

Annex I Tables for Assessing Similarity of Analogues and Category Members for Read-Across^a

^aShaded analogues are considered part of the category. Data for un-shaded analogues reduce uncertainty and add weight-of-evidence.

Table 1: Comparison of Substance Identification, Structure and Chemical Classifications


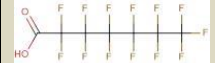
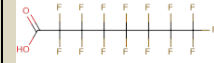

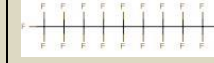
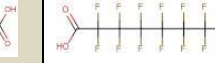
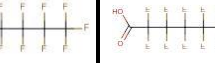
	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUA	PFDoA
Name	Perfluorohexanoic acid	Perfluoroheptanoic acid	Perfluorooctanoic acid	Perfluorononanoic acid	Perfluorodecanoic acid	Perfluoroundecanoic acid	Perfluorododecanoic acid
CAS No:	307-24-4	375-85-9	335-67-1	375-95-1	335-76-2	2058-94-8	307-55-1
SMILES	<chem>FC(F)(C(F)(F)C(=O)O)C(F)(F)C(F)(F)C(F)(F)F</chem>	<chem>O=C(O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F</chem>	<chem>C(=O)(C(C(C(C(C(C(F)(F)F)(F)F)(F)F)(F)F)(F)F)F)F)O</chem>	<chem>C(F)(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(=O)O</chem>	<chem>C(=O)(C(C(C(C(C(C(C(C(F)(F)F)(F)F)(F)F)(F)F)(F)F)F)F)F)O</chem>	<chem>C(=O)(C(C(C(C(C(C(C(C(C(F)(F)F)(F)F)(F)F)(F)F)(F)F)F)F)F)F)O</chem>	<chem>C(=O)(C(C(C(C(C(C(C(C(C(C(F)(F)F)(F)F)(F)F)(F)F)(F)F)F)F)F)F)F)O</chem>
2D Structure							
Molecular Formula:	C ₆ HF ₁₁ O ₂	C ₇ HF ₁₃ O ₂	C ₈ HF ₁₅ O ₂	C ₉ HF ₁₇ O ₂	C ₁₀ HF ₁₉ O ₂	C ₁₁ HF ₂₁ O ₂	C ₁₂ HF ₂₃ O ₂

Table 2: Comparison of Physico-Chemical and Molecular Properties¹

	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUA	PFDoA
Name	Perfluorohexanoic acid	Perfluoroheptanoic acid	Perfluorooctanoic acid	Perfluorononanoic acid	Perfluorodecanoic acid	Perfluoroundecanoic acid	Perfluorododecanoic acid
Molecular Weight¹	314.06	364.06	414.07	464.08	514.09	564.10	614.10
Log Kow^a	3.48	4.14	4.814	5.48	6.15	6.82	7.49
Vapor Pressure^b	263 Pa	149 Pa 10 mmHg (M ³)	139 Pa	11.1 Pa	3.6 Pa	1.21 Pa	0.573 Pa
Density⁴	1.7±0.1 g/cm ³	1.7±0.1 g/cm ³	1.7±0.1 g/cm ³	1.8±0.1 g/cm ³	1.8±0.1 g/cm ³	1.8±0.1 g/cm ³	1.8±0.1 g/cm ³
Melting Point^b	23.07 deg C 14 deg C (M ³)	15.26 deg C 30 deg C (M ³)	55 deg C (M) 59-60 deg C (M ³)	38.94 deg C	50.26 deg C 78-81 deg C (M ³)	61.23 deg C 83-87 deg C (M ³)	71.84 deg C 108 (M ²)
Water Solubility^c	27.12	3.647	5.786	0.06258	0.008043	0.001024	0.0001293
Boiling Point^b	165.08 deg C 157 deg C (M ³)	184.82 deg C 177 (M ²)	203.77 deg C 189 (M ²)	221.92 deg C 218 deg C (M ³)	239.28 deg C 218 deg C (M ³)	255.83 deg C 238 deg C (M ³)	271.58 deg C 249 (M ²)
pKa⁵	0.6	2.4	2.5 (M ⁶)	2.4	2.8 (M ²)	2.4	2.4

Values typically derived from EPISuite v4.1, ^a KOWWIN Program (v1.68), ^b MPBPWIN v1.43, ^c at 25 deg C (mg/L) Kow (WSKOW v1.42); ² values from OECD QSAR Toolbox v3.3; ³ values from ChemSpider; ⁴ ACD/Lab Percepta Platform - PhysChem Module (from ChemSpider); ⁵ Predicted by PERCEPTA; ⁶ measured from Prevedouros, K., Cousins, I.T., Buck, R.C. and Korzeniowski, S.H.2006. Sources, fate and transport of perfluorocarboxylates. Environ. Sci. Technol.40: 32-44.

Table 4: Comparison of Abiotic Transformation and Toxicokinetics

	PFH_xA	PFH_pA	PFOA	PFNA	PFDA	PFUA	PFDoA
Name	Perfluorohexanoic acid	Perfluoroheptanoic acid	Perfluorooctanoic acid	Perfluorononanoic acid	Perfluorodecanoic acid	Perfluoroundecanoic acid	Perfluorododecanoic acid
Abiotic Transformation			Abiotic degradation is slow: atmospheric lifetime = 130 days; hydrolytic half-life > 92 years (most likely 235 years); no photodegradation; DT50 for indirect photolysis is 349 days ¹				
Toxicokinetics²							
Absorption			>92%				
Distribution			PPB>95% serum, liver	liver, no kidneys ²			
Half-life in human	32 days		2.3 years				
half-life in rat (days)	2.5.hours ²	0.05 (F); 0.1 (M) ³		4-2.44 (F) 29.5-130 (M) ²	58.57 (F); 39.92 (M) ²		
half-life in mouse							

	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUA	PFDoA
(days)				25.8-68.4 (F) 34.3- 68.9 (M).			
Tmax	0.3-0.8 hours ²						
Elimination ³	more rapidly cleared by urine ²		poorly eliminated		rapid clearance from plasma was not due to urinary elimination ³		

¹ from <http://echa.europa.eu/documents/10162/1b26b219-6783-4981-9acf-154d620937b4>; ² from Bull, S., Burnett, K., Vassaux, K., Ashdown, L., Brown, T. and Rushton, L. 2014. EFSA supporting publication 2014:EN-572, 345 pp.; ³ from Ohmori, K., Kudo, N., Katayama, K., Kawashima, Y. 2003. Toxicology 184: 135-140.

Table 6: Comparison Toxicophores

	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUA	PFDoA
Name	Perfluorohexanoic acid	Perfluoroheptanoic acid	Perfluorooctanoic acid	Perfluorononanoic acid	Perfluorodecanoic acid	Perfluoroundecanoic acid	Perfluorododecanoic acid
Toxicophores ¹	ISS structural alert for nongenotoxic carcinogenicity, PFOA; H-acceptor-path3-H-acceptor (Micronucleus); Cramer Class III	ISS structural alert for nongenotoxic carcinogenicity, PFOA; H-acceptor-path3-H-acceptor (Micronucleus); Cramer Class III	ISS structural alert for nongenotoxic carcinogenicity, PFOA; H-acceptor-path3-H-acceptor (Micronucleus); Cramer Class III	ISS structural alert for nongenotoxic carcinogenicity, PFOA; H-acceptor-path3-H-acceptor (Micronucleus); Cramer Class III	ISS structural alert for nongenotoxic carcinogenicity, PFOA; H-acceptor-path3-H-acceptor (Micronucleus); Cramer Class III	ISS structural alert for nongenotoxic carcinogenicity, PFOA; H-acceptor-path3-H-acceptor (Micronucleus); Cramer Class III	ISS structural alert for nongenotoxic carcinogenicity, PFOA; H-acceptor-path3-H-acceptor (Micronucleus); Cramer Class III
²			prediction of probability of liver effects>0.7	prediction of probability of liver effects>0.7	prediction of probability of liver effects>0.7		prediction of probability of liver effects>0.7
³			Nuclear receptor alert ^a : PPAR;	Nuclear receptor alert ^a : PPAR;	Nuclear receptor alert ^a : PPAR;	Nuclear receptor alert ^a : PPAR; PPAR γ full agonism prediction ^b	Nuclear receptor alert ^a : PPAR; PPAR γ full agonism prediction ^b

¹ OECD QSAR Toolbox 3.3. ² ACD/Percepta ACD/Labs 2015, acdlabs.com, most common forms of the structures were selected and Probability of Health Effects (Liver) were calculated. ³ COSMOS profilers with positive hits: ^a see Steinmetz et al. (2015), Mellor et al. (2015), ^b see Tsakovska et al. (2014);

Table A.8: Comparison of Toxicologically Relevant *In Vivo*, *In Vitro* and *Ex Vivo* Data

	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUA	PFDoA
Name	Perfluorohexanoic acid	Perfluorooctanoic acid	Perfluorooctanoic acid	Perfluorononanoic acid	Perfluorodecanoic acid	Perfluoroundecanoic acid	Perfluorododecanoic acid
Endpoint: NOAEL (Repeat dose toxicity)	20- 200 (mg/kg/day) [2,10]		0.3-76 (mg/kg/day) 10 (ppm) [2-9]			0.1 (mg/kg/day) [1]	
Endpoint: BMDL10 (Repeat dose toxicity)	13-21 (mg/kg/day) [2]						
Endpoint: NOEL (Repeat dose toxicity)	100 (mg/kg/day) [2]						
Endpoint: NOAEL (reproductive toxicity)			0.3- 150 (mg/kg/day) [12-15]				0.5-0.6 (mg/kg/day) [16]
Endpoint: NOAEL (Developmental and maternal toxicity)	100 (mg/kg/day) [11, 17]					0.3(mg/kg/day) [1]	
Endpoint: BMD5 (Developmental and				0.43- 3.84			

	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUA	PFDoA
maternal toxicity)				(mg/kg) [18]			
Endpoint: BMD5(Teratogenicity)				0.36 (mg/kg) [18]			
Endpoint:BMDL5(Tera togenicity)				0.43 – 3.11 (mg/kg) [18]			
Endpoint:LOAEL(Deve lopmental and maternal toxicity)			0.6-150 (mg/kg/day) [12-15]				
Endpoint: LC50 (Acute oral toxicity)			250 – 680 (mg/kg) [19,20]				
Endpoint: Genotoxicity (AMES, HGPRT assay, Chromosomal abrration)			3x Negative [21-23] 2 x Positive [23]		Negative [28, 29]		
Endpoint: Genotoxicity (SCGE assay)			Induced strand breaks[24]				
Endpoint: Genotoxicity (COS-1 cell assay)			Activated PPAR α in mouse and human plasmids[25]				
Endpoint: Genotoxicity (Comet assay)			Inceased DNA damage [26]				

		PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUA	PFDoA
Toxcast [35]	cytotox.tox21.nhit				1			
	cytotox.tox21.min.uM				43.2			
	cytotox.tox21.max.uM				43.2			
	cytotox.SRB.nhit				1	8	8	
	cytotox.SRB.min.uM				36.3	21.6	7.5	
	cytotox.SRB.max.uM				36.3	35.6	34.3	
	proliferation.nhit		1	1	3	6	6	
	proliferation.min.uM		11.6	123.2	35.3	10.4	3.6	
	proliferation.max.uM		11.6	123.2	113.8	108.9	87.5	

References for Table 8

- [1] Takahashi, M., Ishida, S., Hirata-Koizumi, M., Ono, A. and Hirose, A. 2014. Repeated dose and reproductive/developmental toxicity of perfluoroundecanoic acid in rats. *J. Toxicol. Sci.* 39: 97-108.
- [2] Loveless, S.E., Finlay, C., Everds, N.E, Frame, S.R, Gillies, P.J, O'Connor J.C., Powley, C.R. and Kennedy, G.L. 2006. Comparative responses of rats and mice exposed to linear or branched ammonium perfluorooctanoate (APFO). *Toxicology* 220: 203-217.
- [3] Goldenthal, E.I., Jessup, D.C., Geil, R.G. and Mehring, J.S. 1978. Ninety-day subacute rat toxicity study. 137-085. International Research and Development Corporation.
- [4] Perkins, R.G., Butenhoff, J.L., Kennedy, G.L. and Palazzolo, M. 2004. 13-week dietary toxicity study of ammonium perfluorooctanoate (APFO) in male rats. *Drug Chem. Toxicol.* 27: 361-378.

- [5] Butenhoff, J., Costa, G., Elcombe, C., Farrar, D., Hansen, K., Iwai, H., Jung, R., Kennedy, G., Lieder, P., Olsen, G. and Thomford, P. 2002. Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months, *Toxicol. Sci.* 69: 244-257.
- [6] Sibinski, L.J. 1987. Final report of a two-year oral (diet) toxicity and carcinogenicity study of fluorochemical FC-143 (perfluorooctanane ammonium carboxylate) in rats.. Vol. 1-4, 3M Company/RIKER, Exp. No. 0281CR0012; 8 EHQ-1087-0394, October 16, 1987.
- [7] Palazzolo, M.J. 1993. Thirteen-week dietary toxicity study with T-5180, ammonium perfluorooctanoate (CAS No. 3825-26-1) in male rats. Final report. Laboratory Project Identification HWI 6329-100, Hazelton, Wisconsin Inc.
- [8] Son, H.-Y., Kim, S.-H., Shin, H.-I., Bae, H.-I. and Yang, J.-H. 2008. Perfluorooctanoic acid-induced hepatic toxicity following 21-day oral exposure in mice. *Arch. Toxicol.* 82: 239-246.
- [9] Wolf, D.C., Moore, T., Abbott, B.D., Rosen, M.B., Das, K.P., Zehr, R.D., Lindstrom, A.B., Strynar, M.J. and Lau, C. 2008. Comparative hepatic effects of perfluorooctanoic acid and WY 14,643 in PPAR- α knockout and wild-type mice. *Toxicol. Path.* 36: 632-639.
- [10] Chengelis, C.P., Kirkpatrick, J.B., Radovsky, A. and Shinohara, M. 2009. A 90-day repeated dose oral (gavage) toxicity study of perfluorohexanoic acid (PFHxA) in rats (with functional observational battery and motor activity determinations). *Reprod. Toxicol.* 27: 342-351.
- [11] Loveless, S.E., Slezak, B., Serex, T., Lewis, J., Mukerji, P., O'Connor, J.C., Donner, E.M., Frame, S.R., Korzeniowski, S.H. and Buck, R.C. 2009. Toxicological evaluation of sodium perfluorohexanoate *Toxicology* 264: 32-45.
- [12] Lau, C., Thibodeaux, J.R., Hanson, R.G., Narotsky, M.G., Rogers, J.M., Lindstrom, A.B. and Strynar, M.J. 2006. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. *Toxicol. Sci.* 90: 510-518.
- [13] Butenhoff, J.L., Kennedy, G. L. Jr, Frame, S.R., O'Connor, J.C. and York, R.G. 2004. The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. *Toxicology* 196: 95-116.
- [14] Gortner, E.G. 1982. Oral Teratology Study of T-2998 CoC in Rats. Report prepared by Safety Evaluation Laboratory and Riker Laboratories, Inc. Experiment No. 0681TR0110. AR226-1136. U.S. Environmental Protection Agency, Washington, DC, U.S.A.
- [15] Abbott, B.D., Wolf, C.J., Schmid, J.E., Das, K.P., Zehr, R.D., Helfant, L., Nakayama, S., Lindstrom, A.B., Strynar, M.J. and Lau, C. 2007. Perfluorooctanoic Acid Induced Developmental Toxicity in the Mouse is Dependent on Expression of Peroxisome Proliferator Activated Receptor-alpha. *Toxicol. Sci.* 98: 571-581.

- [16] Kato, H., Fujii, S., Takahashi, M., Matsumoto, M., Hirata-Koizumi, M., Ono, A. and Hirose, A. 2014. Repeated dose and reproductive/developmental toxicity of perfluorododecanoic acid in rats. *Environ. Toxicol.* doi: 10.1002/tox.21996
- [17] Iwai, H. and Hoberman, A.M. 2014. Hiroyuki Iwai and Alan M. Hoberman. Oral (Gavage) Combined Developmental and Perinatal/Postnatal Reproduction Toxicity Study of Ammonium Salt of Perfluorinated Hexanoic Acid in Mice. *International Journal of Toxicology-2014-Iwai-219-37*
- [18] Das, K.P., Grey, B.E., Rosen, M.B., Wood, C.R., Tatum-Gibbs, K.R., Zehr, R.D., Strynar, M.J., Lindstrom, A.B. and Lau, C. 2014. Developmental toxicity of perfluorononanoic acid in mice. *Reprod. Toxicol.* 51: 133-144.
- [19] Dean, W.P., Jessup, D.C., Thompson, G., Romig, G. and Powell, D. 1978. Fluorad Fluorochemical Surfactant FC-95 Acute Oral Toxicity (LD50) Study in Rats. Study No. 137-083, International Research and Development Corporation.
- [20] Glaza, S.M. 1997. Acute oral toxicity study of T-6669 in rats. Corning Hazalton Inc. CHW 61001760. Sponsored by 3M Company, St. Paul, Minnesota. January 10. USEPA AR226- 0420.
- [21] Lawlor, T.E. 1995. Mutagenicity test with T-6342 in the Salmonella-Escherichia coli/mammalian microsome reverse mutation assay. Laboratory Number: 17073-0-409. Corning Hazleton, Inc., Vienna, VA. 3M Company St. Paul, MN. US E.PA. AR226-0436; Lawlor, T.E. 1996. Mutagenicity test with T-6564 in the Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay. with a confirmatory assay. Corning Hazleton Inc., Final Report. CHV Study Number: 17750-0-409R. September 13, 1996. U.S. EPA AR226-0432.
- [22] Sadhu, D. 2002. CHO/HGPRT forward mutation assay. Corning Hazleton Inc., Vienna, VA. Study Number: 17750-0-437CO, September 16, 1996. U.S. EPA AR226-0433.
- [23] Murli, H. 1996. Mutagenicity test on T-6342 measuring chromosomal aberrations in human whole blood lymphocytes with a confirmatory assay with multiple harvests. Corning Hazleton, Inc., Vienna, VA. Study Number: 17073-0-449CO, November 1, 1996. U.S. EPA AR226-0433; Murli, H. 1996e. Mutagenicity test on T-6342 measuring chromosomal aberrations in Chinese Hamster Ovary (CHO) cells with a confirmatory assay with multiple harvests. Corning Hazleton Inc., Vienna VA. Study Number: 17073-0-437CO, September 16, 1996. U.S. EPA AR226-0434; NOTOX, 2000. Evaluation of the ability of T-7524 to induce chromosome aberrations in cultured peripheral human lymphocytes. NOTOX Project Number 292062. Hertogenbosch, The Netherlands.

- [24] Yao, X. and Zhong, L. 2005. Genotoxic risk and oxidative DNA damage in HepG2 cells exposed to perfluorooctanoic acid. *Mutat.Res.* 587, 38-44.
- [25] Wolf, C.J., Takacs, M.L., Schmid, J.E., Lau, C. and Abbott, B.D. 2008. Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. *Toxicol. Sci.* 106: 162-171.
- [26] Eriksen, K.T., Raaschou-Nielsen, O., Sørensen, M., Roursgaard, M., Loft, S. and Mølle, P. 2010. Genotoxic potential of the perfluorinated chemicals PFOA, PFOS, PFBS, PFNA and PFHxA in human HepG2 cells. *Mut. Res./Gen. Toxicol. Environ. Mutagen.* 700: 39-43.
- [27] Huang, Q., Zhang, J., Martin, F.L., Peng, S., Tian, M., Mu, X. and Shen, H. 2013. Perfluorooctanoic acid induces apoptosis through the p53-dependent mitochondrial pathway in human hepatic cells: a proteomic study. *Toxicol. Lett.* 223: 211-220.
- [28] Benigni, R. 2011. CCRIS database, Toxnet databases. CCRIS database, 02.5.2011
- [29] Kazius, J., McGuire, R. and Bursi, R. 2005. Derivation and validation of toxicophores for mutagenicity prediction. *J. Med. Chem.* 48: 312-332.
- [30] Wolf, C.J., Schmid, J.E., Lau, C. and Abbott, B.D. 2012. Activation of mouse and human peroxisome proliferator-activated receptor-alpha (PPAR α) by perfluoroalkyl acids (PFAAs): Further investigation of C4–C12 compounds. *Reprod. Toxicol.* 33: 546-551.
- [31] Watkins, A.M., Wood, C.R., Lin, M.T. and Abbott, B.D. 2015. The effects of perfluorinated chemicals on adipocyte differentiation in vitro. *Mol. Cell. Endocrinology* 400: 90-101.
- [32] Stein, C.R., Wolff, M.S., Calafat, A.M., Kato, K. and Engel, S.M. 2012. Comparison of polyfluoroalkyl compound concentrations in maternal serum and amniotic fluid: A pilot study. *Reprod. Toxicol* 34: 312-316.
- [33] Jiang, W., Zhang, Y., Zhu, L. and Deng, J. 2014. Serum levels of perfluoroalkyl acids (PFAAs) with isomer analysis and their associations with medical parameters in Chinese pregnant women. *Environ. Internat.* 64: 40-47.
- [34] Tsakovska, I., Al Sharif, M., Alov, P., Diukendjieva, A., Fioravanzo, E., Cronin, M.T.D. and Pajeva, I. 2014. Molecular modelling study of the PPAR γ receptor in relation to the Mode of Action/Adverse Outcome Pathway framework for liver steatosis. *Int. J. Mol. Sci.* 15: 7651-7666.
- [35] ToxcastTM data , Provider the USEPA; <http://www.epa.gov/ncct/toxcast/data.html>