHD. The OR was significantly increased in the three upper quartiles of PFHxS (upper limit of Q1 = 2.9 ng/mL) with ORs of 1.3 - 1.5, based on about 1300 cases. The ORs were also increased in Q2 – Q4 (ORs 1.4 – 1.6) for ADHD with medication (about 500 cases). "Learning problems" were not more prevalent in the upper quartiles of PFNA or PFHxS.

In the C8 cohort, cross-sectional associations between PFNA and PFHxS and self-reported memory impairment were examined in 21,000 adults ≥50 years of age (Gallo et al. 2013). The GM PFNA and PFHxS levels in 2005 – 2006 were 1.4 and 3.2 ng/mL. A number of potential confounders were adjusted for. About 20% reported some memory impairment. Statistically significant inverse ("protective") associations were found between PFNA as well as PFHxS and memory impairment. The OR for memory impairment was 0.89 (95% CI 0.80 – 0.99) for Q5 versus Q1 of S-PFNA. For S-PFHxS the OR was 0.89 (95% CI 0.79 – 0.99). The authors discuss anti-inflammatory effects of PFNA/PFHxS (via PPARγ) or residual confounding as possible explanations.

Gump et al. (2011) studied the association between PFNA, PFDA, PFHxS, and FOSA, and impulsivity in a cross-sectional study of 83 children aged 9 – 11 years from north-west USA. Impulsivity was assessed by a procedure reinforcing delayed response in a computer task, which could be learned during the task. Median PFNA and PFDA levels were 0.7 and 0.3 ng/mL. Median PFHxS and FOSA levels were 3.7 and 0.6 ng/mL. A number of potential confounders were taken into account. Higher levels of these four compounds were inversely associated with the (reinforced) response, which was assumed to indicate less ability to inhibit the optimal delay in response, and thus increased impulsivity.

Power et al. (2013) performed a cross-sectional study on the associations between serum levels of PFNA and PFHxS and self-reported memory problems or confusion periods in about 1800 individuals, aged 60 – 85 years, from the U.S. NHANES 1999 – 2008. A number of potential confounders were taken into account. The GM for PFNA was 1.0 ng/mL and for PFHxS 2.1 ng/mL. The authors found no significant associations between PFNA or PFHxS and these cognitive symptoms. The OR for memory problems or confusion was 0.91 (0.79 – 1.04) for a doubling of serum PFNA and 0.93 (95% CI 0.82 – 1.06) for a doubling of serum PFOA. The OR for memory problems was significantly below 1.0 in some sub-analyses of diabetics both for PFNA and PFHxS.

Berk et al. (2014) studied the cross-sectional association between self-reported depressive symptoms and serum levels of FOSA, Me-PFOSA-AcOH, Et-PFOSAAcOH, PFBS, PFHxS, PFOS, PFHpA, PFNA, PFDA, PFUnDA, and PFDoDA in 5400 individuals >18 years in NHANES surveys 2005 – 2010. Levels are not presented. There were relatively strong inverse ("protective") associations between levels of PFNA, PFDA, and PFHxS and depressive symptoms after adjustment for some potential confounders: OR 0.62 (95% CI 0.42 – 0.92) for Q4 versus Q1 of PFNA, and OR 0.62 (95% CI 0.45 – 0.85) for Q4 versus Q1 of PFDA, and 0.66 (95% CI 0.47 – 0.93) for PFHxS.

As mentioned above, Oulhote et al. (2016) studied associations between PFHxS and PFNA at age 5 and 7 and child behavior (parent-reported scales) in 539 Faroese children (born 1997 – 2000) at 7 years of age. No associations between behavioral problems and children's levels at age 7 (PFHxS 0.5, PFNA 1.1 ng/mL) were found. There was, however, a significant association between serum PFNA levels (but not PFHxS) in the children at age 5 and unfavourable behavioral changes.

In a nested case-control from the Danish National Birth Cohort Long et al. (2019) examined associations between concentrations of PFHpS, PFBS, PFHxS, PFDS, PFOSA, PFHxA, PFHpA, PFNA, PFDA, PFUnDA, PFDoDA, PFPeA in amniotic fluid in relation to autism spectrum disorders (75 cases and 135 matched controls). Concentrations in amniotic fluid were much lower (<LOQ up to ~1 ng/mL for the median) compared to levels observed in maternal serum from the same cohort (Ernst et al. 2019). No increase in risk of autism spectrum disorders were observed. If anything, the associations suggested a modest reduction in risk.

J.4. Immune outcomes

Asthma and allergies in children and adults

Prospective studies – New publications not reviewed in the 2018 opinion (EFSA CONTAM 2018)

Manzano-Salgado et al. (2019) examined associations between maternal concentrations (means) of PFOS (6.1 ng/mL), PFHxS (0.6 ng/mL), PFOA (2.4 ng/mL) and PFNA (0.7 ng/mL) and certain outcomes. Blood samples were drawn during the first trimester. Information on the occurrence of lower respiratory tract infections, wheezing, asthma, and eczema was obtained by parental report at ages 1, 1.5, 4 and 7 years (n=1,071 to 1,188). Lung function was also assessed at age 4 years (n=992). Overall, no associations between PFASs and any of the outcomes reported were observed. PFOS was significantly inversely associated with eczema.

Based on the 641 mother-child pairs from the Environment and Childhood Asthma Study from Oslo, Impinen et al. (2018) examined the association between cord blood concentrations (medians) of PFOA (1.6 ng/mL), PFNA (0.2 ng/mL), PFUnDA (0.1 ng/mL), PFOS (5.2 ng/mL), PFHxS (0.2 ng/mL) and PFOSA (0.4 ng/mL) and offspring lung function at birth, and asthma and allergy at 2 and 10 years. Information on offspring asthma, allergy and lung function (spirmotetry test) at ages 2 and 10 years were based on parental report and clinical examination. Of the 100 different comparisons made for asthma and allergies (17 different outcomes with 6 substances), only four comparisons reached formal significance, which is not more than what would be expected by chance.

In a study from the Norwegian Mother and Child Cohort, Impinen et al. (2019) examined associations between maternal concentrations (median) of PFOS (12.3 ng/mL), PFOA (2.5 ng/mL), PFHxS (0.7 ng/mL), PFUnDA (0.2 ng/mL) and PFHpS (0.2 ng/mL) and offspring risk of allergies and asthma (doctor diagnosed and parental reports) at 3 years (n=1,270) and 7 years (n=972). Overall, no association of an association with asthma or allergies was observed.

In a Faroese birth cohort (1997-2000) of 559 mother-child pairs with prospective follow-up, Timmerman et al. (2017) examined the association between maternal concentrations of (medians) PFNA (0.6 ng/mL), PFDA (0.3 ng/mL) and PFHxS (4.2 ng/mL) and asthma and allergies in the offspring assessed by parental report at ages 5 and 13 years. Associations with immunoglobulin E and A (IgE, IgA) levels measured at age 7 years were also explored. In addition, the offspring's own concentrations at age 5 of (medians) PFNA (1.0 ng/mL), PFDA (0.3 ng/mL) and PFHxS (0.6 ng/mL) and age 13 years (similar median concentrations as at age 5) were examined in relation to asthma and allergies at ages 5 and 13 years. In short, neither maternal concentrations of PFNA, PFDA and PFHxS, nor the offspring's own concentrations at age 5 were associated with asthma, allergy or immunoglobulin levels at age 7 and no association was observed cross-sectionally at age 13. The authors also performed secondary analyses among those 22 subjects that had not been vaccinated to measles, mumps, and rubella prior to age 5 and observed elevated risk of asthma and allergy with

higher PFAS concentrations at age 5 and 13 years. Although interesting, those analyses were based on far too few subjects to allow for any meaningful conclusions.

In a cohort of 675 Norwegian adolescents, aged 13 to 19 years, Averina et al. (2019) examined both cross-sectionally and prospectively the association between concentrations (medians) of the sum of 18 PFASs (11 ng/mL), PFOA (2 ng/mL), PFOS (6.5 ng/mL) and PFHxS (1 ng/mL) and asthma and allergies. Outcome assessments were based on both self-report and clinical examinations. Cross-sectionally, both the sum of 18 PFASs and PFOS were positively associated (linear trend test) with self-reported doctor-diagnosed asthma. A positive but non-significant association was observed for PFOA and PFHxS. No consistent associations were observed for other PFASs. Prospectively, similar elevated odds ratios for asthma were observed for the sum of 18 PFASs and PFOS in a follow-up 3 years later, although a test for linear trend did not reach formal significance. In that follow-up, the sum of 18 PFASs and PFHxS were positively associated with eosinophilic airway inflammation (defined as FeNO >25 ppb). For all compounds, no consistent associations were observed for self-reported pollen allergy, food allergy and atopic eczema (cross-sectional analyses).

Prospective studies - Papers previously reviewed only for PFOS and PFOA (EFSA CONTAM Panel, 2018)

Wang et al. (2011) examined the association between serum cord blood concentrations (median) of PFNA (2.3 ng/mL)) with both cord blood levels (cross sectional) and offspring levels (prospective) of IgE and atopic dermatitis at age 2 years (n=244). No associations were observed in cross-sectional or prospective analyses.

Okada et al. (2014) examined 2,063 mother-child pairs from the Hokkaido Study on Environment and Children's Health (2003–2009) for associations between maternal concentrations (medians) of PFHxS (0.3 ng/mL), PFHxA (<0.1 ng/mL), PFHpA (<0.1 ng/mL), PFNA (0.9 ng/mL), PFDA (0.4 ng/mL), PFUnDA (1.0 ng/mL), PFDoDA (0.1 ng/mL), PFTrDA (0.3 ng/mL) and PFTeDA (<0.1 ng/mL) and allergic diseases in the offspring at 12 and 24 months postpartum. Allergic diseases were defined using a modified part of the Japanese version of the International Study of Asthma and Allergies in Childhood (ISAAC) and associations with allergic diseases and eczema were reported. No positive associations between any of the PFAS quantified and allergic disease or exzema were observed. If anything, the observed associations were in some cases inverse (protective).

In a cohort of 1,558 mother-child pairs form Japan with offspring follow-up at 4 years of age, Goudarzi et al. (2016) examined associations between pregnancy concentrations (medians) of PFHxS (0.3 ng/mL), PFNA (0.9 ng/mL), PFDA (0.4 ng/mL), PFUnDA (1.0 ng/mL), PFDoDA (0.1 ng/mL) and PFTrDA (0.2 ng/mL) and prevalence of allergic diseases in the offspring (wheezing, eczema and rhinoconjunctivitis symptoms). Prevalence of offspring allergy was assessed based on maternal report using a modified section of the Japanese version of the International Study of Asthma and Allergies in Childhood (ISAAC). No increase in risk for total allergic diseases or wheeze was observed with higher concentrations of these compounds. If anything, the associations were in the direction of being protective.

Cross sectional studies - Papers previously reviewed only for PFOS and PFOA (EFSA CONTAM Panel, 2018)

Dong et al. (2013) examined associations between serum concentrations (medians) of several PFASs, i.e. PFOS (~31 ng/mL), PFHxS (~2 ng/mL), PFBS (~0.5 ng/mL), PFOA (median ~1 ng/mL), PFDA (~1 ng/mL), PFDoDA (~3 ng/mL), PFHpA (~0.2 ng/mL), PFHxA (~0.2 ng/mL), PFNA (~1 ng/mL) and PFTeDA (~5 ng/mL), and asthma in 10 to 15-year-old Taiwanese children. The study recruited 231 children who had received doctor diagnosis of asthma in the previous year and 225 non-asthmatic controls. Significant and positive dose-response associations with asthma (p for trend<0.05) were observed for PFOA, PFDA, PFDoDA and PFNA. For these acids the odds ratios when comparing the highest to the lowest quartile ranged from 1.6 to 4.1. Significant associations were also observed for the three sulfonates, PFOS, PFHxS and PFBS (corresponding odds ratios ranging between 1.9 to 3.8).

Among asthmatic cases, mean serum concentrations of immunoglobulin E, absolute eosinophil counts and eosinophilic cationic protein concentrations also tended to increase across quartiles of PFAS concentrations. Severity of asthma also appeared to increase with higher concentrations. The Spearman correlation coefficient between individual PFASs ranged from 0.79 (PFDA and PFNA) to 0.02 (PFHpA and PFTeDA).

Using a subset (300 out of 456) of the children from this study, Qin et al. (2017) measured lung function by spirometry in 168 controls and 132 asthma cases. In line with previous findings from this cohort, positive associations between PFOS, PFHxS, PFOA, PFDA and PFNA and asthma were observed. In contrast to findings by Dong et al. (2013), no association was observed for PFBS and a positive association was observed for PFTeDA. Among asthmatic cases, PFOS, PFNA and PFHxS were associated with reduced pulmonary function in terms of forced vital capacity (FVC) and forced expiratory volume in the first second (FEV_1), while PFOA was only associated with FEV_1. Compared to their previous study, these inconsistencies may, at least partly, relate to lower participation rate.

Buser and Scinicariello (2016) examined in adolescents aged 12-19 years from the NHANES (2005–2006) study, the cross-sectional association between serum concentrations (medians) of PFHxS (2.1 ng/mL) and PFNA (0.9 ng/mL) with food sensitisation (defined as having at least one food-specific IgE level ≥0.35 kU/L) and and food allergies (self-reported yes to the question 'What foods are you allergic to')in 637-701 participants. Non-significant associations were observed between PFNA and PFHxS with food sensitisation and self-reported food allergies.

A US study examined associations between serum concentrations of (medians) PFNA (~1.0 ng/mL) and PFHxS (~4 ng/mL) with respiratory conditions among 458 New York State (NYS) employees and National Guard personnel working near the World Trade Centre after its collapse (Tao et al., 2008). Non-significant differences in concentrations for PFNA and PFHxS were observed among subjects classified as having symptomatic and asymptomatic respiratory conditions.

In a study of 12- to 19-year-old children and young adults (n=640) from the US NHANES, Stein et al. (2016) examined the associations between (means) PFNA (0.9 ng/mL) and PFHxS (2.1 ng/mL) with self-reported asthma (n=70), wheeze (n=70), allergy (n=102) and rhinitis (n=164). Sensitization to 19 different allergens was also measured (plants, dust mite, pets, cockroach, shrimp, rodents, mould and food). No consistent associations were observed between the different PFAS quantified and outcomes for asthma and allergies.

In a pregnancy cohort of 1,258 women, Ashley-Martin et al. (2015) examined the cross-sectional association between cord blood concentrations of PFHxS (mean:1.0 ng/mL) and Immunoglobulin E, thymic stromal lymphopoietin, and interleukin-33. No significant associations were observed.

J.4 Endocrine Effects

Thyroid Function and disease

PFHxS and PFNA

PFHxS and PFNA are the most studied compounds (apart from PFOS and PFOA). Therefore, they are summarised first (listed in Table J.1).

Adults

Bloom et al. (2010) compared serum PFHxS/PFNA levels, cross-sectionally, with levels of thyroid hormones in 31 New York anglers. No significant associations were found.

Chan et al. (2011) performed a cross-sectional study of pregnant (15 – 20 weeks) Canadian women subject to prenatal screening. Out of 974 maternal sera, the authors selected all women (N=96 'cases') with normal TSH and low (<10th percentile) free T4. Age-matched controls (N=175), were selected among women with normal TSH and free T4 levels between the 50th and 90th percentiles. PFHxS

was measured in these sera. The a priori hypothesis was that PFHxS would cause hypothyroxinemia (still with normal TSH) but no such association was found.

Ji et al. (2012) examined the association between serum concentrations of PFHxS/PFNA and total T4 and TSH among 633 Koreans aged <12 years. Median serum levels were 1.5 ng/mL for PFHxS and 2.1 ng/mL for PFNA. A number of potential confounders were adjusted for. No associations were found with TSH or T4.

Jain (2013) examined associations between PFHxS/PFNA (levels not reported) and thyroid hormones in 1540 individuals ≥12 years from the NHANES survey 2007 – 2008. Those with thyroid problems or medications, current pregnancy or missing variables had been removed. A number of potential confounders were taken into account. No associations were found with free T3, free T4 or TSH.

Wen et al. (2013) studied associations between PFHxS/PFNA levels and thyroid hormones in adults in US NHANES 2007 – 2010. Subclinical hyper- and hypothyroidism was defined from low and high serum TSH, respectively. There was a positive association between PFHxS and both hyper- and hypothyroidism but this was not supported by any association between PFHxS and TSH, with the latter as a continuous variable.

Wang et al. (2013b) examined the association between PFHxS/PFNA and TSH in 903 pregnant women (about 18th week of gestation) in the Norwegian Mother and Child cohort, MoBa. About half of the women had been selected due to subfecundicity. Those who reported previous thyroid disease were excluded. The GM of serum PFHxS and PFNA were 0.6 and 0.4 ng/mL, respectively. Several potential confounders were adjusted for. PFHxS was positively associated with free T4, but not with TSH. No associations were found between PFNA and thyroid hormones.

Lin et al. (2013a) examined associations between PFNA (GM 1.5 ng/mL) and thyroid hormones (TSH, free T4) in 567 Taiwanese individuals aged 12 to 30 years. A number of potential confounders were adjusted for. PFNA was positively associated with free T4, but not with TSH.

Wang et al. (2014d) performed a cross-sectional study of PFHxS/PFNA and thyroid hormones (TSH, T3, T4 and free T4) in 285 pregnant women (third trimester) in Taiwan with no known thyroid disease, and thyroid hormones were also measured in cord blood in 116 of their neonates. The median maternal PFHxS and PFNA concentrations were 0.8 and 1.5 ng/mL, respectively. Several potential confounders were adjusted for. In women, PFHxS was positively associated with TSH, but not with free T4, total T4 or total T3 and not with thyroid hormones in the newborns. PFNA was inversely associated with free T4 and total T4 but not with TSH. Maternal PFNA was inversely associated with total T4 and total T3, but not with free T4 or TSH.

Webster et al. (2014) examined associations between PFHxS/PFNA and thyroid hormones (free T4 and TSH) in 152 pregnant (measured twice in early 2nd trimester) Canadian nonsmoking women. The median serum PFHxS and PFNA levels were 1.0 and 0.6 ng/mL. Analyses were adjusted for week of gestation and presence of TPO antibodies (positive in 14 women, indicating possible autoimmune thyroid disease). There was a positive association between PFNA and TSH, but no association with free T4, and no associations between PFHxS and thyroid hormones.

Lewis et al. (2015) studied associations between PFHxS/PFNA and thyroid hormones (TSH, T4, free T4, T3, free T3) in 1682 individuals 12 – 80 years enrolled in NHANES 2011 – 2012. Median serum PFHxS and PFNA levels were, respectively, 0.8 – 1.8 ng/mL and 0.7 - 1.1 ng/mL (in various age groups in men and women). There were no overall associations between PFHxS/PFNA and thyroid hormones. In results stratified by sex and four age groups, there were a few significant findings for the most important hormones (TSH, free T4 and free T3): a positive association between PFNA and TSH in male adolescents, and a positive association between PFNA and free T4 in women 20 – 39 years. But many (24) such associations were tested.

Berg et al. (2015) examined associations between PFHxS/PFNA and thyroid hormones in 375 pregnant women in Norway. PFHxS/PFNA were measured around gestation week 18, and thyroid hormones (TSH, free T4, T4, free T3, T3), as well as thyroid hormone binding proteins were measured in the same samples, and also 3 days and 6 weeks postpartum. Median PFHxS and PFNA levels were 0.4 and 0.6 ng/mL. Some potential confounders were adjusted for and also binding proteins in repeated measures analyses (mixed effects models). No significant associations were found with hormone levels.

Berg et al. (2017) also reported on associations between PFHxS/PFNA and thyroid hormones in these pregnant women in Norway and TSH in their infants at birth. Heel prick TSH in infants was measured three days after birth. Some potential confounders were adjusted for. No significant associations were found with maternal thyroid hormones or infant TSH.

Webster et al. (2016) studied associations between PFHxS/PFNA and thyroid hormones (TSH, T4, free T4, T3, free T3) in 1525 adults from NHANES 2007 – 2008, excluding those with a history of thyroid disease. GM PFHxS and PFNA levels were 1.9 and 1.5 ng/mL. Several confounders were adjusted for, and results were also stratified for the presence of thyroid peroxidase antibodies (TPOab, present in 9%, suggesting possible autoimmune thyroid disease) and urinary iodine classified as low (< 100 µg/L) in 26%. In individuals with normal urinary iodine and no TPOab there were no associations between PFHxS/PFNA and thyroid hormones. However, in 26 individuals with TPOab and low urinary iodine, there were positive associations between PFHxS/PFNA and TSH, and free T3, and an inverse association with free T4 (PFHxS only). The authors suggest that the results could support a 'multiple hit' hypothesis with those who have TPO antibodies and low urinary iodine (about 1% of the US population) being a sensitive group.

In a study of 157 mother-infant pairs, Yang et al. (2016) examined cross-sectional associations between maternal concentrations of PFHxS/PFNA and maternal and infant thyroid hormones (TSH, free T4, T4, free T3, T3). Median maternal serum PFHxS and PFNA were 0.5 ng/mL for both compounds, while cord serum levels were 0.2 ng/mL for PFHxS and 1.2 ng/mL for PFNA. In analyses adjusted for potential confounders, there was an inverse association between maternal PFNA and maternal TSH. There were no associations between maternal or infant PFHxS/PFNA and infant thyroid hormones.

Crawford et al. (2017) studied associations between PFHxS/PFNA (GM 1.6 and 0.8 ng/mL) and thyroid hormones (in 99 US women attempting to conceive. There was a positive association between PFNA and free T4, but no association with TSH, and no association between PFHxS and thyroid hormones. Only age was adjusted for.

Li et al. (2017) examined associations between PFHxS (median 0.2 ng/mL) and thyroid hormones in 202 persons (mainly adults, but two infants and ten adolescents, which had been selected in order to include both participants with normal (N=62) and "abnormal" levels (N=140) of thyroid hormones (TSH, free T4 or free T3). In analyses adjusted for age and sex but not for region there were no significant associations between PFHxS and thyroid hormones.

In summary, for **PFHxS**, only one of 13 studies in adults showed an overall positive association with TSH (Wang et al. 2014d) and no studies showed any overall association with free T3 or free T4. In addition, in a small study subgroup with positive TPO antibodies and low iodine (Webster et al., 2016; N=26) there was a positive association with TSH, and free T3, and an inverse association with free T4. There were few studies in newborns/infants and no consistent findings. For **PFNA**, out of 12 studies in adults there was one study in pregnant Chinese women (Yang et al., 2016) sampled just before delivery that showed an inverse association with TSH, while a study in pregnant Canadian women (Webster et al., 2014) showed a positive association with TSH. In addition, the small study subgroup with positive TPO antibodies and low iodine by Webster et al., (2016) showed a positive association with both TSH and free T3. Two small studies (Lin et al., 2013a, Crawford et al., 2017)

showed positive associations with free T4, while another (Wang et al., 2014d) showed an inverse association. Most studies showed no significant associations. There were few studies in newborns/infants and no consistent findings.

Newborns and children

Lopez-Espinosa et al. (2012) examined cross-sectional associations between PFNA (median 1.5 ng/mL) with total T4 and TSH among about 12,000 children in the C8 cohort aged 1-17 years. A number of potential confounders were adjusted for. There was no significant association with parent-reported thyroid disease with serum PFNA, but the number of cases was small (N=39).

As mentioned above, Wang et al. (2014d) found no significant associations between PFHxS/PFNA in maternal serum and cord blood free T4 or TSH.

Shah-Kulkarni et al. (2016) examined associations between PFHxS/PFNA and thyroid hormones (TSH, T4, T3) in cord blood of 279 newborns from Seoul, Korea. GM levels were 0.4 (PFHxS) and 0.2 (PFNA) ng/mL. Several potential confounders were adjusted for. In girls, but not in boys, there was an inverse association between PFNA and TSH. There were no associations between PFHxS and TSH.

Kim et al. (2016) compared serum concentrations of PFHxS/PFNA between 27 cases of infants with congenital hypothyroidism and 13 control infants. Concentrations of PFNA (median: 1.9 versus 0.6 ng/mL) were significantly higher in the cases compared to controls, while PFHxS levels were similar (1.2 ng/mL for both groups). Maternal PFOS/PFOA levels were not available.

As mentioned above, Yang et al. (2016) found no associations between between maternal or infant levels of PFHxS/PFNA and infant thyroid hormones.

The above-mentioned studies are summarised in Table E.1.

Other PFASs

There are eleven studies on associations between PFASs other than PFOS, PFOA, PFHxS, and PFNA and thyroid disease or function, namely Bloom et al. (2010), Ji et al. (2012), Jain (2013), Wang et al. (2013), Lin et al. (2013a), Wang et al. (2014d), Berg et al. (2015), Shah-Kulkarni et al. (2016), Kim et al. (2016), Yang e al. (2016), and Li et al. (2017). For PFASs examined, see Table E.1. There were no consistent associations (reported in more than one study) between these other PFASs and thyroid disease or function.

Table J.1 Reports on associations between serum levels of PFHxS and/or PFNA and thyroid disease or thyroid hormones. In addition, the last column shows if also other PFASs were assessed (apart from PFOS and PFOA, see previous opinion (EFSA CONTAM Panel, 2018)).

Author	Population, country and number of subjects	Type	Serum PFNA/PFHxS levels (ng/mL)	Findings	Comments	Other compounds
Thyroid disease						
Lopez-Espinosa et al., 2012	C8, 12,000 children 1– 17 yrs	CS, L	Median PFNA 1.5	Null for hypo-or hyperthyroidism. PFNA vs. TT4 +, but null for TSH.	Few (39) cases. Adjusted for potential confounders.	-
Wen et al., 2013	NHANES, USA, 1181	CS	PFHxS: median 2.0	PFHxS vs. hypothyroidism + hyperthyroidism + in women only.	Adjusted for potential confounders. Few (24) cases. Diagnoses based	-

			PFNA: median 1.5	PFNA null. PFHxS vs. TT4, TT3, and FT3 + in women, but null vs. TSH.	on cut-offs for TSH. Not consistent with results on continuous scale.	
Thyroid hormones						
Bloom et al., 2010	Anglers, USA, 31	CS	PFHxS GM 0.75 PFNA GM 0.79	Null	Too small study to be informative.	PFDA, PFUnDA
Chan et al., 2011	Pregnant women, Canada, 96+175	CS, analysed as case-control	PFHxS GM 1.1	Cases: high FT4, Controls: normal FT4, Both: normal TSH. No association btw PFHxS and case/ctrl status.	Matched + further adjustment for potential confounders	-
Ji et al., 2012	Adults, Korea, 633	CS	PFHxS median 1.5 PFNA median 2.1	Null for TSH and TT4 for both compounds	Adjusted for potential confounders	PFDA, PFUnDA, PFDoDA, PFTrDA, PFHpS
Jain, 2013	NHANES, USA, ≥12 years, 1540	CS	PFHxS and PFNA measured but mean or median levels not reported	PFHxS vs. TT4 +, but null for FT3, FT4 and TSH for both compounds.	Adjusted for potential confounders.	Several, but data not presented
Wang et al., 2013b	Pregnant women, Norway, 903	CS	PFHxS GM 0.6 PFNA GM 0.4	Null for TSH for both compounds	Adjusted for potential confounders.	PFDA, PFUnDA, PFHpDA
Lin et al., 2013a	Young individuals, Taiwan, 567	CS	GM PFNA 1.0	PFNA vs. FT4 +, but null for PFHxS. Null for TSH vs. both compounds.	Adjusted for potential confounders.	PFUnDA
Wang et al., 2014d	Pregnant women, 285 newborns, Taiwan, 116	CS	PFHxS median 0.8, PFNA median 1.5	PFHxS: In women TSH +, but null for FT4, TT4, and TT3. In newborns null. PFNA: In women FT4 - and TT4 -, but null for TSH. In newborns – for TT3 and TT4, but null for FT4 and TSH.	Adjusted for potential confounders.	PFDA, PFUnDA, PFDoDA
Webster et al., 2014	Pregnant women, Canada, 152	CS	PFHxS median 1.0 PFNA median 0.6	PFNA vs. TSH + in all women and in 14 women with TPO	Adjusted for gestational week and TPO antibodies.	-

				antibodies. Null for FT4. PFHxS vs. TSH and FT4 null.		
Lewis et al., 2015	NHANES, USA, 1682, results by sex and age groups	CS	PFHxS: medians 0.8 – 1.8 PFNA: medians 0.7 – 1.1	Overall null for both compounds. Some significant findings in subgroups by age and sex)	Adjusted for potential confounders.	-
Berg et al., 2015	Pregnant women, Norway, 375	CS, but repeated sampling	PFHxS: median 0.4 PFNA: median 0.6	Null for both compounds vs. TSH, FT4, T4, FT3, T3.	Hormones measured three times. Adjusted for some potential confounders.	PFDA, PFUnDA, PFHpS
Webster et al., 2016	Adults NHANES, USA, 1525	CS	PFHxS GM 1.9 PFNA GM 1.5	No TPOab and normal U-I: Null for both compounds vs. FT4, TSH, T3, T4, FT4. TPOab and low U-I: Several sign. assoc for PFHxS and PFNA.	Adjusted for potential confounders. 26 out of 1525 had TPO antibodies and low U-iodine.	-
Shah-Kulkarni et al., 2016	Newborns, Korea, 279	CS	PFHxS GM 0.4 and PFNA 0.2 in cord blood	PFHxS and PFNA vs. TSH, T4, T3: overall and in boys null. In girls PFHxS vs. TT3 +, and PFNA vs. TSH -,	Adjusted for potential confounders.	PFPeA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA
Kim et al., 2016	27 infants with congenital hypothyroidism, 13 controls	CS	PFHxS: Medians 1.2 and 1.2 PFNA: Medians 1.9 and 0.6.	PFNA higher in cases. No difference for PFHxS.	No confounding adjustment Maternal levels not available.	PFBA, PFPeDA, PFHpDA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFHpS, PFDS
Yang et al., 2016	Mother-infant pairs, Beijing, 157	CS	PFHxS: median maternal 0.5, infant 0.2 PFNA: median maternal 0.5, infant 1.2	Maternal PFNA vs maternal TSH: - Infant PFHxS and PFNA vs: infant FT3, FT4, TSH: null	Adjusted for potential confounders.	PFDA, PFUnDA, PFDoDA
Crawford et al., 2017	Healty women in a time to	CS	GM PFHxS 1.6 and PFNA 0.8	PFNA vs. FT4 +, null for TT3, TT4, TSH.	Adjusted only for age.	-

	pregnancy study, US, 99			PFHxS vs. FT4, TT3, TT4 and TSH: null.		
Li et al., 2017	140 persons with "abnormal thyroid hormones" and 62 with normal levels, China	CS	Median PFHxS 0.2	PFHxS vs. FT4, FT3, TSH, antibodies: null	Unadjusted	PFPrPA, PFBA, PFPeDA, PFPrA, PFBS,
Berg et al. 2017	370 mother-infant pairs from Norway	CS	Median PFHxS 0.4 and PFNA 0.6	Maternal PFHxS and PFNA vs. infant TSH: null	Adjusted for some confounders and for other persistent OPs	PFDA, PFUnDA, PFHpS

CS: cross-sectional (study); FT4: free T4; FT3: free T3; GM: geometric mean; L: longitudinal (study); N: number of subjects; PFHxS: perfluorohexane sulfonic acid; PFNA: perflurononoic acid; T3: triiodothyronine; TT3: total T3, T4: thyroxine; TT4: total T4; TPO: thyroid peroxidase; TSH: thyroid stimulating hormone. A '+' sign denotes a positive association and a '–' denotes an inverse association

Male fertility and puberty

Di Nisio et al (2019) examined the association between exposure PFOA and fertility males from the Veneto region in Italy. The study was conducted within routine reproductive health survey and included 383 subjects (median age 18 y, range 18-24 y), which were clinically examined and provided semen samples. A Sub-set of these participants (n=212) were living in areas with known environmental PFOA contamination. To verify differences in exposure serum samples from 50 subjects from non-contaminated area (controls) and 50 subjects form the contaminated areas (exposed) were quantified. The median concentrations for PFOA among exposed versus controls was 7.4 and 4.7 ng/mL, respectively. For the full sample anogenital distance, testicular volume and penis length were smaller among subjects in the exposed versus controlled area ($p<0.001$). Sperm concentrations and subjects with % normal morphology were also lower among subjects form the exposed area. When restricting the analyses to subjects with quantified PFOA levels (n=50/50) similar differences were observed for genital development while significant differences were not observed for semen quality. Serum PFOA concentrations were also positively associated with testosterone. Even through these results suggests that residential exposure may affect semen quality this simple ecological comparison between subjects presumed to be exposed versus controls is prone to confounding. The exposure gradients between controls and exposed as reported from a sub-sample of study participants was also modest, and no associations based on actual individual measured concentrations were reported for comparison. Differences in penis length, testicular volume and anogenital distance reflect different genital development during earlier years and are difficult to interpret in relation to current adult exposure to PFOA.

In a sample of 105 Danish men from the general population Joensen et al. (2009) examined associations between several PFAS and semen quality and reproductive hormones. Serum PFAS concentrations were (medians in ng/mL) 6.6 for PFHxS, 0.2 for PFHpA, 0.8 for PFNA, 0.9 for PFDA, 0.1 for PFUnDA, 0.08 for PFDoDA, <0.01 for PFTrDA, and 0.06 for PFOSA. As reviewed previously (EFSA 2008) the sum of PFOS and PFOA was associated with reduced normal spermatozoa. No results were, however, reported for other PFAS. Concerning reproductive hormones, PFOSA was associated with higher testosterone concentrations while no associations were observed for other PFASs.

Toft et al. (2012) examined associations between PFHxS (1.1 ng/mL) and PFNA (1.2 ng/mL) and semen quality in a cohort of 588 men from Greenland, Poland and Ukraine. Overall no consistent associations with semen quality were observed.

In cross-sectional analyses among 103 Chinese males living in an area with high industrial activity Song et al (2018) examined correlations between several PFAS measured in blood and semen quality. Blood concentrations (medians in ng/mL) were 3.0 for PFBA, 0.6 for PFPrA 3.0 for PFPeA, 29 for PFHxA, 0.2 for PFHBS, 0.4 for PFHpA and 3.9 for PFHxS. PFAS concentrations in blood and semen were not correlated with sperm concentrations. Blood concentrations for individual PFAS showed varying concentrations (positive and inverse) with progressive sperm motility. Overall no consistent associations were observed between PFBA, PFPrA, PFPeA, PFHxA, PFHBS, PFHpA and PFHxS and semen quality.

In a sub-cohort within the Danish National birth cohort Ernst et al. (2019) examined the associations between maternal concentrations of PFAS and pubertal development in 455 mother-child pairs. Median (in ng/mL) serum concentrations (1st or 2nd trimester samples) were ~1 for PFHxS, ~0.5 for PFNA and ~0.2 for PFDA. Outcomes assessment was based on self-report and covered questions on Tanner stages 2-5 for pubic hair, breast and genital development, as well as axillary hair growth and acne. In addition, information on age at first ejaculation and voice break (for boys) and age at menarche (for girls) was reported Overall maternal concentrations of different PFAS appeared to suggest slightly slower pubertal development in both boys and girls. However, few associations reached formal statistical significance, consistency with different outcomes measures was in many cases absent, and clear dose response was not observed.

Female fertility, menstrual cycle and puberty

In a cohort of 495 women aged 18-44 scheduled for laparoscopy in Salt Lake City US, Buck Louis et al. (2012) examined associations between PFNA, PFDA and PFHxS and endometriosis (mean or median concentrations not reported). The same associations were also examined among 131 women from the same area who were not seeking clinical care. After adjustment for covariates a non-significant increased risk was observed for PFNA and PFDA among women seeking clinical care (n=495). In the smaller sub-sample of women not seeking clinical care (n=131), similar non-significant associations were also observed but with much more imprecise/wide confidence intervals.

With a case-control design, Zhang et al. (2018) examined associations between (medians in ng/mL) PFNA (~2), PFDA (1.8), PFUnDA (1.3), PFDoDA (0.2), PFHpA (0.2), PFHxS (0.3) and PFBS (0.05) and primary ovarian insufficiency among 120 diagnosed cases and 120 healthy controls. A significant and positive association was observed between PFHxS and primary ovarian insufficiency. No association was observed for other PFASs. Associations between individual PFASs and reproductive hormones (FSH, luteinizing hormone, estradiol and prolactin) were also explored among cases only. In those analyses an inverse association was observed between PFHxS and estradiol.

In a cohort of 2731 women form the NHANES study Taylor et al. (2014) examined associations between PFNA (median: ~1.1 ng/mL) and PFHxS (median: 1.3 ng/mL) with age at natural menopause among women aged 20-65 years. Positive association with PFHxS and PFNA for age at natural menopause was observed. Much stronger association was also observed between these two substances and rate of hysterectomy. In addition, the authors noted that the concentrations of these two substances increased with time since natural menopause. Those findings strongly suggest that the association between PFNA and PFHxS and natural age at menopause are driven by reverse causation.

Singer et al. (2018) examined in a cohort of 1977 Norwegian pregnant women, the associations between several PFAS and menstrual cycle characteristics. Medians (in ng/mL) levels of PFAS in maternal serum drawn around gestation week 17 was 0.33 for PFNA, 0.09, 0.13 for PFDA, 0.21 for PFUnDA, 0.65 for PFHxS, and 0.15 for PFHpS. Overall no consistent associations were observed between individual PFASs and irregular menstrual cycles.

Using a case-control design, Heffernan et al. (2018) examined the associations between several PFAS and polycystic ovarian syndrome (PCOS) among 29 cases and 30 controls matched for age and BMI. Mean serum concentrations (in ng/mL) were 1.0 for PFHxS, 0.6 for PFNA and 0.41 for PFDA. No associations were observed for these substances. In this study associations with biomarkers of glucose homeostasis (fasting glucose, insulin HbA1 and HOMA-IR) and reproductive hormones (testosterone, SHBG, FAI, androstenedione and oestradiol) were also explored for cases and controls separately. Some inconsistent associations were observed among cases and controls. Given the modest statistical power (n~30) limited conclusions can be drawn.

Using a case-control design Wang et al. (2019b) examined the associations between PFASs and polycystic ovarian syndrome (PCOS) in Chinese women. A total of 180 infertile PCOS cases and 187 healthy controls were recruited into the study. Medium (ng/mL) plasma PFAS concentrations were 0.11 for PFBS, 0.08 for PFHpA, 0.24 for PFHxS, 0.52 for PFNA, 0.45 for PFDA, 0.40 for PFUnDA, 0.24 for PFDoDA and 12.16 for the sum of all PFASs, including PFOS and PFOA. PFDoDA was significantly associated with increased odds of PCOS-related infertility, while PFUnDA showed a significant inverse association.

With a nested design the UK ALSPAC cohort was used by Christensen et al. (2011) to examine the association between different PFAS and age at menarche. Maternal pregnancy samples among 218 female cases who had age at menarche before the age of 11.5 y and 230 randomly selected controls were quantified. Median concentrations (in ng/mL) in pregnancy serum were 0.2 for PFOSA, 0.6 for Et-PFOSA-AcOH, 0.4 for Me-PFOSA-AcOH, 19.8 for PFOS, 1.6 for PFHxS, 3.7 for PFOA, 0.6 for PFNA. Overall no consistent associations with age at menarche were observed.

Regarding puberty in girls, see also the study by Ernst et al. (2019) in section 3.3.4.5.2.

Other endocrine effects

In a cohort of 349 Chinese pregnant women, Yao et al. (2019) examined cross sectional associations between several PFAS in cord blood and reproductive hormones (estradiol and testosterone) and steroidogenic enzymes (P450arom, 3β-HSD1, 17β-HSD1). Median cord blood concentrations (ng/mL) were 0.2 for PFBS, 0.2 for PFDA, 0.1 for PFDoDA, 0.1 for PFHpA, 0.2 for PFHxS, 0.3 for PFNA, 0.1 for PFOSA, and 0.1 for PFUnDA. PFHxS was positively associated with estradiol while PFUnDA and PFNA were positively associated with testosterone. PFHxS was positively associated with P450arom, 3β - HSD1 and 17β-HSD1. Sum of all PFASs (incl. PFOS ~1 ng/mL and PFOA ~36 ng/mL), PFUnDA, PFNA and PFDA also showed a positive association with P450arom. On their own, the biological relevance of these cross-sectional association in cord-blood is far from clear. Replication in an independent data would be needed to draw any meaningful conclusions on causality.

In a study of 80 mother child pairs form the Faroese Islands, Shelly et al. (2019) examined the association between maternal and childhood (age 5, 7 and 9 y) exposures to PFHxS, PFDA and PFNA (median or mean concentrations not reported) and adipokine hormone levels at these same ages. For these substances no consistent associations were observed.

Limited conclusions can be taken on the basis of these two studies.

J.5 Metabolic Outcomes

Diabetes

Lin et al. (2009) examined cross-sectional associations between PFOS, PFOA, PFNA (GM 0.7 ng/mL), and PFHxS (GM 2.6 ng/mL) and glucose homeostasis and metabolic syndrome in 1443 adults and adolescents (12 – 19 years) from US NHANES samples 1999 – 2004. Data were available on fasting plasma glucose and insulin, and beta cell function and insulin resistance were assessed. Plasma glucose, blood lipids, waist, blood pressure and medications were used to define metabolic syndrome.

There was an inverse association between PFNA and metabolic syndrome in adolescents, but null findings in adults. Also for PFHxS the point estimates for the odds ratio of metabolic syndrome were below 1.

The study by Nelson et al. (2010) (see also Section 3.3.4.6.1. above), found no consistent associations between PFNA (median 1.0 ng/mL) or PFHxS (median 1.8 ng/mL) and body weight, BMI or insulin resistance (HOMA-IR).

The study by Lin et al. (2011) (see Section 3.3.4.6.1) found no consistent associations between PFNA or PFUnDA and indicators of metabolic syndrome, but few details were given.

The study by Fisher et al. (2013), see Section 3.3.4.6.1, found no consistent associations between PFHxS and indicators of metabolic syndrome.

Lind et al. (2014) studied the association between diabetes, insulin secretion, and insulin resistance, and serum levels of PFOS, PFOA, PFHpA, PFNA, PFUnDA, PFHxS, FOSA (all of these with detection rates >90%; seven other PFASs were determined but had lower detection rates and were not considered). For PFNA, but not for the other PFASs, there was a significant (nonlinear) association with prevalent diabetes. There were no associations between PFASs and insulin resistance.

A cross-sectional study of associations between PFOS, PFOA, PFNA (mean 3.8 ng/mL) and PFUnDA (mean 6.4 ng/mL) and glucose homeostasis among 571 Taiwanese adults from outpatient cardiology clinics (but free of self-reported coronary heart disease, stroke or diabetes) was reported by Su et al. (2016). The odds ratio for diabetes was decreased with increasing PFNA and PFUnDA (i.e. "protective" association).

The association between PFOS, PFOA, and PFHxS (GM 1.0 ng/mL) levels in early pregnancy and development of GDM and impaired glucose tolerance (IGT) at the end of pregnancy was examined in a prospective study of 1259 women in Canada (Shapiro et al., (2016). The odds ratio for IGT and GDM was increased in quartile 2 of PFHxS, but not significantly increased in Q3 or Q4.

Cardenas et al. (2017) examined associations between PFNA (GM 0.5 ng/mL), PFHxS (GM 2.4 ng/mL) N-methyl-perfluorooctane sulfonamido acetic acid (Et-FOSA-AcOH) (GM 1.1 ng/mL), N-methyl-perfluorooctane sulfonamido acetic acid (Me-FOSA-AcOH) (GM 0.9 ng/mL), and glycemic indicators at baseline and during 5 years of follow-up in 957 individuals with high risk of diabetes. PFHxS, Et-PFOSA-AcOH, and Me-PFOSA-AcOH were positively associated with insulin resistence (HOMA-IR) and insulin response (HOMA-beta), and HbA1c at baseline, but three were no associations between baseline levels of any of the afore-mentioned PFASs and incident diabetes changes or in changes in glycemic indicators during follow-up.

J.6 Kidney and Uric acid

The association between PFHxS and PFNA and eGFR (Schwarz formula) was examined in 9660 children and adolescents aged 1 – 18 years (mean 12 years) from the C8 cohort by Watkins et al. (2013). The median serum levels of PFHxS and PFNA were 5.2 and 1.5 ng/mL in 2005 - 2006. There was a significant inverse association with eGFR both for PFHxS and PFNA. For PFNA most of this difference was found already between Q1 and Q2. The change in eGFR per interquartile range of PFHxS and PFNA was about 1 mL/min/1.73 m^2, which was similar to what was found (per IQR) for PFOS and PFOA. When the analysis for PFOA was repeated but using estimated S-PFOA (from drinking water), there was no association between estimated S-PFOA and eGFR. The authors conclude that reverse causation is a likely cause of the association between (measured) S-PFOA and eGFR, i.e. that lower GFR causes higher S-PFOA, rather than vice versa, and this argument is valid also for PFHxS and PFNA.

Kataria et al. (2015) studied, cross-sectionally, the association between PFHxS/PFNA and kidney function (and uric acid) among 1960 adolescents in the US NHANES 2003 – 2010. Median PFHxS and

PFNA levels were 2.0 and 1 ng/mL. A number of potential confounders were adjusted for. No significant associations were found with eGFR (Schwartz formula) or uric acid.

Lin et al. (2013b) examined associations between PFNA and PFUnDA and serum uric acid in a cross-sectional study of 644 young people from Taiwan, who had been subject to a mass urine screening. The focus of the study was carotid intima-media thickness. Most of them were in the age 20 – 30 years and the GM PFNA and PFUnDA levels were about 1.1 and 5.9 ng/mL. No significant associations were found between PFNA/PFUnDA and urinc acid in adjusted models including a number of potential confounders.

In the cross-sectional study by Gleason et al. (2015), mentioned above in the Liver section (Section 3.3.4.6.3), associations between serum PFHxS/PFNA and serum uric acid were examined in about 4300 individuals from US NHANES surveys 2007 – 2010. The median levels of PFHxS and PFNA were 1.8 ng/mL and 1.2 ng/mL. A number of potential confounders were adjusted for. A significant positive association was found between ln serum PFNA (but not ln PFHxS) and uric acid.

Associations between a number of PFAS (PFBS, PFHxS, PFHxA, PFNA, PFDA, PFDoDA, PFTeDA), and serum uric acid were examined in a cross-sectional study of 225 Taiwanese children (12 – 15 years) by Qin et al. (2016). There was a significant positive association between ln serum PFHxS (but not the other PFASs) and uric acid in a model adjusted for potential confounders.

J.7. Carcinogenicity outcomes

In a nested case-control study frrm France, Mancini et al (2019) examined the association between serum PFOS (median ng/mL) and PFOA (median ng/mL) with breast cancer risk. Blood samples were drawn between 1994-1999 and cancer cases were prospectively identified until 2013. PFOS and PFOA were not associated with overall breast cancer risk (all cases). PFOS was, however, positively associated with estrogen receptor-positive and progesterone receptor-positive tumors. However, the effect estimates did not indicate a clear dose response as all estimates were similarly elevated above the referent quartile. No consistent associations were observed for PFOA.

In another nested case-control design Cohn et. al. (2019) examined the association between pregnancy concentration of PFAS with breast cancer risk in the female offspring after 54 years of follow-up. Out of 9300 daughters born in 1959 to 167 archived maternal samples drawn during pregnancy whose daughters had been diagnosed (cases: n=102) and matched controls (n=310) were selected. Maternal concentrations were (median in ng/mL) 0.3 for EtFOSAA, and 2.0 for PFHxS. No association with breast cancer was found for these two substances. In a sub-set of women with high maternal cholesterol, EtFOSAA was positively associated with later offspring risk of breast cancer. Without further replication this study provides limited evidence for a link between in utero exposure to EtFOSAA and PFHxS and later breast cancer risk.

In a nested case-control (902 breast cancer cases and matched 858 controls) design of US women Hurley et al (2018) examined association between baseline concentrations (median in ng/mL) of PFNA (0.9), PFUnDA (0.1), PFHxS (1.6) and MeFOSAA (0.2) and later breast cancer risk over 10-19 years of follow-up time. No increased risks of breast cancer were observed. If anything, the observed associations tended to show a modest reduction in risk.

J.8 Cardiovascular disease and mortality

Lin et al. (2013b) studied, cross-sectionally, the association between intima-media thickness (as a measure of atherosclerosis) in the carotid artery (CIMT) and serum levels of PFNA and PFUnDA (in addition to PFOS and PFOA) in 644 individuals from Taiwan, aged 12 – 30 years, recruited at a mass-screening of urine for glucose and protein. S-PFNA and S-PFUnDA were about 1.1 and 5.9 ng/mL. No positive associations were found between PFNA or PFUnDA and CIMT after adjustment for potential confounders Similar results were found be the same authors (Lin et al., 2016) when analysing some additional subjects and performing some subanalyses.

Mattson et al. (2015) performed a case-control study (nested in a Swedish cohort) of the association between several PFAS (apart from PFOS/PFOA): PFHpDA (median at recruitmen 1990 – 1991 0.05 ng/ml), PFNA (median 0.5 ng/mL), PFDA (median 0.2 ng/mL), PFUnDA (median 0.2 ng/mL), PFDoDA (median 0.02 ng/mL), and PFHxS (median 1.6 ng/mL) and incident coronary heart disease (CHD, fatal or non-fatal). Cases and age-matched controls (231 pairs) were obtained from a cohort of 1782 men living in rural Sweden. For part of the age-control pairs still alive, blood samples were collected also in 2002 – 2003, and then levels had decreased about 10%. An association with risk of CHD was found only for PFHpA (OR for Q3 of S-PFHpA 2.6 (95%CI 1.4 – 4.8) and for Q4 OR=1.7, 95% CI 0.9 – 3.2).

Lind et al. (2017) studied cross-sectional associations between several PFASs (apart from PFOS and PFOA): PFHxS (mean 2.1 ng/mL), PFHpA (mean 0.05 ng/mL), PFNA (mean 0.7 ng/mL), PFDA (mean 0.3 ng/mL), PFUnDA (mean 0.3 ng/mL), and PFOSA (mean 0.1 ng/mL) in serum in about 1000 elderly Swedish individuals (see Lind et al. 2014, Section 3.3.4.6.2 on diabetes, obesity, and metabolic syndrome. The authors found a positive association between ln PFNA and echogenicity (a marker of plaque vulnerability) of the intima-media complex, but only in women, and a positive association between PFUnDA and prevalence of carotid atherosclerotic plaques, but again only in women.

Bao et al. (2017) examined associations between PFASs in serum and blood pressure among 1612 adult government employees or residents in Shenyang, China. Apart from PFOS and PFOA, also PFBA (0.2 ng/mL), PFHxDA (0.03 ng/mL), PFNA (median 1.2 ng/mL), PFDA (median 0.8 ng/mL), PFUnDA (median 0.5 ng/mL), PFDoDA (median 0.1 ng/mL), PFTrDA (median 0.4 ng/mL), PFTeDA (median 0.1 ng/mL), and PFHxS (median 0.7 ng/mL) could be quantified in >50% of the participants. After adjustment for relevant potential confounders, statistically significant positive associations were found between PFNA and PFBA and prevalence of hypertension as well as blood pressure. For a three-fold increase in PFNA the adjusted OR was 1.2 (95% CI 1.04 – 1.36). For PFBA the corresponding adjusted OR was 1.1 (95% CI 1.04-1.17). Adjusted models indicated increase in systolic and diastolic blood pressure with 3 mm Hg for a three-fold increase in PFNA and 1 mm Hg for PFBA. Associations with hypertension and blood pressure were stronger (and statistically significant) for PFOS and PFOA.Huang et al. (2018) studied associations between a number of PFASs in serum and self-reported physician-diagnosed occurrence of cardiovascular disease (about 1200 cases) in seven rounds of the US NHANES (1999 – 2014) including about 11 000 participants. Apart from PFOS and PFOA, PFHpA (median 0.2 ng/mL), PFNA (median 1.0 ng/mL), PFDA (0.2 ng/mL), PFUnDA (0.2 ng/mL), PFDoDA 0.1 ng/mL), PFHxS (1.6 ng/mL) could be quantified in >50% of participants. A large number of potential confounders and cardiovascular risk factors (including serum cholesterol) were adjusted for. There were significant associaitons between PFNA, PFUnDA, and PFDoDA and the odds ratios for any cardiovascular disease. This was also the case when all PFASs (including PFOS and PFOA) were summed up. Regarding specific cardiovascular diseases, positive associations were found for coronary heart disease and associatoons were also found between PFASs and cardiovascular risk factors, such as serum cholesterol.

Mastrantonio et al. (2018) studied mortality in an ecological study of 24 municipalities with drinking water contaminated by PFASs (from a manufacturing company operating since 1964), and 56 municipalities with non-contaminated drinking water, all of them in the Veneto region of Italy. Selection of municipalities with contaminated drinking water was based on water levels of PFOS>30 ng/L, PFOA>500 ng/L or other PFAS>500 ng/L, while municipalities with uncontaminated water had PFAS levels <LOQ = 10 ng/L. Other PFASs than PFOS and PFOA included PFBA, PFPeDA, PFHxA, PFHpDA, PFNA, PFDA, PFUnDA, PFDoDA, PFBS, and PFHxS. The contaminated municipalities had 144 000 inhabitants and the uncontaminated had 588,000 inhabitants. Age- and sex-standardized mortality (SMR) rates for 1980 – 2013 were based on mortality for Italy. The specific causes of death examined were selected a priori, based on previous literature. Apart from sex and age, no individual potential confounders could be assessed, but a deprivation index and municipality-based smoking habits were adjusted for. Increased relative risks (RR) in contaminated municipalities were found for total mortality (RR = 1.11, 95% CI 1.10 – 1.12), myocardial infarction (RR 1.13, 95% CI 1.09 – 1.18),

cerebrovascular disease (RR=1.21, 95% CI 1.17 – 1.25), diabetes (RR 1.27, 95% CI 1.17 – 1.37), and Alzheimers disease (RR 1.24, 95% CI 1.08 – 1.43). The RR for liver cancer was significantly decreased (RR 0.84, 95% CI 0.74 – 0.94).

In summary, six studies report findings on cardiovascular disease in relation to exposure to other PFASs than PFOS and PFOA. Regarding the study by Lin et al. (2013b), it should be noted that CIMT is not a good marker of atherosclerosis at this young age. The findings by Lind et al. (2017) on atherosclerosis (PFNA and PFUnDA) were restricted to women and have not yet been replicated. For associations with blood pressure/hypertension (Bao et al., 2017), replication in other studies is lacking. The same is true for the longitudinal study by Mattsson et al. (2015) – PFHpA has rarely been studied in relation to cardiovascular disease. The study by Huang et al. (2018) is large and has good confounder-control. Self-reported diseases is a limitation, but on the other hand information bias is not likely since the parricipants were unaware of their PFASs levels. Therefore, it supports the hypothesis that the aforementioned PFASs (PFNA, PFUnDA, and PFDoDA) contribute to the risk of, especially coronary heart disease, the major outcome reported. The findings have, however, not been replicated.

The longitudinal ecological study by Mastrantonio et al. (2018) is relevant for all PFASs since it used drinking water contamination as exposure instead of serum levels. Without individual data, it is, however, difficult to know if established cardiovascular risk factors differ between municipalities (apart from smoking and socioeconomy, which could be taken into account on area level). The large size, and the fact that results were averaged over many (contaminated and uncontaminated) municipalities are strengths of this study and it does provide some support for associations between PFASs and cardiovascular disease. This is true also for PFOS and PFOA (this paper was published after the literature deadline for the PFOS/PFOA opinion, (EFSA CONTAM Panel, 2018). For Alzheimers disease and diabetes, data on morbidity are more appropriate than data from death certificates, and there is no corresponding support from morbidity studies (see sections 3.3.4.6.2 (Diabetes) and 3.3.4.3 (Neurotoxic effects)).

Appendix K – Additional information on the study from Abraham et al. (2020)

Table K.1. Results of the statistical evaluation of the influence of PFAS sum (PFOA, PFNA, PFHxS and PFOS) on vaccine antibodies against Hib, tetanus and diphtheria in children vaccinated at least two times. Yellow colour marks the quantile corresponding to the NOAECs for the PFAS sum derived as the quantile below thequantile significantly different from the first quantile. Anova results (p values) of the group evaluations are given in green.

	PFASsum																	
Groups	**Hib**						**Tetanus IgG1**						**Diphtheria**					
	Quantile	grp.mean	N	mean	sd	p.value	Quantile	grp.mean	N	mean	sd	p.value	Quantile	grp.means	N	mean	sd	p.value
	Q1	10	20	1.92	0.75	0.025	Q1	10	20	1.06	0.32	0.041	Q1	10	20	0.44	0.4	0.06
	Q2	19.8	20	1.67	0.59	0.247	Q2	19.8	20	0.99	0.41	0.573	Q2	19.8	20	0.57	0.36	
5	Q3	31.9	20	1.78	0.74	0.547	Q3	30.9	20	1.06	0.44	0.955	Q3	30.9	20	0.56	0.4	
	Q4	40.5	20	1.38	0.79	0.034	Q4	39.8	20	1.01	0.35	0.677	Q4	39.8	20	0.46	0.42	
	Q5	50.4	18	1.49	0.63	0.065	Q5	49.7	20	0.77	0.35	0.01	Q5	49.7	20	0.2	0.55	
	Quantile	grp.mean	N	mean	sd	p.value	Quantile	grp.mean	N	mean	sd	p.value	Quantile	grp.means	N	mean	sd	p.value
	Q1	8.4	10	1.8	0.82	0.045	Q1	8.4	10	1.1	0.4	0.043	Q1	8.4	10	0.54	0.52	0.05
	Q2	11.5	10	2.04	0.71	0.482	Q2	11.5	10	1.01	0.22	0.575	Q2	11.5	10	0.35	0.21	0.3
	Q3	15.7	10	1.7	0.75	0.79	Q3	15.7	10	1.01	0.43	0.643	Q3	15.7	10	0.63	0.31	0.65
	Q4	23.9	10	1.63	0.4	0.58	Q4	23.9	10	0.97	0.42	0.493	Q4	23.9	10	0.52	0.41	0.92
	Q5	29.6	10	1.6	0.69	0.574	Q5	28.7	10	0.97	0.45	0.507	Q5	28.7	10	0.47	0.42	0.74
10	Q6	34.2	10	1.95	0.78	0.675	Q6	33.1	10	1.16	0.42	0.748	Q6	33.1	10	0.65	0.37	0.59
	Q7	38.7	10	1.14	0.66	0.063	Q7	38	10	1.05	0.25	0.742	Q7	38	10	0.51	0.19	0.88
	Q8	42.3	10	1.63	0.86	0.658	Q8	41.6	10	0.98	0.44	0.525	Q8	41.6	10	0.42	0.58	0.62
	Q9	46.3	10	1.58	0.75	0.543	Q9	45.1	10	0.78	0.29	0.062	Q9	45.1	10	0.28	0.62	0.34
	Q10	55.5	8	1.38	0.47	0.196	Q10	54.3	10	0.75	0.42	0.077	Q10	54.3	10	0.12	0.49	0.08

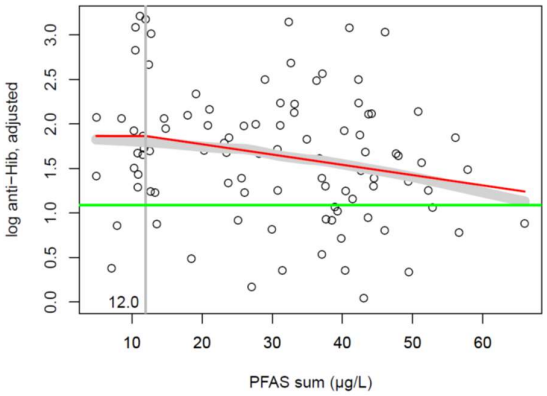


Figure K1

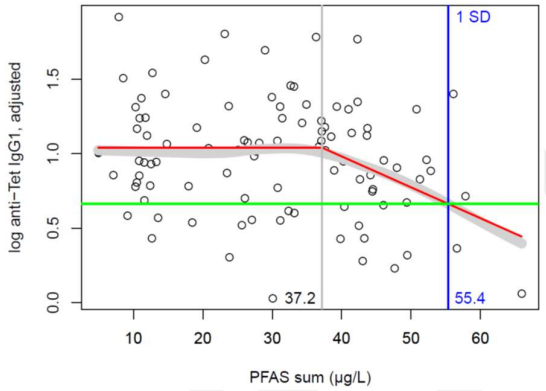


Figure K2

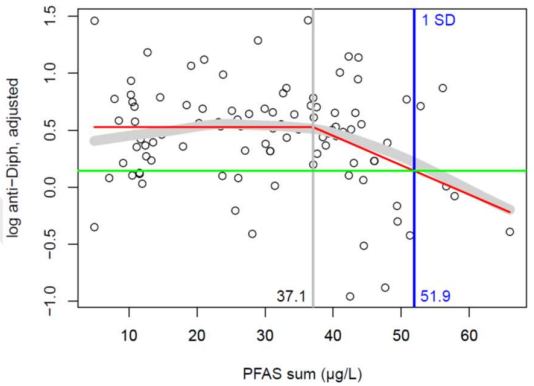
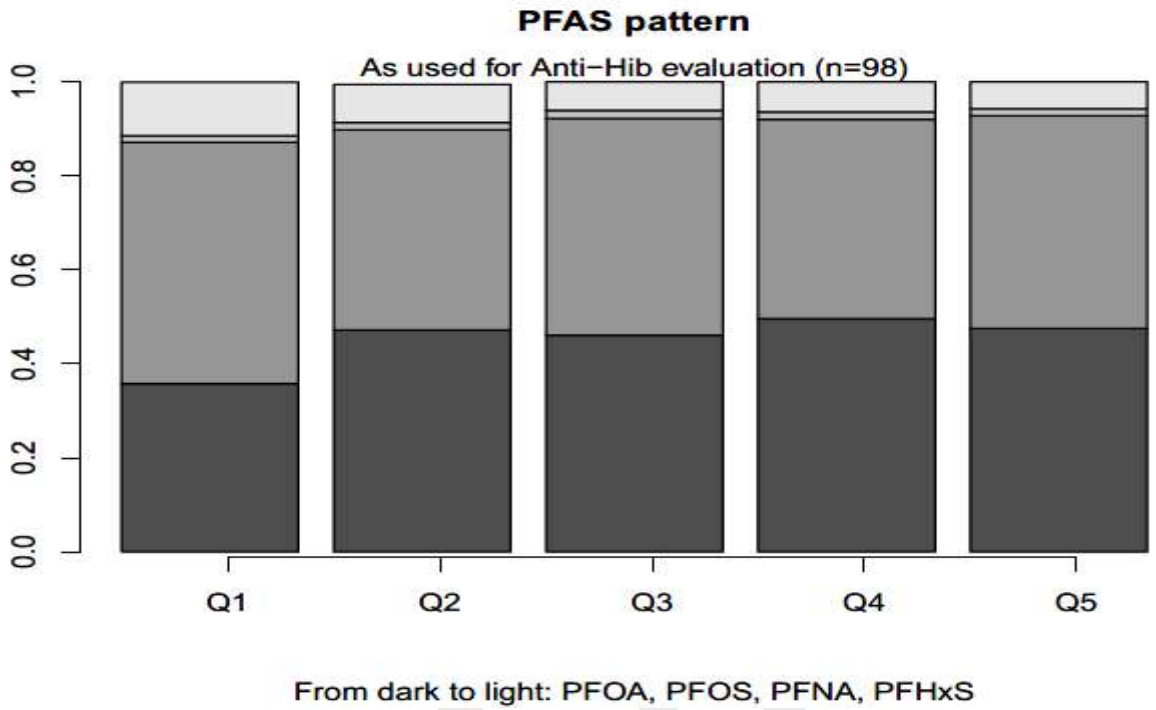


Figure K.3

Figures K.1 – K.3: Scatter plot of levels of vaccine antibodies (K.1 Hib, K.2 Tetanus, K.3 Diphtheria) adjusted for the number of vaccinations (in the case of tetanus only) and for the time since the last vaccination for Hib (K.1, n=98), tetanus IgG1 (K.2, n=100) and diphtheria (K.3, n=100), in relation to the PFAS sum (PFOA, PFNA, PFHxS and PFOS) levels. Broad gray band: moving average; red line: Fitted 'knee' function; horizontal green line: mean minus one standard deviation of the antibody levels below the 'knee'; vertical gray line: PFAS sum level of the 'knee'; vertical blue line: PFAS sum level of the 'knee' function with antibody levels averagely diminished by one standard deviation.

10736



10737

10738 **Figure K 4** Relative contribution of PFOA, PFOS, PFNA and PFHxS to the PFAS sum in the quintiles

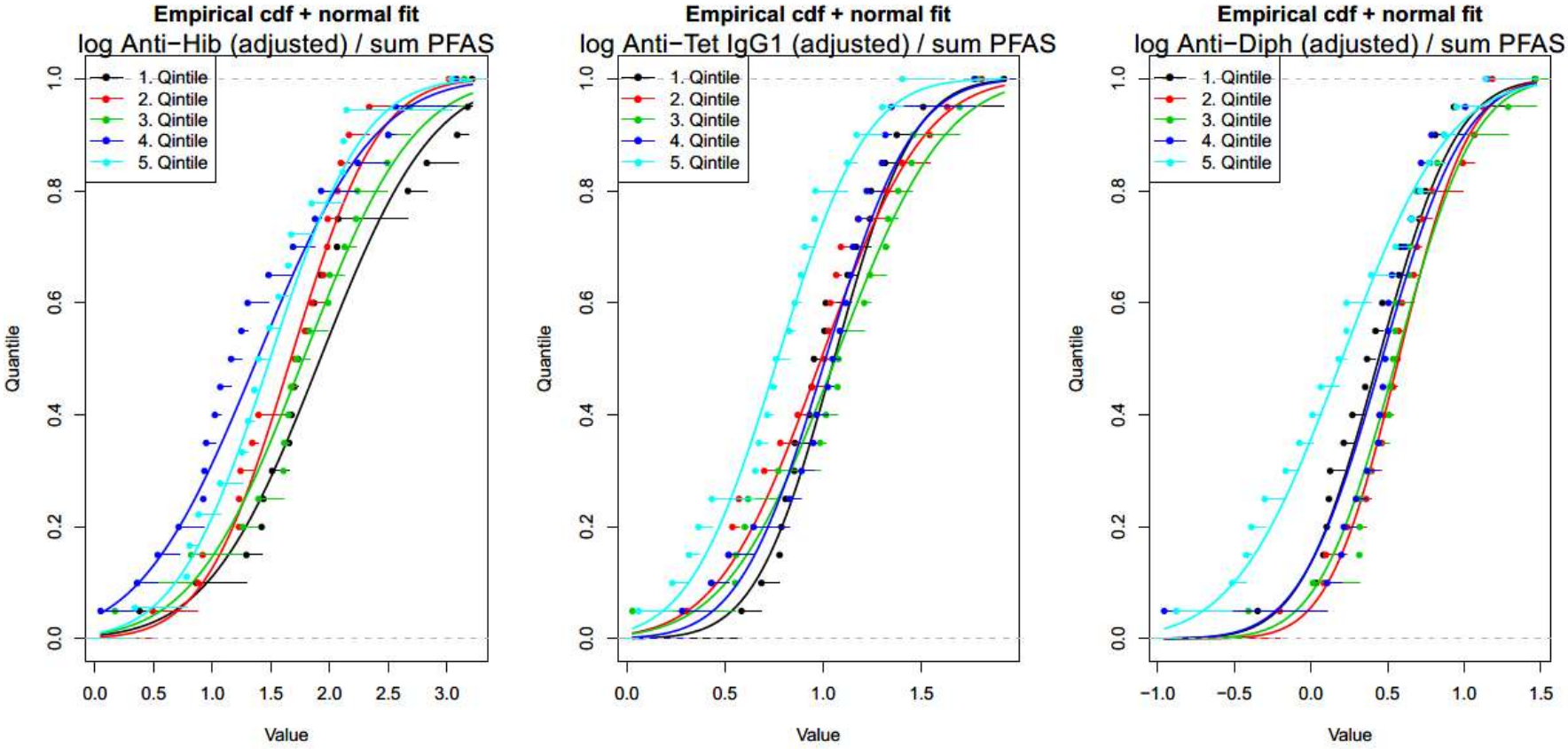


Figure K 5 Empirical cumulative distribution functions of PFAS sum (PFOA, PFNA, PFHxS and PFOS) quintiles together with the respective fitted normal distribution curves

Appendix L – Additional information from the study of Grandjean et al. (2012)

10742

10743 **Table L.1. PFOS at age 5 versus diphtheria at age 7**

10744

					t-test	
Deciles	**PFOS (ng/mL)**	**N**	**mean**	**SD**	**P-value**	**%change**
1	10.3	43	0.17	1.7	referent	
2	12.7	43	0.33	1.9	0.69	11
3	14.2	43	-0.41	2.1	0.17	-33
4	15.6	43	0.27	1.7	0.79	7
5	16.6	43	-0.42	1.9	0.12	-34
6	18.2	43	-0.54	1.9	0.07	-39
7	19.8	43	0.09	1.8	0.22	-6
8	21.3	43	-0.41	2.0	0.16	-33
9	23.6	43	-0.19	2.1	0.37	-22
10	28.4	43	-0.59	1.8	0.048	-41

					t-test	
Quintiles	**PFOS (ng/mL)**	**N**	**mean**	**SD**	**P-value**	**%change**
1	11.5	86	0.25	1.81	referent	
2	14.9	86	-0.07	1.91	0.32	-20
3	17.4	86	-0.48	1.89	0.01	-40
4	20.6	86	-0.16	1.90	0.15	-25
5	26.0	86	-0.39	1.94	0.03	-36

10745 Yellow highlighted text = NOAEC; Blue highlighted text = LOAEC
10746 Total number of individuals in these analyses = 431. Divided into deciles: ~43 and quintiles ~86. Mean and standard
10747 error provided on log-2 scale SD derived. P-value: t-test (assuming log-normal distribution). % change estimated
10748 based on mean difference relative to the lowest (referent) decile corresponds to % shift on median concentration
10749 on the original (back-transformed scale). Mean and SD pooled (from deciles) to estimate NOAEC in quintiles.

10750 **Table L.2. PFOA at age 5 versus diphtheria at age 7**

10751

						t-test	
Deciles	**PFOA (ng/mL)**	**N**	**mean**	**stderr**	**SD**	**P**	**%change**
1	2.5	43	0.23	0.24	1.58	referent	
2	3	43	0.06	0.25	1.61	0.61	-11
3	3.3	43	-0.11	0.32	2.12	0.4	-21
4	3.6	43	-0.35	0.28	1.82	0.11	-33
5	3.9	43	-0.51	0.31	2.04	0.06	-40
6	4.2	43	-0.05	0.27	1.74	0.48	-18
7	4.6	43	0.26	0.31	2.05	0.95	2
8	4.9	43	0.00	0.33	2.14	0.71	-15
9	5.5	43	-0.52	0.26	1.73	0.04	-41
10	6.7	43	-0.69	0.31	2.04	0.02	-47

						t-test	
Quintiles	**PFOA (ng/mL)**	**N**	**mean**	**stderr**	**SD**	**P**	**%change**
1	2.75	86	0.15	0.17	1.60	0.17	
2	3.45	86	-0.23	0.21	1.97	0.22	-23
3	4.05	86	-0.28	0.20	1.89	0.11	-26
4	4.75	86	0.13	0.23	2.09	0.94	-1
5	6.1	86	-0.60	0.20	1.9	0.007	-41

10752 Yellow highlighted text = NOAEC; Blue highlighted text = LOAEC
10753 Total number of individuals in these analyses = 431. Divided into deciles: ~43 and quintiles ~86. Mean and standard error
10754 provided on log-2 scale SD derived. P-value: t-test (assuming log-normal distribution). % change estimated based on mean
10755 difference relative to the lowest (referent) decile corresponds to % shift on median concentration on the original (back-
10756 transformed scale). Mean and SD pooled (from deciles) to estimate NOAEC in quintiles.

10757 **Table L.3. SUM PFAS at age 5 versus diphtheria at age 7**

10758

Decile	Sum_PFAS (ng/mL)	N	mean	stderr	SD	t-test P	%change
1	14.7	43	0.14	0.25	1.63	referent	
2	17.5	43	0.49	0.25	1.67	0.33	27
3	19.5	43	-0.31	0.29	1.88	0.24	-27
4	20.8	43	-0.44	0.28	1.80	0.12	-33
5	22.5	43	-0.38	0.36	2.35	0.23	-30
6	24.2	43	-0.06	0.29	1.90	0.60	-13
7	25.8	43	-0.17	0.31	2.02	0.43	-19
8	28.2	43	-0.01	0.27	1.76	0.68	-10
9	31.2	43	-0.74	0.27	1.80	0.02	-46
10	38.5	43	-0.66	0.30	1.98	0.04	-43

Quintiles	Sum_PFAS	N	mean	SD	t-test P	%change
1	16.1	86	0.31	1.65	referent	
2	20.2	86	-0.37	1.84	0.02	-38
3	23.4	86	-0.22	2.14	0.08	-31
4	27.0	86	-0.09	1.90	0.16	-24
5	34.8	86	-0.70	1.89	0.0005	-50

10759 Yellow highlighted text = NOAEC; Blue highlighted text = LOAEC

10760 Total number of individuals in these analyses = 431. Divided into deciles: ~43 and quintiles ~86. Mean and standard error
10761 provided on log-2 scale SD derived. P-value: t-test (assuming log-normal distribution). % change estimated based on mean
10762 difference relative to the lowest (referent) decile corresponds to % shift on median concentration on the original (back-
10763 transformed scale). Mean and SD pooled (from deciles) to estimate NOAEC in quintiles.

10764

10765 **Table L.4. SUM PFASs at age 5 versus tetanus at age 7**

10766

Decile	Sum_PFAS (ng/mL)	N	mean	stderr	SD	t-test P	%change	
1	14.7	43	1.225	0.282	1.85	referent		no NOAEC for deciles
2	17.5	43	1.343	0.335	2.20	0.79	9	
3	19.5	43	1.205	0.287	1.88	0.96	-1	
4	20.8	43	0.534	0.293	1.92	0.09	-38	NOAEC identified in quintile below
5	22.5	43	1.232	0.263	1.72	0.99	0	
6	24.2	43	1.163	0.32	2.10	0.88	-4	
7	25.8	43	0.852	0.321	2.10	0.47	-23	
8	28.2	43	0.608	0.317	2.08	0.19	-35	
9	31.2	43	0.51	0.321	2.10	0.10	-39	
10	38.5	43	0.57	0.372	2.44	0.16	-36	

Quintiles	Sum_PFAS	N	mean	SD	t-test P	%change
1	16.1	86	1.28	2.03	referent	
2	20.2	86	0.87	1.90	0.17	-25
3	23.4	86	1.20	1.92	0.79	-6
4	27.0	86	0.73	2.09	0.08	-32
5	34.8	86	0.54	2.28	0.03	-40

10767 Yellow highlighted text = NOAEC; Blue highlighted text = LOAEC.
10768 Total number of individuals in these analyses = 431. Divided into deciles: ~43 and quintiles ~86. Mean and standard error provided
10769 on log-2 scale SD derived. P-value: t-test (assuming log-normal distribution). % change estimated based on mean difference
10770 relative to the lowest (referent) decile corresponds to % shift on median concentration on the original (back-transformed scale).
10771 Mean and SD pooled (from deciles) to estimate NOAEC in quintiles.

Appendix M - PBPK Modelling

The model codes were based on the supplementary material in Loccisano et al. (2011), with some modifications provided by a co-author of the original paper (see EFSA CONTAM Panel, 2018)

Model description

The original Loccisano model was slightly modified by integrating a growth equation based on a French survey. This study (EAT for French total Diet Study) includes 4,078 subjects with age between 3 and 60 years, and 703 subjects of less than 3 years). The reported data (weight, age) from this study allow building an equation describing the increase in weight according to age.

Briefly, PFOA and/or PFOS are taken up into the plasma (i.v.) or into the gut (oral). From the gut, PFOA and/or PFOS are transported to the liver by the portal blood. Only the free fractions of PFOA and/or PFOS in plasma are assumed to be available for partitioning into tissues. PFOA and/or PFOS is eliminated through the filtrate compartment to storage into urine, while in the filtrate compartment, PFOA and/or PFOS can be reabsorbed back into the plasma through a saturable process with a transporter maximum constant (Tmc) and affinity constant (Kt). The Qs indicate blood flows into and out of tissues. Qfil is not a blood flow – it is a clearance (L/h) from the plasma to the filtrate compartment.

Model validation

The model was applied in a case study of human individuals living in Little Hocking (Ohio, USA) and Arnsberg (Germany), and exposed to relatively high concentrations of PFOA through consumption of drinking water. For PFOS data from occupational exposure were used. The result was a PBPK model reasonably capable to estimate the concentration of PFOS and PFOA in the human body. Coding and simulations for both the PFOA and PFOS models was performed in the Berkeley Madonna program (Macey et al., 2000).

Sensitivity analysis

Sensitivity analysis (SA) provides a quantitative assessment of the degree of influence of input parameters on the model results. According WHO guidance (WHO/IPCS, 2010), sensitivity analysis results can be summarised as:

- high (absolute value greater than or equal to 0.5)
- medium (absolute value greater than or equal to 0.2 but less than 0.5)
- low (absolute value greater than or equal to 0.1 but less than 0.

The highest values of sensitivity analysis results ($p > 0.1$) were obtained for the following parameters: Cardiac output, elimination parameters (glomerular filtration rate and resorption maximum parameters), the free fraction and the haematocrit. For PFOA and PFOS, the parameter with the highest contribution to the SA was free fraction, and Kt followed by oral intake, Tm, Q. kidney. In general terms, the cardiac output and volumes of tissues showed the smallest contribution, with the only exception of Q. kidney. The reason of the high sensitivity of Q. kidney might be due to the fact that kidney is the elimination tissue, and it has been reported that Q. kidney is a physiological parameter with a low uncertainty.

Model codes applied in the PBPK model used in this Opinion

The codes are based on model codes in Loccisano et al. (2011), with slight modifications by the EFSA CONTAM Panel in 2018. Explanation of the modifications from the original model were given in notes in the respective model codes (see EFSA opinion 2018).

In the present opinion, the PFOA and PFOS human PBPK models were used to estimate maternal exposure (daily intake) to PFOA/PFNA and PFOS/PFHxS for women corresponding to a critical serum and corresponding milk level at 35 years, and were also able to simulate PFAS concentrations in the infant at 1 years after 12 months of breastfeeding. These models were run for 35 years in order to calculate the serum and milk concentrations at 35 years which were used as starting concentrations at the end of pregnancy (at delivery); then these concentrations were used as starting concentrations for the new born infant and the breastfeeding. To perform these simulations, constants (e.g. placental transfer, transfer from blood to milk and decline of PFASs in milk per month) have been added to the model code to allow estimation of the serum level of PFASs at birth, and exposure during breastfeeding. They are indicated in red in the text code.

General constant

These line codes represent the placental transfer, ratio for milk concentration/maternal serum PFAS concentration, the decline of PFASs in milk due to transfer to the infant, and these allow the calculation of initial amount at birth and intake via breastfeeding:

BWbirth=3.68 ; Body weight at birth in kg new add opinion 2020
PT=; placental transfer, new add opinion 2020
Ratio=; Milk concentration/maternal serum concentration, new add opinion 2020; for PFOA a ratio of 0.03 was used based on previous studies
DECLINE =; decline of PFAS concentration in milk per month, new add opinion 2020

Line code for the initial amount at birth (example for PFOA)

These line codes allow the calculation of the initial amount of PFOA in several compartments at birth according to the maternal level at delivery and the placental transfer. The number (e.g. AGbirth= APlasbirth***0.99)** represent the ratio between the initial amount at birth in a specific compartment (here the gut) /initial amount in plasmatic compartment. They have been calculated by running a simple PFOA PBPK model with different exposure, and are constant. The initial amount at birth in the plasma is calculated with the following formula:
Aplas=CA x free x Vplas= CA x Free x Vplac x BW

PFOAmaternal=0 ; maternal concentration ng/mL at delivery new add opinion 2020
CONCbirth= PFOAmaternal*PT; PFOA concentration at birth, new add in 2020
APlasbirth= CONCbirth *Free*VPlasC*BWbirth; initial amount at birth in the plasma
AGbirth= APlasbirth*0.99; initial total amount at birth in gut
ALbirth= APlasbirth*67; initial total amount at birth in liver
AFbirth= APlasbirth*10; initial total amount at birth in fat
AKbirth= APlasbirth*5.9; initial total amount at birth in kidney
ARbirth= APlasbirth*75; initial total amount at birth in rest of body

Line code for Breastfeeding 12-month (example for PFOA)

These line codes allow the calculation of amount in milk per month according the serum PFOA maternal concentration/milk concentration ratio and the decline of PFOA in milk per month. Based on studies a value of 7.7% per month was used for PFOA

```
PFOAmaternal=0;  maternal concentration ng/mL at delivery new add opinion 2020
PFOaMilkconcentration=PFOAmaternal*ratio;initialMilkconcentrationatbirthµg/L
Milkconsumption=0.8;milkconsumptioninl
Intakemilka=PFOaMilkconcentration*Milkconsumption;initialintakeviabreastfeedingfirstmonth
Intakemilkb=Intakemilka*(1-DECLINE);intakeviabreastfeedingsecondmonth
Intakemilkc=Intakemilkb*(1-DECLINE);intakeviabreastfeeding3month
Intakemilkd=Intakemilkc*(1-DECLINE);intakeviabreastfeeding4month
Intakemilke=Intakemilkd*(1-DECLINE);intakeviabreastfeeding5month
Intakemilkf=Intakemilke*(1-DECLINE);intakeviabreastfeeding6month
Intakemilkg=Intakemilkf*(1-DECLINE);intakeviabreastfeeding7month
Intakemilkh=Intakemilkg*(1-DECLINE);intakeviabreastfeeding8month
Intakemilki=Intakemilkh*(1-DECLINE);intakeviabreastfeeding9month
Intakemilkj=Intakemilki*(1-DECLINE);intakeviabreastfeeding10month
Intakemilkk=Intakemilkj*(1-DECLINE);intakeviabreastfeeding11month
Intakemilkl=Intakemilkk*(1-DECLINE);intakeviabreastfeeding12month
```

From the previous calculation above (calculation of amount in milk per month), these line codes allow to calculate the intake via breastfeeding and the corresponding PFAAS serum concentrations at different age:

```
Oralconc=IF year <0.083 THEN Intakemilka/BW ELSE IF year>= 0.083 AND year <0.167 THEN
Intakemilkb/BW ELSE IF year>=0.167 and year <0.250 THEN Intakemilkc/BW ELSE IF year >=0.250
AND year <0.333 THEN Intakemilkd /BW ELSE IF year>=0.333 AND year <0.417 THEN
Intakemilke/BW ELSE IF year>=0.417 AND year <0.500 THEN Intakemilkf/BW ELSE IF year>=0.500
AND year <0.583 THEN Intakemilkg/BW ELSE IF year>=0.583 AND year <0.667 THEN
Intakemilkh/BW ELSE IF year>=0.667 AND year <0.750 THEN Intakemilki/BW ELSE IF year>=0.750
AND year <0.833 THEN Intakemilkj/BW ELSE IF year>=0.833 AND year <0.917 THEN
Intakemilkk/BW ELSE IF year>=0.917 AND year <1 THEN Intakemilkl/BW ELSE IF year>=1 THEN
oralexpo ELSE 0.0
```

Model code for PFOA

```
METHOD Stiff
STARTTIME = 0
STOPTIME=438000       ;end of simulation (h); 50 years
DT = 0.01
TOLERANCE = 0.01  ; default tolerance
DTMAX = 10.0
DTMIN = 0.000001
year= TIME/(24*365)
month=TIME/(12*24*365)

;  Physiological parameters (from Brown, et al. 1997)
;fractional blood flows
QCC = 12.5              ; Cardiac blood output (L/h/kg^0.75)
QFC = 0.052             ; Fraction cardiac output going to fat
QLC = 0.069             ; Fraction cardiac output going to liver, through hepatic artery
QKC = 0.175             ; Fraction cardiac output going to kidney
QSkC = 0.058            ; Fraction cardiac output going to skin
QGC = 0.181             ; Fraction of cardiac output going to gut and in the liver via portal artery
; Not used ;QfilC = 0.035                ; Fraction cardiac output to the filtrate compartment (20% of
;kidney blood flow)

;weight algorithm based on french survey (French total Diet Study)
BW=3.68+4.47*year-0.093*year^2+0.00061*year^3

;fractional tissue volumes
VLC = 0.026             ; Fraction liver volume
VFC = 0.214             ; Fraction fat volume
VKC = 0.004             ; Fraction kidney volume
VfilC = 0.0004          ; Fraction filtrate compartment volume (10% of kidney volume)
VGC = 0.0171            ; Fraction gut volume
VPlasC = 0.0428         ; Fraction plasma volume (58% of blood)
Htc = 0.44                ; hematocrit

BWbirth=3.68 ; Body weight at birth in kg new add opinion 2020
PT= 0.74 ; placenta transfer, new add opinion 2020
Ratio= 0.03 ; milk concentration/maternal serum concentration during breastfeeding, new add opinion
2020
DECLINE = 0.077 ; decline of PFOA in milk was 7.7% per month, new add opinion 2020

;dermal uptake
SkinTarea = 9.1*((BW*1000)**0.666)   ; Total area of skin (cm^2)
Skinthickness = 0.1                    ; Skin thickness (cm)

; Chemical-specific parameters (PFOA)
Tmc = 6000                    ; Maximum resorption rate
Kt = 55                 ; Resorption affinity; same as monkey
```

```
10940
10941
10942   Free = 0.02                      ; Free fraction of PFOA in plasma; same as monkey
10943   PL = 2.2                        ; Liver/plasma partition coefficient
10944   PF = 0.04                       ; Fat/plasma partition coefficient
10945   PK = 1.05                       ; Kidney/plasma partition coefficient
10946   PSk = 0.1                       ; Skin/plasma partition coefficient
10947   PR = 0.12                       ; Rest of the body/plasma partition coefficient
10948   PG = 0.05                          ; Gut/blood plasma coeff.
10949   kurinec = 0.0003                ; ; urinary elimination rate constant (/h/kg^-0.25); estimated
10950   ;from Harada, et al 2005
10951
10952   kurine = kurinec*BW**(-0.25)
10953
10954   ; Free fraction of chemical in tissues
10955   FreeL = Free/PL                  ;liver
10956   FreeF = Free/PF                 ;fat
10957   FreeK = Free/PK                  ;kidney
10958   FreeSk = Free/PSk               ;skin
10959   FreeR = Free/PR                 ;rest of tissues
10960   FreeG = Free/PG                 ;gut
10961
10962   ; Exposure parameters
10963   tchng = 525600           ; Duration of exposure (h); 30 years
10964   year= TIME/(24*365)
10965   ;turn dose on/off
10966   DoseOn = IF time<tchng THEN 1.0 ELSE 0.0
10967
10968    ;direct input to plasma (IV dose)
10969   ;IVconc = 0              ;iv uptake (ug/kg/day)
10970   ;IVdose = IVconc*BW   ;(ug/day)
10971
10972   ; Dermal exposure
10973   Dermconc = 0.0                           ; Dermal concentration (mg/mL)
10974   Dermvol = 0.001                         ; Dermal exposure volume (mL)
10975   Dermdose = Dermconc*Dermvol*1000     ; (ug)
10976   Skinarea = 972                           ; Exposed area on skin (cm^2)
10977
10978   ; Oral exposure
10979   oralexpo= 0 ; µg/kg/day new add opinion 2020
10980   ;oralconc =0                        ; Oral uptake (µg/kg/day) not used in opinion 2020
10981   Oraldose = Oralconc*BW         ; (µg/day)
10982
10983   PFOAmaternal=0; maternal concentration ng/mL at delivery new add opinion 2020
10984   PFOaMilkconcentration=PFOAmaternal*ratio; initial Milk concentration at birth ng/mL
10985   Milkconsumption=0.8; milkconsumption L per day
10986   Intakemilka=PFOaMilkconcentration*Milkconsumption; initial intake via breastfeeding first month
10987   Intakemilkb=Intakemilka*(1-DECLINE);intakeviabreastfeedingsecondmonth
10988   Intakemilkc=Intakemilkb*(1-DECLINE);intakeviabreastfeeding3month
10989   Intakemilkd=Intakemilkc*(1-DECLINE);intakeviabreastfeeding4month
10990   Intakemilke=Intakemilkd*(1-DECLINE);intakeviabreastfeeding5month
```

```
Intakemilkf=Intakemilke*(1-DECLINE);intakeviabreastfeeding6month
Intakemilkg=Intakemilkf*(1-DECLINE);intakeviabreastfeeding7month
Intakemilkh=Intakemilkg*(1-DECLINE);intakeviabreastfeeding8month
Intakemilki=Intakemilkh*(1-DECLINE);intakeviabreastfeeding9month
Intakemilkj=Intakemilki*(1-DECLINE);intakeviabreastfeeding10month
Intakemilkk=Intakemilkj*(1-DECLINE);intakeviabreastfeeding11month
Intakemilkl=Intakemilkk*(1-DECLINE);intakeviabreastfeeding12month

Oralconc=IF year <0.083 THEN Intakemilka/BW ELSE IF year>= 0.083 AND year <0.167 THEN
Intakemilkb/BW ELSE IF year>=0.167 and year <0.250 THEN Intakemilkc/BW ELSE IF year >=0.250
AND year <0.333 THEN Intakemilkd /BW ELSE IF year>=0.333 AND year <0.417 THEN
Intakemilke/BW ELSE IF year>=0.417 AND year <0.500 THEN Intakemilkf/BW ELSE IF year>=0.500
AND year <0.583 THEN Intakemilkg/BW ELSE IF year>=0.583 AND year <0.667 THEN
Intakemilkh/BW ELSE IF year>=0.667 AND year <0.750 THEN Intakemilki/BW ELSE IF year>=0.750
AND year <0.833 THEN Intakemilkj/BW ELSE IF year>=0.833 AND year <0.917 THEN
Intakemilkk/BW ELSE IF year>=0.917 AND year <1 THEN Intakemilkl/BW ELSE IF year>=1 THEN
oralexpo ELSE 0.0

CONCbirth= PFOAmaternal*PT; PFOA concentration at birth, new add in 2020
APlasbirth= CONCbirth *Free*VPlasC*BWbirth; initial amount at birth
AGbirth= APlasbirth*0.99; initial total amount at birth
ALbirth= APlasbirth*67; initial total amount at birth
AFbirth= APlasbirth*10; initial total amount at birth
AKbirth= APlasbirth*5.9; initial total amount at birth
ARbirth= APlasbirth*75; initial total amount at birth



;Drinking water exposure
Drinkconc = 0                          ; Drinking water concentration (ug/L or ppb)
Drinkrate = 13                                 ; Drinking water rate (mL/kg/day)
Drinkdose = (Drinkconc*Drinkrate/1000)*BW   ; (ug/day)


;Tinput = 0.6            ; duration of dose (drinking water or oral) (h)
Tinput = 24             ; duration of dose (h) the CONTAM Panel increased the Tinput to 24h
(instead ;of 0.6) considering continuous exposure from food.


;oral
Input1 = IF MOD(time,24) <=Tinput THEN Oraldose/Tinput  ELSE 0.0

;drinking water
Input2 = IF MOD(time,24) <= Tinput THEN Drinkdose/Tinput ELSE 0.0


QC = QCC*BW**0.75 ; Cardiac output (L/h)
QCP = QC*(1-Htc) ; adjust for plasma flow
QL = QLC*QCP ; Plasma flow to liver (L/h)
QF = QFC*QCP ; Plasma flow to fat (L/h)
```

```
11042  QK = QKC*QCP ; Plasma flow to kidney (L/h)
11043  Qfil = 0.2*QK ; Plasma flow to filtrate compartment (L/h); 20% of QK
11044  QG = QGC*QCP ; Plasma flow to gut (L/h)
11045  QSk = IF Dermconc >0.0 THEN QSkC*QCP*(Skinarea/SkinTarea) else 0.0 ;plasma flow to skin
11046  QR = QCP - QL - QF - QK - QG -QSk ; Plasma flow to rest of the body (L/h)
11047  Qbal = QCP - (QR+QL+QF+QK+QG+QSk) ; balance check--better be 0
11048
11049  VL = VLC*BW ; Liver volume (L)
11050  VF = VFC*BW ; Fat volume (L)
11051  VK = VKC*BW ; Kidney volume (L)
11052  Vfil = VfilC*BW ; Fitrate compartment volume (L)
11053  VG = VGC*BW ; Gut volume (L)
11054  VPlas = VPlasC*BW ; Plasma volume (L)
11055  VSk = (Skinarea*Skinthickness)/1000 ; Skin volume (L)
11056  VR = 0.84*BW - VL - VF - VK - Vfil - VG - VPlas - VSk ; Rest of the body volume (L)
11057  Vbal = (0.84*BW)-(VL+VF+VK+VFil+VG+VPlas+VSk) ; Balance check--better be 0
11058  Tm = Tmc*BW**0.75 ;transporter maximum
11059
11060
11061
11062
11063
11064  ;>>>>>>>>>>>>>>>Model equations<<<<<<<<<<<<<<<<<<
11065
11066  ;Plasma compartment
11067
11068  APlas' = QF*CF*FreeF+(QL+QG)*CL*FreeL+QR*CR*FreeR+QSk*CSk*FreeSk+QK*CK*FreeK -
11069  QCP*CA*Free - Qfil*CA*Free
11070
11071  init APlas = 0 +   APlasbirth
11072
11073  CAFree = APlas/VPlas              ;free concentration of chemical in plasma; µg/L (ng/mL)
11074  CA = CAFree/Free                  ;total concentration of chemical in plasma
11075
11076  ; Gut compartment
11077  AG' = QG*(CA*Free-CG*FreeG) + Input1*DoseOn + Input2*DoseOn
11078  init AG = 0.0 + AGbirth
11079  CG = AG/VG                                ; Concentration in gut (µg/L)
11080  CVG = CG/PG                               ; Concentration leaving gut (µg/L)
11081
11082
11083  ; Liver compartment
11084  AL' = (QL*(CA*Free))+(QG*CG*FreeG) - ((QL+QG)*CL*FreeL)   ; Rate of change in liver (ug/h)
11085  init AL = 0.0 + ALbirth
11086  CL = AL/VL                                ; Concentration in liver (µg/L)
11087  CVL = CL/PL                               ; Concentration leaving liver (µg/L)
11088
11089  ; Fat compartment
11090  AF' = QF*(CA*Free-CF*FreeF)               ; Rate of change in fat (µg/h)
11091  init AF = 0.0 + AFbirth
11092  CF = AF/VF                                ; Concentration in fat (µg/L)
```

```
11093   CVF = CF/PF                                          ; Concentration leaving fat (µg/L)
11094
11095   ; Kidney compartment
11096   AK' = QK*(CA*Free-CK*FreeK) + (Tm*Cfil)/(Kt+Cfil)     ; Rate of change in kidneys (µg/h)
11097   init AK = 0.0 + AKbirth
11098   CK = AK/VK                                           ; Concentration in kidneys (µg/L)
11099   CVK = CK/PK                                          ; Concentration leaving kidneys (µg/L)
11100
11101   ; Filtrate compartment
11102   Afil' = Qfil*(CA*Free-Cfil) - (Tm*Cfil)/(Kt+Cfil)    ; Rate of change in filtrate compartment (ug/h)
11103   init Afil = 0.0
11104   Cfil = Afil/Vfil                                     ; Concentration in filtrate compartment (µg/L)
11105
11106   ; Storage compartment for urine
11107   Adelay' = Qfil*Cfil-kurine*Adelay
11108   init Adelay = 0.0
11109
11110   ; Urine
11111   Aurine' = kurine*Adelay
11112   init Aurine = 0.0
11113
11114
11115   ; Skin compartment
11116   ASk' = QSk*(CA*Free-CSk*FreeSk)    ; Rate of change in skin(µg/h)
11117   init ASk = 0.0
11118   CSk = ASk/VSk                                         ; Concentration in skin compartment (µg/L)
11119   CVSk = CSk/PSk                                        ; Concentration leaving skin compartment (µg/L)
11120
11121   ; Rest of the body
11122   AR' = QR*(CA*Free-CR*FreeR)                          ; Rate of change in rest of the body (µg/h)
11123   init AR = 0.0 + ARbirth
11124   CR = AR/VR                                           ; Concentration in rest of the body (µg/L)
11125   CVR = CR/PR                                          ; Concentration leaving rest of the body (µg/L)
11126
11127   Display  Oralconc,  TmC, Kt, Free, PL,PK,PF,PR,PSK,PG,
11128   tchng,BW,QCC,QFC,QLC,QKC,QGC,QSkC,VFC,VLC,VKC,VGC,VFilC,VPlasC,Dermvol,Drinkrate, Qfil,
11129   APlas, AG,  AL, AF, AK, AR ,PFOaMilkconcentration, Oralconc , Oraldose, oralexpo, PFOAmaternal, PT,
11130   ratio, DECLINE      ;for parameters window
11131
11132   Display CA, year, BW ,CVK, APlas, AG,  AL, AF, AK, AR, PFOaMilkconcentration, Oralconc , Oraldose,
11133   oralexpo , PFOAmaternal, Qbal                        ;for plotting
11134
11135
```

MODEL code for PFOS

```
METHOD Stiff

STARTTIME = 0
STOPTIME=438000       ;end of simulation (h); 50 years
DT = 0.01
TOLERANCE = 0.01  ; default tolerance
DTMAX = 10.0
DTMIN = 0.000001
year= TIME/(24*365)
month=TIME/(12*24*365)









; Physiological parameters (from Brown, et al 1997)
;fractional blood flows
QCC = 12.5              ; Cardiac blood output (L/h/kg^0.75)
QFC = 0.052             ; Fraction cardiac output going to fat
QLC = 0.069             ; Fraction cardiac output going to liver, through hepatic artery
QKC = 0.175             ; Fraction cardiac output going to kidney
QSkC = 0.058            ; Fraction cardiac output going to skin
QGC = 0.181             ; Fraction of cardiac output going to gut and in the liver via portal artery
; Not used ;QfilC = 0.035                ; Fraction cardiac output to the filtrate compartment (20% of
kidney blood flow)

;BW = 70                        ; Body weight (kg) for men; 58 kg for women
;weight algorithm based on french survey (French total Diet Study)
BW=3.68+4.47*year-0.093*year^2+0.00061*year^3

;fractional tissue volumes
VLC = 0.026             ; Fraction liver volume
VFC = 0.214             ; Fraction fat volume
VKC = 0.004             ; Fraction kidney volume
VfilC = 0.0004          ; Fraction filtrate compartment volume (10% of kidney volume)
VGC = 0.0171            ; Fraction gut volume
VPlasC = 0.0428         ; Fraction plasma volume (58% of blood)
Htc = 0.44                ; hematocrit
BWbirth=3.68 ; Body weight at birth in kg new add opinion 2020
PT= 0.36 ; placental transfer, new add opinion 2020
Ratio= 0.015 ; milk concentration/maternal serum concentration during breastfeeding, new add
opinion 2020
DECLINE = 0.031 ; decline of PFOS in milk was 3.1% per month, new add opinion 2020

;for dermal exposure
SkinTarea = 9.1*((BW*1000)**0.666)     ; Total area of skin (cm^2)
```

```
Skinthickness = 0.1                    ; Skin thickness (cm)

; Chemical-specific parameters (PFOS)
Tmc =3500.                  ; Maximum resorption rate, Changed from 3.5 in the original
Loccisano 2011 model and expressed in µg, to be consistent with other parameters
Kt = 23.0                   ; Resorption affinity, Changed from 0.023 in the original Loccisano
2011 model and expressed in µg, to be consistent with other parameters
Free = 0.025                 ; Free fraction of PFOS in plasma
PL = 3.72                   ; Liver/plasma partition coefficient
PF = 0.14                   ; Fat/ plasma partition coefficient
PK = 0.8                    ; Kidney/ plasma partition coefficient
PSk = 0.29                  ; Skin/ plasma partition coefficient
PR = 0.2                    ; Rest of the body/ plasma partition coefficient
PG = 0.57                     ; Gut/ plasma partition coeff.
kurinec = 0.001              ; urinary elimination rate constant  (/h/kg^-0.25); estimated from
;Harada, et al 2005
kurine = kurinec*BW**(-0.25)

; Free fraction of chemical in tissues
FreeL = Free/PL             ;liver
FreeF = Free/PF             ;fat
FreeK = Free/PK             ;kidney
FreeSk = Free/PSk           ;skin
FreeR = Free/PR             ;rest of tissues
FreeG = Free/PG             ;gut

; Exposure parameters
tchng =438000               ;Duration of exposure (h); 50 years

;turn dose on/off
DoseOn = IF time<tchng THEN 1.0 else 0.0


; Dermal exposure
Dermconc = 0.0                                  ; Dermal concentration (µg/mL)
Dermvol = 0.0                                   ; Dermal exposure volume (mL)
Dermdose = Dermconc*Dermvol*1000               ; (ug)
Skinarea = 5                                    ; Exposed area on skin (cm^2)

; Oral exposure
oralexpo= 0 ; µg/kg/day new add opinion 2020

PFOSmaternal=0 ;  maternal concentration µg/L at delivery and during pregnancy new add opinion
2020
PFOSMilkconcentration=PFOSmaternal*ratio;initialMilkconcentration at birth µg/L
Milkconsumption=0.8;milk consumption L per day
Intakemilka=PFOSMilkconcentration*Milkconsumption;initialintakeviabreastfeedingfirstmonth
Intakemilkb=Intakemilka*(1-DECLINE);intakeviabreastfeedingsecondmonth
Intakemilkc=Intakemilkb*(1-DECLINE);intakeviabreastfeeding3month
Intakemilkd=Intakemilkc*(1-DECLINE);intakeviabreastfeeding4month
Intakemilke=Intakemilkd*(1-DECLINE);intakeviabreastfeeding5month
```

```
Intakemilkf=Intakemilke*(1-DECLINE);intakeviabreastfeeding6month
Intakemilkg=Intakemilkf*(1-DECLINE);intakeviabreastfeeding7month
Intakemilkh=Intakemilkg*(1-DECLINE);intakeviabreastfeeding8month
Intakemilki=Intakemilkh*(1-DECLINE);intakeviabreastfeeding9month
Intakemilkj=Intakemilki*(1-DECLINE);intakeviabreastfeeding10month
Intakemilkk=Intakemilkj*(1-DECLINE);intakeviabreastfeeding11month
Intakemilkl=Intakemilkk*(1-DECLINE);intakeviabreastfeeding12month

Oralconc=IF year <0.083 THEN Intakemilka/BW ELSE IF year>= 0.083 AND year <0.167 THEN
Intakemilkb/BW ELSE IF year>=0.167 and year <0.250 THEN Intakemilkc/BW ELSE IF year >=0.250
AND year <0.333 THEN Intakemilkd /BW ELSE IF year>=0.333 AND year <0.417 THEN
Intakemilke/BW ELSE IF year>=0.417 AND year <0.500 THEN Intakemilkf/BW ELSE IF year>=0.500
AND year <0.583 THEN Intakemilkg/BW ELSE IF year>=0.583 AND year <0.667 THEN
Intakemilkh/BW ELSE IF year>=0.667 AND year <0.750 THEN Intakemilki/BW ELSE IF year>=0.750
AND year <0.833 THEN Intakemilkj/BW ELSE IF year>=0.833 AND year <0.917 THEN
Intakemilkk/BW ELSE IF year>=0.917 AND year <1 THEN Intakemilkl/BW ELSE IF year>=1 THEN
oralexpo ELSE 0.0

CONCbirth= PFOSmaternal *PT; PFOA concentration at birth, new add in 2020
APlasbirth= CONCbirth *Free*VPlasC*BWbirth; initial amount at birth
AGbirth= APlasbirth*9; initial total amount at birth
ALbirth= APlasbirth*89; initial total amount at birth
AFbirth= APlasbirth*27.5; initial total amount at birth
AKbirth= APlasbirth*3.5; initial total amount at birth
ARbirth= APlasbirth*99; initial total amount at birth









                                        ; Oral uptake (µg/kg/day)
Oraldose = Oralconc*BW                  ; (µg/day)

 ;Drinking water exposure
Drinkconc = 0.0                         ; Drinking water concentration (µg/L or ppb)
Drinkrate = 13                          ; Drinking water rate (mL/kg/day)
Drinkdose = (Drinkconc*Drinkrate/1000)*BW     ; (µg/day)

; Inhalation exposure
Inhalation = 0.0          ; Inhalation dose (ppm)

Tinput = 24               ; duration of dose (h) the CONTAM Panel increased the Tinput to 24h
(instead of 0.6) considering continuous exposure from food.


;oral dose
Input1 = IF MOD(time,24) <=Tinput THEN Oraldose/Tinput  ELSE 0.0

;drinking water
```

```
11289  Input2 = IF MOD(time,24) <= Tinput THEN Drinkdose/Tinput ELSE 0.0
11290
11291  ; Scaling parameters
11292  QC = QCC*BW**0.75              ; Cardiac output (L/h)
11293  QCP = QC*(1-Htc)               ; adjust for plasma flow
11294  QL = QLC*QCP                   ; Plasma flow to liver (L/h)
11295  QF = QFC*QCP                   ; Plasma flow to fat (L/h)
11296  QK = QKC*QCP            ; Plasma flow to kidney (L/h)
11297  Qfil = 0.2*QK                   ; Plasma flow to filtrate compartment (L/h); 20% of QK
11298  QG = QGC*QCP           ; Plasma flow to gut (L/h)
11299
11300  QSk = IF Dermconc >0.0 THEN QSkC*QCP*(Skinarea/SkinTarea) else 0.0  ;plasma flow to skin
11301
11302  QR = QCP - QL - QF - QK - QG -QSk      ; Plasma flow to rest of the body (L/h)
11303  Qbal = QCP - (QR+QL+QF+QK+QG+QSk)       ; balance check--better be 0
11304
11305  VL = VLC*BW                    ; Liver volume (L)
11306  VF = VFC*BW                    ; Fat volume (L)
11307  VK = VKC*BW                    ; Kidney volume (L)
11308  Vfil = VfilC*BW                ; Fitrate compartment volume (L)
11309  VG = VGC*BW                    ; Gut volume (L)
11310  VPlas = VPlasC*BW              ; Plasma volume (L)
11311
11312  VSk = (Skinarea*Skinthickness)/1000                          ; Skin volume (L)
11313  VR = 0.84*BW - VL - VF - VK - Vfil - VG - VPlas - VSk        ; Rest of the body volume (L)
11314  Vbal = (0.84*BW)-(VL+VF+VK+VFil+VG+VPlas+VSk)                ; Balance check--better be 0
11315
11316  Tm = Tmc*BW**0.75              ;transporter maximum
11317
11318  ;>>>>>>>>>>>>>>>>>>>> Model equations <<<<<<<<<<<<<<<<<<<<<<<<<<<
11319
11320  ; Plasma compartment
11321  APlas' = QF*CF*FreeF+(QL+QG)*CL*FreeL+QR*CR*FreeR+QSk*CSk*FreeSk+QK*CK*FreeK -
11322  QCP*CA*Free  -  Qfil*CA*Free
11323  init APlas = 0 +   APlasbirth
11324
11325  CAFree = APlas/VPlas                    ; free concentration of PFOS in plasma in µg/L (ng/mL)
11326  CA = CAfree/Free                         ; total concentration in plasma
11327
11328  ; Gut compartment
11329  AG' = QG*(CA*Free-CG*FreeG) + Input1*DoseOn + Input2*DoseOn
11330  init AG = 0.0 + AGbirth
11331  CG = AG/VG                                                   ; Concentration in gut (µg/L)
11332  CVG = CG/PG                                                  ; Concentration leaving gut (µg/L)
11333
11334  ; Liver compartment
11335  AL' = (QL*(CA*Free))+(QG*CG*Freeg) - ((QL+QG)*CL*FreeL)   ;Rate of change in liver (µg/h)
11336  init AL = 0.0 + ALbirth
11337  CL = AL/VL                                                   ; Concentration in liver (µg/L)
11338  CVL = CL/PL                                                  ; Concentration leaving liver (µg/L)
11339
```

```
11340   ; Fat compartment
11341   AF' = QF*(CA*Free-CF*FreeF)                                  ; Rate of change in fat (µg/h)
11342   init AF = 0.0 + AFbirth
11343   CF = AF/VF                                                   ; Concentration in fat (µg/L)
11344   CVF = CF/PF                                                  ; Concentration leaving fat (µg/L)
11345
11346   ; Kidney compartment
11347   AK' = QK*(CA*Free-CK*FreeK) + Tm*Cfil/(Kt+Cfil)                    ; Rate of change in kidneys
11348   (ug/h)
11349   init AK = 0.0 + AKbirth
11350   CK = AK/VK                                                  ; Concentration in kidneys (ug/L)
11351   CVK = CK/PK                                                  ; Concentration leaving kidneys
11352   (µg/L)
11353
11354   ; Filtrate compartment
11355   Afil' = Qfil*(CA*Free-Cfil) - Tm*Cfil/(Kt+Cfil)        ; Rate of change in filtrate compartment (µg/h)
11356   init Afil = 0.0
11357   Cfil = Afil/Vfil                                  ; Concentration in filtrate compartment (µg/L)
11358
11359   ; Storage compartment for urine
11360   ;Adelay' = Qfil*Cfil-kurine*Adelay
11361   ;init Adelay = 0.0
11362
11363   ; Urine
11364   ;Aurine' = kurine*Adelay
11365   Aurine' = Qfil*Cfil - kurine*Aurine
11366   init Aurine = 0.0
11367
11368   ; Skin compartment
11369   ASk' = QSk*(CA*Free-CSk*FreeSk)                          ; Rate of change in skin (µg/h)
11370   init ASk = DermDose
11371   CSk = ASk/VSk                                  ; Concentration in skin compartment (µg/L)
11372   CVSk = CSk/PSk                                 ; Concentration leaving skin compartment (ug/L)
11373
11374   ; Rest of the body
11375   AR' = QR*(CA*Free-CR*FreeR)                    ; Rate of change in rest of the body (µg/h)
11376   init AR = 0.0 + ARbirth
11377   CR = AR/VR                                     ; Concentration in rest of the body (µg/L)
11378   CVR = CR/PR                                    ; Concentration leaving rest of the body (µg/L)
11379   Display  Oralconc, Tmc, Kt, Free, PL,PK,PF,PR,PSK,PG, tchng,
11380   BW,QCC,QFC,QLC,QKC,QSkC,QGC,VLC,VFC,VKC,VFilC,VGC,VPlasC,kurinec, year , APlas, AG, AL, AF,
11381   AF, AK, ASK, AR, Oraldose, PFOSmaternal,oralexpo, Oralconc , oralexpo, PT, ratio, DECLINE
11382   ;for parameters window
11383
11384   Display CA, CG, CL, CF, CR, CK, CAFREE, Qbal , year, month,   Oralconc , Oraldose, PFOSmaternal
11385   ,oralexpo ,Qbal                                  ;for plotting
11386
11387
11388
11389
11390
```

Appendix N - Literature search

11391 **Table N.1. Search terms**

Human observations	
Search terms	perfluoro* OR pfos OR pfoa OR pfas OR 'fluorotelomer alcohol' AND 'human health' OR 'adverse effect*' OR 'occupational case*' OR occupational OR epidemiol* OR biomarker OR 'biological marker' OR poison* OR 'incidental poison*' OR 'case stud*' OR adverse OR 'case control*' OR 'case report*' OR human OR adult OR man OR woman OR men OR women OR female OR male OR child OR children OR infant OR neonate OR maternal OR cohort OR prenatal
Biomonitoring	
Search terms	perfluoro* OR pfos OR pfoa OR pfas OR 'fluorotelomer alcohol' AND teeth OR tooth OR skin OR bone OR sperm or semen OR tissue OR level* OR concentration* OR 'time trend' OR milk OR blood OR 'whole blood' OR serum OR plasma OR 'breast milk' OR biomarker OR 'human milk' OR 'cord blood' OR urine OR 'amniotic fluid' OR faeces OR placenta OR meconium OR hair OR nail* OR sweat OR saliva OR level* OR concentration*
Toxicity	
Search terms	perfluoro* OR pfos OR pfoa OR pfas OR 'fluorotelomer alcohol' AND toxicity OR toxi* OR acute OR subacute OR subchronic OR chronic OR mutagen* OR carcino* OR cardiotox* OR genotox* OR reprotox* OR nephrotox* OR neurotox* OR hepatotox* OR immune OR immuno* OR hematotox* OR haematotox* OR cytotox* OR 'developmental tox*' OR thyroid OR endocri* OR endocrine OR estrogen OR oestrogen OR fertility OR tumour OR tumor OR gestat* OR lactat* OR 'DNA damage' OR mortality OR adverse OR 'adverse effect' OR 'blood lipid*' OR 'serum lipid*' OR PPAR OR 'ex vivo' OR 'in vitro' OR 'in vivo' OR exvivo OR invitro OR invivo OR cell* OR tissue* OR rodent* OR mouse OR animal* OR rat* OR mice OR rabbit* OR dog* OR monkey* OR 'experimental animal*' OR 'lab* animal*'
Toxicokinetics	
Search terms	perfluoro* OR pfos OR pfoa OR pfas OR 'fluorotelomer alcohol' AND toxicokinetic* OR absorption OR distribution OR metabolism OR excretion OR ADME OR biotransformation OR pharmacokinetic* OR disposition OR fate OR transfer OR conjugat* OR hydroxylation OR 'half-life' OR 'half life' OR PBPK OR 'physiologically based pharmacokinetic modelling*' OR uptake OR elimination OR urine OR bile OR faeces OR feces OR milk
Chemistry and analysis	
Search terms	perfluoro* OR pfos OR pfoa OR pfas OR 'fluorotelomer alcohol' AND chemistry OR analysis OR determination OR detection OR spectroscopy OR chromatography OR TLC OR GC OR GC-MS OR HPLC OR LC-MS OR ICP-MS
Occurrence in Food	
Search terms	perfluoro* OR pfos OR pfoa OR pfas OR 'fluorotelomer alcohol' AND occurrence* OR level* OR concentration* OR amount*OR food OR beverage OR 'drinking water' OR 'bottled water' OR vegetable* OR legume* OR fruit* OR grain* OR cereal* OR poultry OR chicken OR beef OR turkey OR meat OR egg* OR milk OR seafood OR fish OR

	shrimp OR prawn* OR mollusc* OR feed OR feedstuff OR beef OR pork OR livestock OR bivalve*
Food Processing	
Search terms	perfluoro* OR pfos OR pfoa OR pfas OR 'fluorotelomer alcohol' AND process* OR cook* OR roast* OR fry* OR boil* OR bak* OR 'thermal processing' OR sterilisation OR sterilization OR sterilise OR sterilize OR freez* OR heat*
(Dietary) Exposure	
Search terms	perfluoro* OR pfos OR pfoa OR pfas OR 'fluorotelomer alcohol' AND 'exposure assessment*' OR 'dietary exposure assessment*' OR 'human dietary exposure assessment*'
(Non-dietary) Exposure	
Search terms	perfluoro* OR pfos OR pfoa OR pfas OR 'fluorotelomer alcohol' AND 'non-dietary exposure assessment*' OR 'human non-dietary exposure assessment*' OR 'exposure pathway*' OR 'indoor exposure' OR 'dermal exposure' OR occupational OR dust OR air OR 'in-utero' OR inutero OR 'in utero' OR skin
Production/Use	
Search terms	perfluoro* OR pfos OR pfoa OR pfas OR 'fluorotelomer alcohol' AND source* OR application OR use* OR production OR 'production volume' OR application
Environmental fate	
Search terms	perfluoro* OR pfos OR pfoa OR pfas OR 'fluorotelomer alcohol' AND 'environmental fate' OR 'environmental monitoring' OR soil OR biosolid OR manure OR sediment OR sewage OR sludge OR water OR 'waste water*' OR 'ground water*' OR wastewater* OR groundwater* OR river OR land OR lake OR grass OR vegetation

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Appendix O - EFSA guidance documents applied for the risk assessment

EFSA (European Food Safety Authority), 2010a. Standard sample description for food and feed. EFSA Journal 2010;8(1):1457, 54 pp. https://doi.org/10.2903/j.efsa.2010.1457

EFSA (European Food Safety Authority), 2010b. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal 2010;8(3):1557, 96 pp. https://doi.org/10.2903/j.efsa.2010.1557

EFSA (European Food Safety Authority), 2011a. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2011b. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal 2011;9(3):1970, 27 pp. https://doi.org/10.2903/j.efsa.2011.1970

EFSA (European Food Safety Authority), 2011c. Report on the development of a Food Classification and Description System for exposure assessment and guidance on its implementation and use. EFSA Journal 2011;9(12):2489, 84 pp. https://doi.org/10.2903/j.efsa.2011.2489

EFSA (European Food Safety Authority), 2012. Perfluoroalkylated substances in food: occurrence and dietary exposure. EFSA Journal 2012;10(6):2743. 55 pp. https://doi.org/10.2903/j.efsa.2012.2743

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data.

EFSA (European Food Safety Authority), 2015. The food classification and description system FoodEx2 (revision 2). EFSA supporting publication 2015:EN-804, 90 pp. https://doi.org/10.2903/sp.efsa.2015.EN-804

EFSA Scientific Committee, More SJ, Bampidis V, Benford D, Bennekou SH,Bragard C, Halldorsson TI, Hernandez-Jerez AF, Koutsoumanis K, Naegeli H, Schlatter JR, Silano V,Nielsen SS, Schrenk D, Turck D, Younes M, Benfenati E, Castle L, Cedergreen N, Hardy A, Laskowski R,Leblanc JC, Kortenkamp A, Ragas A, Posthuma L, Svendsen C, Solecki R, Testai E, Dujardin B, Kass GEN,Manini P, Jeddi MZ, Dorne J-LCM and Hogstrand C, 2019. Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA Journal 2019;17(3):5634, 77 pp.https://doi.org/10.2903/j.efsa.2019.5634

Annex A – Occurrence and exposure data

– available as excel file on EFSA Knowledge Junction on zenodo at: https://doi.org/10.5281/zenodo.NNNNNNN

Annex B – Distribution of analytical results

- available as excel file on EFSA Knowledge Junction on zenodo at: https://doi.org/10.5281/zenodo.NNNNNNN

Annex C – Comparison of PFOA and PFOS occurrence and exposure data with previous assessment (EFSA CONTAM Panel, 2018)

- available as excel file on EFSA Knowledge Junction on zenodo at: https://doi.org/10.5281/zenodo.NNNNNNN