Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

8:2 Fluorotelomer alcohol (8:2 FTOH)

EC number: 211-648-0
CAS number: 678-39-7

CLH-O-0000002460-84-03/A1

Adopted

06 March 2013

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	8:2 Fluorotelomer alcohol (3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecan-1-ol)
EC number:	211-648-0
CAS number:	678-39-7
Annex VI Index number:	
Degree of purity:	99 %
Impurities:	Impurities: < 1%; 8:2 Fluorotelomer α-β unsaturated alcohol ($F(CF_2)_7CF=CHCH_2OH$)

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation		
Current proposal for consideration by RAC	Repr. 1B; H360 D	Repr. Cat. 2; R 61
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Pictogram: GHS08, Signal word: Danger Hazard statement codes: H360 D	Class of danger: Toxic (T)

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Proposed classification and labelling based on DSD criteria:

Class of danger: Toxic (T), R 61

Repr. Cat. 2; R 61 May cause harm to the unborn child.

Proposed classification based on CLP criteria:

Pictogram: GHS08, Signal word: Danger

Hazard statement codes: H360 D May damage the unborn child

Repr. 1B; H360 D

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				
2.2.	Flammable gases				
2.3.	Flammable aerosols				
2.4.	Oxidising gases				
2.5.	Gases under pressure				
2.6.	Flammable liquids				
2.7.	Flammable solids				
2.8.	Self-reactive substances and mixtures				
2.9.	Pyrophoric liquids				
2.10.	Pyrophoric solids				
2.11.	Self-heating substances and mixtures				
2.12.	Substances and mixtures which in contact with water emit flammable gases				
2.13.	Oxidising liquids				
2.14.	Oxidising solids				
2.15.	Organic peroxides				
2.16.	Substance and mixtures corrosive to metals				
3.1.	Acute toxicity - oral				
	Acute toxicity - dermal				
	Acute toxicity - inhalation				
3.2.	Skin corrosion / irritation				
3.3.	Serious eye damage / eye irritation				
3.4.	Respiratory sensitisation				
3.4.	Skin sensitisation				
3.5.	Germ cell mutagenicity				
3.6.	Carcinogenicity				
3.7.	Reproductive toxicity	Repr. 1B; H360	-	-	-

		D			
3.8.	Specific target organ toxicity –single exposure				
3.9.	Specific target organ toxicity – repeated exposure				
3.10.	Aspiration hazard				
4.1.	Hazardous to the aquatic environment				
5.1.	Hazardous to the ozone layer				

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Danger
Hazard statements: H360 D
Precautionary statements: -

Proposed notes assigned to an entry:

Table 4: Proposed classification according to DSD

Hazardous property	**Proposed classification**	**Proposed SCLs**	**Current classification [1)]**	**Reason for no classification [2)]**
Explosiveness				
Oxidising properties				
Flammability				
Other physico-chemical properties				
Thermal stability				
Acute toxicity				
Acute toxicity – irreversible damage after single exposure				
Repeated dose toxicity				
Irritation / Corrosion				
Sensitisation				
Carcinogenicity				
Mutagenicity – Genetic toxicity				
Toxicity to reproduction – fertility				
Toxicity to reproduction – development	Rep. Cat. 2; R 61	-	-	-
Toxicity to reproduction – breastfed babies. Effects on or via lactation				
Environment				

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger: Toxic (T),
R-phrases: R 61
S-phrases: -

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

No current classification in annex VI

2.2 Short summary of the scientific justification for the CLH proposal

Using a weight-of–evidence approach based on 1) ADME consideration of female rats exposed to FTOH, and their low levels of serum metabolites combined with 2) supportive information concerning developmental effects of one major serum metabolite (PFOA), 3) the long half-life of PFOA in humans (2-4 years) and bioaccumulation and 4) coherent developmental effects following exposure to 8:2 FTOH and PFOA, we propose a classification for 8:2 FTOH equal to PFOA.

The classification for PFOA was agreed in the former TC C&L group in October 2006 for reproductive toxicity with Repr. Cat.2 R61 according to DSD criteria (classification according to CLP: Repr. 1B, H360D).

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

8:2 Fluorotelomer alcohol is currently not included in Annex VI, Table 3.1 in the CLP Regulation.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

8:2 Fluorotelomer alcohol is currently not included in Annex VI Table 3.2 in the CLP Regulation.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

8:2 Fluorotelomer alcohol is currently not self-classified based on the CLP Regulation criteria

2.4.2 Current self-classification and labelling based on DSD criteria

8:2 Fluorotelomer alcohol is currently not self-classified based on the DSD criteria

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

No need for justification.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

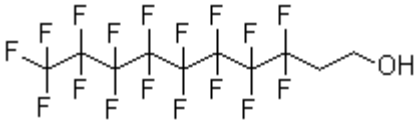
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	211-648-0
EC name:	3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecan-1-ol
CAS number (EC inventory):	
CAS number:	678-39-7
CAS name:	1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-
IUPAC name:	3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecan-1-ol
CLP Annex VI Index number:	-
Molecular formula:	$C_{10}H_5F_{17}O$ $F(CF_2)_8CH_2CH_2OH$
Molecular weight range:	464.12

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
8:2 Fluorotelomer alcohol	> 99%		

Current Annex VI entry: -

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
8:2 Fluorotelomer α-β unsaturated alcohol ($F(CF_2)_7CF=CHCH_2OH$)	Impurities: < 1%		

Current Annex VI entry: -

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks

Current Annex VI entry:

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Waxy solid		
Melting/freezing point	47°C	DuPont, 2010	
Boiling point	201,3°C at 760 mm Hg	Kaiser et al., 2004	
Relative density	1.7 g/mL	DuPont, 2010	
Vapour pressure	31 Pa at 25 ºC (Retention time method) 29 Pa at 45ºC (Headspace GC/AED method) 254 Pa ved 25 ºC , volatile, 99.9 % detected mainly in the gassousphase in the atmosphaere 0.227 kPa 0.023 mmHg	Vapour pressure seem sensitive to choice of method. Cobranchi et al., 2006 Stock et al., 2004 Lei et al., 2004 Berti WR DuPont EMSE Report No 92-02	
Surface tension	No information available		
Water solubility	1.4 x 10^{-4} g/l or 140 µg/l at 25 °C	Berti WR DPont EMSE Report No 92-02	
Partition coefficient n-octanol/water	No information available		
Flash point	110°C [closed cup]	DuPont, 2010	
Flammability	No information available		
Explosive properties	No information available		
Self-ignition temperature	No information available		
Oxidising properties	No information		

	available		
Granulometry	No information available		
Stability in organic solvents and identity of relevant degradation products	No information available		
Dissociation constant	No information available		
Viscosity	No information available		

2 MANUFACTURE AND USES

2.1 Manufacture

Manufacture of raw material for the synthesis of fluorotelomer-based surfactants and polymeric products.

2.2 Identified uses

8:2 FTOH is mainly used for coating of textiles, paper and carpets to achieve oil, stain and water repellent properties, cleaning agents and is present as residual raw materials (Dinglasan-Panlilio and Mabury, 2006). The reaction of fluorotelomer alcohol to make fluorotelomer acrylates or methacrylate esters leaves 0.1-0.5 wt% unreacted residual FTOH. The FTOHs are present in the ultimate sales products unless removed. A Norwegian study by Berger and Thomsen (Berger and Thomsen, 2006) showed that by extraction of a Norwegian “all weather coat” with ethylacetate and methanol there were measured nearly 1000 µg/m^2 8:2 FTOH and about 20 µg/m^2 PFOA.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not considered in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Oral:

Species	Dose (mg/kg)	Observations and Remarks	Ref.
Cr:CD® (SD) Rats	0, 5, 125 mg/kg bw 8:2 FTOH (99.2% purity with 0.8% 8-2 unsaturated FTOH ($C_8F_{17}CH=CHOH$) Gavage	Rats were exposed daily by oral gavage to 8:2 FTOH (0, 5 and 125 mg/kg bw) for 45 days, as a conditioning phase, to identify and obtain steady-state plasma kinetics, followed by a recovery period with sacrifice at day 53 to study kinetics and metabolism of 8:2 FTOH along with liver GSH status. Rats were sacrifice in groups of 3, first 3/sex of the unexposed group at day 0 followed by 3/sex/dose level at days 1, 8, 25 and 45. At day 46 after conditioning, 3/sex for the 5 and 125 mg 8:2 FTOH/kg bw groups were given radioactive 8:2 FTOH except from the control group (3/sex), after which they were sacrificed at day 53 to study ADME. The metabolites were quantitatively analyzed by LC-MS/MS, and a radiochromatographic method (liquid chromatography-accurate radioisotope counting LC-ARC) along with LC-MS and GC-MS methods. Liver GSH-status did not change during the experiment Most radioactive 8:2 FTOH was found in the faeces (mice were not bile duct cannulated), relatively more at the higher dose. Elimination via urine was minor (2.48-8.98% of recovered dose), with females eliminating more than males (2-3 times). The major metabolites in urine were PFOA, 7-2 sFTOH-gluc (females only) and 8-2 uFTOH-SCysNAcetyl (females only). The net systemic uptake of 8:2 FTOH was 3-1.5 fold higher in females than in males. In the liver PFOA, PFNA and 8:2 FTOH-sulf was detected in both sexes whereas 7-3 acid was detected in males only. In the kidney PFOA was detected in males only and 8:2 FTOHSulf in females only. In plasma 8:2 FTOHSulf was detected in females only. The maximum concentration of the parent 8:2 FTOH in plasma (C_{max}) was higher in female rats (271 ng/ml) than in male rats (88 ng/ml) at the high dose level. Independent of dose level, C_{max} for PFOA in plasma from male rats observed over the 45-day conditioning period at the low (1610 ng/ml) and high dose level (36,100 ng/ml) was much greater than in female rats at the low (102 ng/ml) and high dose levels (1673 ng/ml). C_{max} in liver for 8:2 FTOH was comparable for males and	Fasano et al., 2009

		females at the low dose (309 vs. 202 ng/g, respectively) and high dose level (5843 vs. 7590 ng/g, respectively). Independent of dose level, C_{max} for PFOA in liver of male rats observed over the 45-day conditioning period at the low (4033 ng/g) and high dose level (28,900 ng/g) were found to be much greater than the female rats (82 and 878 ng/g, low and high dose level, respectively). As was observed in plasma from control rats, the concentration of PFOA in liver from control males increased with time.	
Sprague-Dawley rats (Crl:CD (SD)IGS BR rats)	5 and 125 mg/kg 8:2 FTOH in Methylcellulose (0.5% wt/vol) Gavage	Single oral dose of 8:2 FTOH was given to bile duct cannulated females and males (4 rats per sex) with 168 h collection period of urine, bile and faeces. The total recovered dose at termination was 77-89 % of administrated dose. The total absorbed dose (sum of levels in urine, bile, cage wash, residual feed and tissues) varied between 27 to 57%, with highest absorption level at the lowest dose. The ^{14}C concentrations in bile were 39-45% at the lowest dose and 19- 21% at the highest dose. The majority of 8:2 FTOH (>70%) was excreted in the faeces and 37-55% was identified as parent compound. Less than 4% of the administered dose was excreted in urine which contained low concentrations of PFOA (~1% of total ^{14}C). Females eliminated significantly more than males. No sex-differences in oral absorption or bile excretion were observed. Metabolites detected in the bile were principally composed of glucuronide and glutathione conjugates, 8:2 FTOH glucuronide conjugate, 8:2 uFTOH GSH conjugate and 8:2 FTUA GSH conjugate. Perfluorohexanoate (PFHxA) and perfluoroheptanoate (PFHpA) in addition to other metabolites was identified in excreta and plasma. The maximum concentration of 8:2 FTOH in plasma occurred at 1 h post dosing and cleared rapidly with a half-life of less than 5 h. The internal dose of 8:2 FTOH increased in a dose-dependent manner. At 7 days postdose, 4–7% of the administered radioactivity was present in tissues, and for the majority, ^{14}C concentrations were greater than whole blood with the highest concentration in fat, liver, thyroid, and adrenals. The $t_{1/2}$ for PFOA was longer in males (112-217 h) than in female rats (5.6 – 17.5h) at both dose levels.	Fasano et al., 2006
Sprague-Dawley rats (Crl:CD (SD)IGS BR rats)	0, 5, 25, 125 mg/kg/day 8:2 FTOH Gavage	Young adult male (10/group) and female (5/group) were exposed by oral gavage to 8:2 FTOH in an 84 day extended range-finding study to determine the length of time needed to reach a steady state blood fluorine level. Some animals (males and females) were allowed to recover for 75 days post-dosing. Total fluorine and 8:2 FTOH levels as well as levels of the metabolites 8:2 acid, PFOA and PFNA were measured. Steady state blood fluorine level was reached between day 60 and day 84. The plasma level of 8:2 FTOH did not exceed 0.074 ppm at steady state in neither males nor females. The	Ladics, 2001

		plasma total fluorine level was 53 ppm (males) and 23 ppm (females) in the high dose groups, whereas the level of PFOA was 65.60 ppm and 1.85 ppm in the high dose group in males and females respectively. Thus, PFOA accounts for a large portion of the fluorine present in plasma in males, which was not the case for females following 84 days of 8:2 FTOH administrations. In males, the highest concentration of PFOA was found in the liver at the lower doses and in plasma at the highest dose. Significant levels of PFOA were also found in kidneys. The levels of 8:2 acid and PFNA were low in the analysed tissues. The major metabolite in females was not identified in this study. The measurements of fluorinated compounds detected in plasma at steady state (day 84) showed that PFOA accounts for the majority of the fluorinated substances in males (85%) at 125 mg/kg /day. No information of the amount of 8:2 FTOH and PFNA in these samples were given. However, the content of 8:2 FTOH, PFOA and PFNA was measured in males and females in various tissues at day 81 from the doses of 5, 25 and 125 mg/kg/day. There were major increases of PFOA in the liver, kidney and plasma and a slight increase in the fat. There was a slight increase of PFNA in the liver and kidney but no change in the fat levels. The level of 8:2 acid showed a slight increase in the fat and liver while the kidney levels were almost unchanged.	
Sprague-Dawley rats (S-D rats)	400 mg/kg of 8:2 FTOH in corn oil i.p.	As an *in vivo* pre-study to the subsequent *in vitro* study, one male SD rat (275-300g) was exposed i.p. to 8:2 FTOH and one was exposed to the vehicle (corn oil) by intraperitoneal injection. Blood, liver and kidney samples were taken 6 h after exposure. Samples were analysed by HPLC/MS/MS for the parent compound and the various metabolites. In the in vitro studies rat hepatocytes was exposed and the metabolites were measured by HPLC/MS/MS analysis as well. Rat tissue (blood, liver and kidney) showed metabolites of 8:2 FTOH like PFOA and 8:2 FTCA and 8:2 FTUCA. A novel metabolite, PFNA was also confirmed both from *in vivo* and *in vitro* samples (rat hepatocytes) though at 10-fold lower levels than the aforementioned acids. Based on in vivo and in vitro data, this study indicates the presence of metabolites corresponding to O-glucuronide and O-sulfate in addition to GSH conjugates and dehydrofluorination to yield α,β-unsaturated GSH conjugates. These species may play a role in excretion or enterohepatic recirculation of FTOHs *in vivo*.	Martin, Mabury, and O'Brien, 2005
CD-1 mice	30 mg/kg bw 8:2 FTOH solved in deionized water:	Male CD-1 mice were exposed to 8:2 FTOH by oral gavage. The animals were sacrificed 6 h after treatment and blood and liver samples were collected. Several PFCAs were detected and quantified by LC/MSMS analyses and the volatile precursor compounds were detected	Henderson et al., 2007

	polypropylene glycol (1:1) Gavage	by GC/MS analyses. Six hours after treatment 97 ± 26 ng/ml 8:2 FTOH was detected in serum and 134 ± 42 ng/g in liver. In serum the concentration of PFOA was 972 ± 44 ng/ml and in liver 277 ± 29 ng/g while the PFNA concentrations were 65 ± 15 ng/ml in serum and 60 ± 22 ng/g in liver.	
CD-1 mice	30 mg/kg bw 8:2 FTOH in propylene glycol/water (1:1) vehicle	Timed-pregnant CD-1 mice received a single dose of 8:2 FTOH or vehicle by gavage on gestation day 8 (GD8). After birth, pups were cross-fostered with dams that had been treated at GD8 with 8:2 FTOH or vehicle Resulting in four treatment groups. Maternal and neonatal serum and liver as well as foetal and neonatal homogenate extracts were analysed by GC/MS. During gestation (GD9 to GD18), maternal serum and liver concentration 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 foetuses as early as 24 h post-treatment with increasing concentration 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 concentration decreased from 451 ± 21 ng/ml postnatal-day (PND) 1 to 52 ± 19 ng/ml PND15 and PFNA concentrations although fivefold less, exhibited a similar trend. Pups treated on GD8 and fostered by dams treated during lactation and pups exposed on GD8 showed an average PFOA concentration of 200 ± 26 ng/g at PND1 that decreased to 149 ± 19 ng/g at PND3. This decrease continued in both neonatal liver and serum from PND3 to PND15. The group of pups only exposed via lactation had a PFOA concentration of 57 ± 11 ng/ml at PND3 and 58 ± 3 ng/ml at PND15. This leads to the conclusion that neonates were exposed through lactation. The exposure of both PFOA and PFNA occurred both pre- and postnatally following maternal exposure to 8:2 FTOH on GD8.	Henderson and Smith, 2007
ddY mice	0, 0.025, 0.05, 0.1 and 0.2% (W/W) 8:2 FTOH Gavage	Male ddY mice were exposed in the diet to 8:2 FTOH for 7, 14, 21 and 28 days. These treatments induced liver enlargement in a dose - and duration - dependent manner. Peroxisome proliferation in the liver was confirmed by electron microscopic examination. They showed that peroxisomal acyl-CoA oxidase was induced by these treatments in a dose – and time – dependent manner. The concentration of PFOA and related compounds were determined in the liver and plasma. Five metabolites, PFOA, PFNA, 8:2 telomer acid and two unidentified metabolites were present in the liver and serum. There is a strong indication that the increased peroxisome proliferation is not induced by 8:2 FTOH itself but by the metabolite PFOA since there were detected an accumulation of PFOA in the liver. A high correlation (r^2 = 0.86) was observed between hepatic concentration of PFOA and the acyl-CoA oxidase activity (a peroxisomal enzyme). This was	Kudo et al., 2005

		not observed for the other metabolites.	

Inhalation:

Species	Dose	Observation and Remarks	Ref.
Cr1:CD (SD) rats	3 or 30 mg/m^3 99.2 wt.% 8:2 FTOH, 0.08% unsaturated 8:2 FTOH (8:2 uFTOH)	Two groups of 5 male and 5 female Cr1:CD (SD) rats were exposed to 8:2 FTOH by nose-only inhalation at 3 or 30 mg/m^3 for 6h. Exposure concentrations were estimated to correspond to inhaled doses of 0.7 and 7 mg/kg bw. Inhaled amounts were calculated as 366.5 and 3665 nmoles per rat. Blood samples were collected at 1, 3, 6, 12, and 24h from initiation of exposure, after which all animals were euthanized. Plasma samples were analyzed by liquid chromatography-tandem mass spectrometry. The predominant metabolites in plasma were 8:2 FTA, 7:3 Acid, PFOA, and PFNA. The unsaturated acids 8:2 FTUA and 7:3 UA and shorter chain PFCAs (PFHxA and PFHpA) occurred at lower concentrations. The major metabolites expressed as percent of dose in males were PFOA (0.09-0.12%) > 7:3 Acid (0.08%) > 8:2 FTA (0.02-0.03%) > PFNA (0.01-0.03%). The percentage of plasma PFOA in females (0.02%) was 4-6X lower than that of males (0.09-0.12%) whereas the amount of plasma 7:3 Acid was similar in females and males (0.05-0.06%) Generally, plasma concentrations of the other metabolites were lower in the females. Rapid clearance of the parent chemical was observed. The plasma concentrations of 8:2 FTOH were below the limit of quantification (<LOQ,15 ng/ml = 32 nM) at 3 mg/m^3 and the high (30 mg/m^3) exposure had a relatively low mean ranging from 37-69 nM and declined to <LOQ at 6h post exposure. The plasma metabolite concentrations were nearly directly proportional with the 10-fold increase in exposure concentration.	Himmelstein, 2011

Dermal:

Species	Dose (mg/kg)	Observations and Remarks	Ref.
Sprague-Dawley rats (Crl:CD (SD)IGS BR rats)	125 mg/kg (10 µl/cm^2). 8:2 FTOH in	Single dermal dose of radioactive (3-^{14}C) 8:2 FTOH in 3 females and 3 males. The substance was dosed on a clipped, shaved and washed hair free area on the shoulder (5.3 cm^2). The substance was glued to the area by Scotch Brand Crazy Glue gel and covered with gauze and wrapped with Coban. Immediately after exposure the area was covered by airtight,	Fasano et al., 2006

	Methylcellulose (0.5% wt/vol)	organic volatile trap containing untreated activated charcoal. After 6h exposure the test substance were removed and washed. The majority of the dermal dose either volatilized from the skin (37%) or was removed by washing (29%). Following the 6h dermal exposure and a 7-day collection period, excretion of total radioactivity via urine (< 0.1%) and faeces (< 0.2%) was minor, and radioactivity concentrations in most tissues were below detection limit. Based on average amount of 8:2 FTOH applied (~31,250 µg), total radioactive residues at termination were detected at site of application (21-58 µg equiv/g), in fat (0.25-0.36 µg equiv/g) and in liver (0.09-0.24 µg equiv/g). The total absorbed dose following a single 6 h exposure and a 7- day recovery collection period was low, ranging from 0.5 to 1% and was primarily due to the radioactivity at the application site. The conclusion from the study was that systemic availability of 8:2 FTOH following dermal exposure was negligible.	

In vitro metabolism:

Species	**Dose (mg/kg)**	**Observations and Remarks**	**Ref.**
Sprague-Dawley rats (Crl:CD (SD)IGS BR rats), CD mice (Crl:CD1 (ICR) and human hepatocytes	0, 5, 20 and 100 µM 8:2 FTOH for 2h at 37°C.	In vitro metabolism of [14C] 8:2 FTOH and selected acid metabolites were measured in male rat, mouse and human hepatocytes, and in male rat, mouse and human liver microsomes and cytosol of commercial origin. Hepatocytes from male rats and mice at age 8-12 weeks and human hepatocytes of commercial origin from three male donors aged 54-80 years old were used. The viability for all cell types measured by Trypan blue exclusion were >85%. The cells were exposed to [^{14}C] 8:2 FTOH for 2h at 37°C. The human cells cultures were run in duplicate, whereas the rodent cells were run in triplicate. Rodent hepatocytes eliminated 8:2 FTOH ($T_{1/2}$ = 9.9–12.9 min) about 3 times faster than human hepatocytes ($T_{1/2}$ = 35.9 min). A number of metabolites were identified. PFOA was generated in human hepatocytes, although at low rates (0.012 pmol/min /10^6 cells) compared to rat and mouse hepatocytes that produced 5-fold and 11.9-fold more PFOA than human hepatocytes, respectively. Neither PFOA nor PFNA were detected from incubation with human microsomes. The activities of the microsomal fractions used were however not tested. The percentages of 8:2 FTOH metabolized to PFOA, based on the molar concentration detected at the end of the incubation periods, were small, 0.47, 0.24, and 0.02% for mouse, rat and human hepatocytes, respectively. The overall	Nabb et al., 2007

		results indicated that 8:2 FTOH is extensively metabolized in rats and mice and to a lesser extent in humans. Metabolism of 8:2 FTOH to perfluorinated acids was small and likely mediated by enzymes in the microsomal fraction.	
Sprague-Dawley rats (S-D rats)	20-200 µM 8:2 FTOH	Hepatocytes were isolated from young (275-300g) male SD rat liver perfused with collagenase. The isolated hepatocytes were exposed to 8:2 FTOH and the samples were analysed by HPLC/MS/MS for the parent compound and the various metabolites for 1-4 h. Rat hepatocytes showed metabolites of 8:2 FTOH like PFOA and PFNA and others after 1-3 h of exposure. After 4 h with 18 µM 8:2 FTOH, 78% of the parent compound had been biotransformed; of the transformed fraction there were 2.9 % 8:2 FTCA, 4.1 % 8:2 FTUCA, 1.4 % PFOA, and <0.2 % PFNA. Two other perfluorinates acids were detected in isolated rat hepatocytes: tetrahydroperfluoroalkyl carboxylate (THPFCA) and dihydroperfluoroalkyl carboxylate (DHPFCA). In addition aldehyde metabolites were detected. These substances are unstable except under special treatment conditions were they form stable hydrazone derivatives. Using 2,4-dinitrophenylhydrazine (DNPH) trapping, the immediate oxidation product of 8:2 FTOH was identified as 8:2 fluorotelomer aldehyde (8:2 FTAL;CF3(CF2)7CH2C(H)O). 8:2 FTAL was transient and eliminated HF non-enzymatically to yield 8:2 fluorotelomer, unsaturated aldehyde (8:2 FTUAL; CF3(CF2)6CF CHC(H)O) which was also short-lived and reacted with GSH and perhaps other endogenous nucleophiles. Four polyfluorinated acid intermediates were also detected, including 8:2 fluorotelomer carboxylate (8:2 FTCA; CF3(CF2)7CH2C(O)O−), 8:2 fluorotelomer ,-unsaturated carboxylate (8:2 FTUCA; CF3(CF2)6CFCHC(O)O−), tetrahydroperfluorodecanoate (CF3(CF2)6(CH2)2CO2−), and dihydroperfluorodecenoate (CF3(CF2)6CH CHCO2−). The pathways leading to 8:2 FTCA and FTUCA involve oxidation of 8:2 FTAL, however, the pathways leading to the latter two polyfluorinated acids remain inconclusive. Although significant, the low molar balance for the quantifiable acid metabolites, including PFOA, indicates that they are not the major metabolic fate of FTOH in rat hepatocytes *in vitro*.	Martin, Mabury, and O'Brien, 2005
Sprague-Dawley rats (Crl:CD (SD)IGS BR rats)	[3-^{14}C] 8-2 FTOH at 50µM for 2 h at 37°C	Rat hepatocytes were prepared by a two-stage collagenase perfusion method. The isolated cells were exposed to [3-^{14}C] 8-2 FTOH at 50µM and incubated at 37°C for 2 h. In extract samples from rat hepatocytes several metabolites were detected, including 8:2 FTOH*Gluc, 8:2 uFTOH*GS and PFOA.	Fasano et al., 2006

4.1.2 Human information

See 4.1.3 subheading 'Other relevant information'.

4.1.3 Summary and discussion on toxicokinetics

***In vivo*, oral:**

Absorption and excretion

Rats: The 8:2 FTOH was rapidly absorbed and excreted, mainly via faeces (>70%). The total absorbed dose within a 168h collection period (sum of levels in urine, bile, cage wash, residual feed and tissues) in rats orally dosed with 5 or 125 mg 8:2 FTOH /kg bw varied between 27 to 57%, with the highest absorption level at the lowest dose. The ^{14}C concentrations in bile were 39-45% at the lowest dose and 19-21% at the highest dose. Less than 4% was excreted via urine, and females eliminated more than males. The total recovered dose in the study was > 77% (Fasano et al., 2006). The net systemic uptake of 8:2 FTOH was 3-1.5 fold higher in females compared to males (Fasano et al., 2009).

Distribution

Rats: At day 7 following 8:2 FTOH exposure 4-7% of the oral administered dose was present in the tissues; highest in fat, liver, thyroid and adrenals (Fasano et al., 2006).

Metabolism

Rats: Four studies in rats are included (Martin et al., 2005; Ladics, 2001; Fasano et al., 2006; Fasano et al., 2009) showing that metabolites of 8:2 FTOH, including PFOA, were quickly formed in rats and were distributed to blood, liver, kidney and to a lower degree in fat.

Ladics (2001) measured total fluorine and parent/metabolite levels to examine steady state levels. They found that males had higher steady state levels of total fluorine in the blood than females. Moreover, PFOA accounted for a large portion of the fluorine present in the plasma in males, which was not the case for females. In females the plasma levels of 8:2 FTOH and other upstream metabolites such as 8-2 FTA and 7-3 acid were higher compared to plasma levels in males. A similar result was observed in Fasano et al., 2009. Moreover, C_{max} levels of PFOA in the liver were higher in males than in females. Levels of PFOA in plasma in males were 22-35 times higher than in females (Fasano et al., 2009; Ladics et al., 2001).

8:2 FTOH and most metabolites were rapidly or completely cleared from the tissues ($t_{1/2}$=5h) (Fasano et al., 2006), with the exception of PFOA (particularly in males), which exhibited significant slower rates of clearance. The major metabolites in urine were PFOA, 7-2 sFTOH-gluc (females only) and 8-2 uFTOH-SCysNAcetyl (females only; Fasano et al., 2009).

Mice: In male mice 8:2 FTOH was rapidly biotransformed to a number of metabolites, including PFOA. The metabolites were detected in the urine, serum, liver and kidney. After 6h following 30 mg 8:2 FTOH/kg bw, 972 mg PFOA/ml serum was measured (Henderson et al., 2007). 8:2 FTOH was preferentially distributed to the fat tissue, while the metabolites were generally found at greater concentrations in highly perfused lean tissues, like the liver.

Both male (Kudo et al., 2005; Henderson et al., 2007) and female (Henderson and Smith, 2007) mice have been studied. Although measured in two different studies in which males (Henderson, 2007) and females (Henderson and Smith, 2007) were exposed to the same dose of 8:2 FTOH, male serum PFOA level 6 h after exposure was 972 ± 44 ng/ml in serum whereas the pregnant female serum PFOA levels were relatively stable shortly after exposure (789 ± 41 to 668 ± 23 ng/ml at GD9 to GD18). This indicates that there are no major sex differences in mice.

Moreover, Henderson and Smith (2007) showed that when pregnant mice were administrated 8:2 FTOH the metabolites PFOA and PFNA were detected in the neonates, and that exposure also occurred via lactation.

In vivo inhalation rat: In plasma samples collected from nose-only 8:2 FTOH exposed rats rapid clearance of the parent chemical was observed. The major metabolites in males

expressed as percent of dose were PFOA (0.09-0.12%) > 7:3 Acid (0.08%) > 8:2 FTA (0.02-0.03%) > PFNA (0.01-0.03%). The percentage of plasma PFOA in females (0.02%) was 4-6X lower than that of males (0.09-0.12%) whereas the amount of plasma 7:3 Acid was similar in females and males (0.05-0.06%). Generally, plasma concentrations of the other metabolites were lower in the females.

***In vivo* dermal rat**: The systemic availability of 8:2 FTOH following dermal exposure was considered as negligible (Fasano et al., 2006).

***In vitro* metabolism:**

Three studies have been included to describe metabolism of 8:2 FTOH *in vitro*: Nabb et al., 2007; Martin, Mabury and O'Brien, 2005 and Fasano et al., 2006.

Nabb et al. (2007) studied the *in vitro* metabolism of [^{14}C] 8:2 FTOH and selected acid metabolites in rat, mouse, trout and human hepatocytes and in rat, mouse and human liver microsomes and cytosol. Cells were incubated with 8:2 FTOH for 2 h. The clearance rate of 8:2 FTOH in hepatocytes were highest in rats followed by mice>humans. The human hepatocytes do generate PFOA following exposure to 8:2 FTOH, and the rodents seem to have a higher biotransformation of 8:2 FTOH to PFOA than humans. The percentages of 8:2 FTOH metabolized to PFOA, based on the molar concentration detected at the end of the incubation periods, were small, 0.47, 0.24, and 0.02% for mouse, rat and human hepatocytes.

Martin and co-workers determined that at 4 h post-treatment of rat hepatocytes *in vitro*, 78% of the parent FTOH had been metabolized; however, 8:2 FTCA (2.9%), 8:2 FTUCA (4.1%), PFOA (1.4%) and PFNA (<0.2%) only accounted for approximately 8.5% total molar mass (Martin, Mabury and O'Brien, 2005). This indicates that metabolites such as PFOA are not the major metabolic fate of FTOHs in rat hepatocytes *in vitro*.

The incubation time for the cells with 8:2 FTOH was 2 h in the study by Nabb et al., (2007) and 4 h in the study by Martin and co-workers (2005) which lead to 0.24% PFOA and 1.4% PFOA, respectively. The percentages of PFOA thus seem to depend on the time of incubation, and thus quantification based on results from the *in vitro* studies is not recommended. Moreover only two duplicates were included in the study with human hepatocytes. From the *in vitro* studies one can conclude that all species, including humans, have the ability to metabolise 8:2 FTOH to PFOA, and that the conversion to PFOA is only a few percentages of the parent.

In conclusion, rats rapidly absorbed orally administrated 8:2 FTOH and mainly excreted it via the bile. The absorption rate was between 27-57%. Metabolites of 8:2 FTOH, including PFOA, were quickly formed and distributed to blood, liver, kidney and to a lower degree in fat. The major urine metabolite in rats is PFOA. Major sex differences exist; females have a higher net uptake of 8:2 FTOH than males, while males showed higher levels of PFOA in plasma and liver than females. The metabolites generated differed between males and females in an organ specific manner. A novel study involving exposure by inhalation (Himmelstein, 2011) generally confirmed the results obtained after oral exposure.

In mice orally administered 8:2 FTOH was rapidly biotransformed to a number of metabolites, including PFOA that was detected in urine, serum, liver and kidney. No major sex differences were observed. After exposure to 8:2 FTOH, the metabolite PFOA was found to cross both the placenta and to be excreted into milk.

The *in vitro* data suggest that hepatocytes from rats, mice and humans have the ability to biotransform 8:2 FTOH into several metabolites including PFOA. Under the conditions used in the *in vitro* studies rat hepatocytes metabolised 0.24-1.4% PFOA from 8:2 FTOH, following 2 h and 4 h incubation with 8:2 FTOH respectively. In general, humans seemed to poorly biotransform 8:2 FTOH to PFOA compared to rodent cells, but the significantly longer half-life

of PFOA in humans (2-4 years) compared to rodents (hours to days) suggests that the low levels of biotransformation may contribute to bioaccumulation of PFOA in humans.

Toxicokinetics parameters of 8:2 FTOH and the metabolite PFOA for rats, mice and humans:

Species	**Sex differences after FTOH administration**	**Sex differences after APFO/PFOA administration**	**Half life for PFOA**	**Bio-transformation of FTOH to PFOA**
Rats	Oral: *8:2FTOH:* ↑♀: C_{max} 271 ng/ml (plasma) ↓♂: C_{max} 88 ng/ml (plasma) (Fasano et al., 2009) *PFOA :* ↓♀: C_{max} 1673 ng/ml (plasma) ↑♂: C_{max} 36100 ng/ml (plasma) (Fasano et al., 2009) Tot. plasma fluorine level in blood: ♂ > ♀ Inhalation: PFOA in plasma: ↓♀: 0.02% ↑♂: 0.09-0.12% (Himmelstein et al., 2011)	Oral: Excretion after 120h: ↓♀: >99% ↑♂: 39 % (Hundley et al., 2006)	Oral administration of 8:2 FTOH: ♀$t_{1/2}^{=}$ 17.5h ♂$t_{1/2}^{=}$ 217h (Fasano et al., 2006) Injection of PFOA: ♀$t_{1/2}^{=}$ 2-4 h ♂$t_{1/2}^{=}$ 6-7 d (Ohmori et al., 2003)	*in vitro*: % metabolism of 8:2 FTOH to PFOA in hepatocytes (2h): 0.24% (Nabb et al., 2007) *in vitro*: % metabolism of 8:2 FTOH to PFOA in hepatocytes (4h): 1.4% (Martin, Mabury and O'Brien et al., 2005)
Mice	Oral: Level of PFOA in serum shortly after exposure: ♂=♀ (Henderson, 2007; Henderson and Smith, 2007)	Oral: Excretion after 120h: ♂=♀: 21% (Hundley et al., 2006)	Oral administration of PFOA: ♀$t_{1/2}^{=}$ 15.6 d ♂$t_{1/2}^{=}$ 21.7 d (Lou et al., 2009)	*in vitro*: % metabolism of 8:2 FTOH to PFOA in hepatocytes (2h): 0.47% (Nabb et al., 2007)
Human	No data	No observed sex-difference in children, but significant lower levels in females after breastfeeding.	2-4 year (Olsen et al. 2007; Bartell et al. 2009; Brede et al. 2010)	*in vitro*: % metabolism of 8:2 FTOH to PFOA in hepatocytes (2h): 0.02% (Nabb et al., 2007)

Other relevant information: Bioaccumulation of the metabolite PFOA in humans

8:2 FTOH is metabolised to PFOA which is an end product. Due to the long half-life of PFOA in humans bioaccumulation of PFOA is considered as an important factor in the toxicity of FTOH.

Based on *in vitro* studies with hepatocytes it was shown that human hepatocytes exhibit the ability to biotransform 8:2 FTOH to PFOA (Nabb et al., 2007). In a study by Martin et al. (2005) using rat hepatocytes the conversion of 8:2 FTOH to PFOA was found to be 1.4% compared to 0.24% in the study by Nabb et al. (2007). This discrepancy indicates that there is some variations in the measured portion of PFOA converted from of 8:2 FTOH which probably depends on the study design. Hence, to use of the exact conversion portion of PFOA for humans based on one *in vitro* study is not recommended.

In two recent studies from Sweden and Norway significantly elevated PFOA levels have been reported in professional ski waxers, in comparison to general populations (Freberg et al. 2010, Nilsson et al. 2010a). In the Swedish study, blood samples were collected before the skiing season, i.e., pre-season, the next collection was at four FIS World Cup competitions in cross country skiing, and the final collection was after an unexposed 5-month post-season period. The exact composition of fluorinated additives are rarely disclosed by producers. Fluorinated organic components are added to most glide waxes due to their unique surfactant properties. The precursor molecules have not been identified in this study nor is it known if they are present in the ski wax as a byproduct or are formed during the process of heating the wax upon application (Nilsson et al. 2010a). The PFOA levels in three technicians with “low” initial levels of PFOA (<10.0 ng/mL in pre-season blood) increased from before season to pre-season by 254, 134, and 120% respectively, whereas five technicians with initial “high” initial levels (>100 ng/mL in preseason sample) seemed to be at steady state. In the Norwegian study, serum samples from 13 professional male ski waxers were collected at three occasions (Freberg et al. 2010). The first blood sample was drawn at the end of season I, the second at the beginning of season II and the third at the end of season II. The median concentration of PFOA was 50 ng/mL (range; 15-174 ng/mL), which is approximately 25 times higher than the background level. A statistically significant positive association between the numbers of years as a ski waxer and concentration of PFOA in serum was observed, suggestive of accumulation.

In a follow up study by Nilsson et al 2010 the objective was to determine concentrations of PFCAs, PFSAs and FTOHs in air collected in the respiratory zone of ski wax technicians' during work. The results show daily inhalation exposure of 8:2 FTOH (range = 830-250000 ng/m^3) in air which is 800 times higher than levels of PFOA (range = 80-4900 ng/m^3) in air. This suggests internal exposure of PFOA through biotransformation of 8:2 FTOH to PFOA and PFNA in humans (Nilsson et al 2010b).

For the people living in the vicinity of a fluoropolymer production facility in Ohio, a median serum PFOA concentration of 354 ng/mL has been reported (Emmett et al. 2006). From the dependence of serum levels on the person’s use of water, it was concluded that drinking water was the major route of exposure. In the same study group, markedly higher levels of PFOA were associated with working at the chemical plant that was the source of the contamination (Steenland et al. 2009). Workers who no longer worked at the plant had much higher levels than did non-workers but lower levels than those who continued working there.

In Germany, PFC contaminated material had been applied on a large agricultural area leading to the contamination of drinking water sources. Plasma PFOA levels were around 24 ng/mL in adult residents from the contaminated area which was 4.4 (males) and 8.3 (females) times higher than PFOA levels from a control region (Wilhelm et al. 2008).

Very high serum concentrations have been reported in fluorochemical production workers with mean concentrations of PFOA in the range of 500 to 7,000 ng/mL depending on the type of job (Fromme et al. 2009). The highest serum level reported for PFOA was 114,100 ng/mL in 1995.

In three publications addressing human blood levels of PFOA with life time, no correlation between PFOA concentration and age was reported (Calafat et al., 2007; Olsen 2003; 2004). However, in the US NHANES study Calafat and co-workers (2007) found higher levels of PFOA in males at age 26 and 39 (fertile age), but not at age 55, compared to females. Similar finding has also been observed in a Japanese study (Harada et al., 2004). In a study by Thomsen and co-workers relatively high levels of PFOA was found in breast milk. After breast-

feeding for a year, the concentration of PFOA in the breast milk was reduced with more than 90%. This demonstrates a significant transfer of PFOA to breast-fed children and a significantly reduced PFOA level in the mothers (Thomsen et al., 2010). A highly reduced PFOA level in breast-feeding women may at least partly explain the lower levels of PFOA in females compared to males at fertile age (26 and 39 year) shown in the NHANES study. Also, PFOA in diet is an important exposure source. It has been shown that people eating more shrimps have statistically significant higher levels of PFOA than people eating less (Haug et al., 2010). Lifestyle factors like ski-waxing, prolonged use of protective coating of fabrics, carpets and paper may also be important indoor environmental exposure sources. In a previous study, levels of PFOA in dust samples was highly correlated to serum levels in humans and the study indicated that inhalation of PFOA in the indoor environment may be a significant contributing source to total PFOA exposure (Haug et al., 2011). As a result of different activities and age of fabrics and furniture exposure via indoor environment may also vary between age groups. Taken together, breastfeeding, differences in diet, life style and indoor environment are important exposure factors not addressed in the studies by Calafat (2007) and Olsen (2003;2004) and are factors that most likely will hide the measurable accumulation of PFOA with age. This is further supported by two Norwegian studies using multiple linear regression analyses to adjust for different contributing factors. In the Norwegian Fish and Game Study (n=175) levels of PFOA in men and women serum increase statistically significant with age (Haug et al., 2010). Also in a study with 41 women in the age of 25-45 years a statistically significant increase in the PFOA levels with age was found (Haug et al., 2011). These two studies strongly indicate that PFOA levels increase with age, but that breast feeding, diet and indoor environment are important factors for PFOA exposure that need to be addressed in the evaluation of PFOA human exposure and accumulation.

In conclusion, these studies shows that PFOA have the ability to accumulate in humans as elevated concentrations are seen following specific exposure to PFOA, either via the environment (e.g contaminated drinking water) or occupationally. In addition, lack of adjustment for PFOA determinants might hide human accumulation of PFOA with age.

4.2 Acute toxicity

Tables of relevant acute toxicity studies are included below.

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Species	LD50 (mg/kg)	Observations and Remarks	Ref.
		Acute toxicity:	
Sprague-Dawley rats (Crl:CD® (SD)IGS BR rats)	> 2000 mg/kg	Range finding study Single intragastric intubation to male and female rats according to OECD Test Guideline 420. Female rats were administered 500 mg/kg and males 2000 mg/kg. No toxicity was reported. Main study Five male and female rats were given single intragastric intubation of 2000 mg/kg according to OECD Test Guideline 420. No mortality or clinical signs of toxicity were reported. There were no body weight loss and no gross lesions were present at necropsy. Minimum lethal dose = 2000 mg/kg	Finlay C, 2008

4.2.1.2 Acute toxicity: inhalation

A summary of a DuPont study on acute toxicity by inhalation of a mixture containing approximately 30 wt% 8:2 FTOH was made available by DuPont with an acute inhalation LC50 of 35.3 mg/L in rats (DuPont Co., 1980).

4.2.1.3 Acute toxicity: dermal

No data available.

4.2.1.4 Acute toxicity: other routes

No data available.

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

An oral acute toxicity study with 8:2 FTOH was available. The LD50 value from the acute toxicity study was ≥ 2000 mg/kg (limit dose).

An acute inhalation toxicity study with a mixture containing approximate 30 wt % 8:2 FTOH was made available by DuPont. The LC50 value from the acute toxicity study was 35.3 mg/L).

4.2.4 Comparison with criteria

DSD: For acute toxicity, oral, classification with R-22 is defined as 200 < LD50 ≤ 2000 mg/kg. If LD50 is > 2000 there is no category for acute toxicity, oral.

CLP: For acute toxicity, oral, Category 4 is defined as 300 < ATE ≤ 2000, where ATE is the LD50/LC50 where available. If LD50 is > 2000 there is no category for acute toxicity, oral.

DSD: For acute toxicity, inhalation, classification with R-22 is defined as 1 < LC50 ≤ 5 mg/l/4 h (aerosols) and 2 < LC50 ≤ 20 mg/l/4 h (vapours/gases). When LC50 is > 5 or 20 there is no category for acute toxicity, inhalation.

CLP: For acute toxicity, inhalation, Category 4 is defined as 1 < Category 4 ≤ 5 mg/l (dust/mist) and 10 < Category 4 ≤ 20 mg/l (vapours). When LC50 is > 5 or 20 there is no category for acute toxicity, inhalation.

4.2.5 Conclusions on classification and labelling

No classification for oral acute toxicity is proposed.

No classification for inhalation acute toxicity is proposed.

4.3 Specific target organ toxicity – single exposure (STOT SE)

No data available.

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

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4.3.2 Comparison with criteria

-

4.3.3 Conclusions on classification and labelling

No conclusion can be drawn on classification of 8:2 FTOH for STOT SE.

4.4 Irritation

4.4.1 Skin irritation

4.4.1.1 Non-human information

No data available

4.4.1.2 Human information

No data available

4.4.1.3 Summary and discussion of skin irritation

No data were available on the irritating properties of 8:2 FTOH following skin exposure.

4.4.1.4 Comparison with criteria

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4.4.1.5 Conclusions on classification and labelling

No conclusion can be drawn on classification of 8:2 FTOH for skin irritation.

4.4.2 Eye irritation

4.4.2.1 Non-human information

No data available.

4.4.2.2 Human information

No data available.

4.4.2.3 Summary and discussion of eye irritation

No data were available on the irritating properties of 8:2 FTOH following eye exposure.

4.4.2.4 Comparison with criteria

-

4.4.2.5 Conclusions on classification and labelling

No conclusion can be drawn on classification of 8:2 FTOH for eye irritation.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

No data available

4.4.3.2 Human information

No data available

4.4.3.3 Summary and discussion of respiratory tract irritation

No data were available on the irritating properties of 8:2 FTOH following respiratory tract exposure.

4.4.3.4 Comparison with criteria

-

4.4.3.5 Conclusions on classification and labelling

No conclusion can be drawn on classification of 8:2 FTOH for respiratory tract irritation.

4.5 Corrosivity

4.5.1 Non-human information

No data available

4.5.2 Human information

No data available

4.5.3 Summary and discussion of corrosivity

No data were available on the corrosive properties of 8:2 FTOH.

4.5.4 Comparison with criteria

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4.5.5 Conclusions on classification and labelling

No conclusion can be drawn on classification of 8:2 FTOH for corrosivity.

4.6 Sensitisation

4.6.1 Skin sensitisation

4.6.1.1 Non-human information

No data available.

4.6.1.2 Human information

No data available.

4.6.1.3 Summary and discussion of skin sensitisation

No data were available on the skin sensitisation properties of 8:2 FTOH.

4.6.1.4 Comparison with criteria

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4.6.1.5 Conclusions on classification and labelling

No conclusion can be drawn on classification of 8:2 FTOH for skin sensitisation.

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

No data available

4.6.2.2 Human information

No data available.

4.6.2.3 Summary and discussion of respiratory sensitisation

No data were available on respiratory sensitization following exposure to 8:2 FTOH.

4.6.2.4 Comparison with criteria

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4.6.2.5 Conclusions on classification and labelling

No conclusion can be drawn on classification of 8:2 FTOH for respiratory sensitization

4.7 Repeated dose toxicity

Assessment provided for information only.

Tables of relevant repeated dose toxicity studies are included below.

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Species	Dose mg/kg body weight, mg/kg diet	Duration of treatment	Observations and Remarks	Ref.
Sprague-Dawley rats (Crl:CD®(SD)IGS BR rats)	0, 5, 25, 125 mg/kg bw/day 8:2 FTOH Gavage	84 days of exposure, 75 days recovery	Extended range finding study. The toxicokinetic information is given in 5.1. Young adult rats were administered 8:2 FTOH by oral gavage. 10 males/group and 5 females/group. The animals were exposed to various doses of 8:2 FTOH until steady state of fluorine in blood, around 60-84 days post-dosing. At steady state blood fluorine levels, the liver and kidneys of 5 males/group were weighed. At the end of the study liver, kidneys and fat were analysed for 8:2 FTOH, PFOA and PFNA from both males and females. In addition, pooled plasma samples obtained from males and females over the duration of the in-life phase of the study were analysed for 8:2 FTOH, 8:2 acid, PFOA and PFNA. At steady state (day 84) pooled plasma were analysed for 8:2 FTOH, PFOA and total fluorine levels. A recovery period of 75 days was included. No test related mortality was reported. A test substance-related statistically significant increase was observed in the striated teeth at 125 mg/kg/day in both males and females. There was a decrease in body weight and body weight gains in male rats at 125 mg/kg/day at day 81. Body weight was similar to control at the end of the recovery period. In females there were no changes in either body weight or bodyweight gains. There were a test substance related and statistically significant increase in relative liver weight in males administered 125 mg/kg/day at day 81 (females not sacrificed at this time point). After 75 days of recovery	Ladics, 2001

			in males, liver weights were reversed to normal whereas relative kidney weights seemed to be increased in a dose-dependent manner (non significant increases). After 75 days of recovery a test substance related and statistically significant increases in liver and kidney weights were observed in females administered 125 mg/kg/day. No histopathological examination was performed.	
Sprague-Dawley rats (Crl:CD®(SD)IGS BR rats)	0, 1, 5, 25, 125 mg/kg bw/day8:2 FTOH in 0,5 % aqueous methylcellulose	90 days, 3 month recovery group	8:2 FTOH: subchronic toxicity. 90-day oral gavage study in rats (15 rats/sex/dose). A 3 month recovery group were included for animals in control and high-dose groups only. In addition, 5 rats/sex/dose were designated for hepatic biochemical analysis following a 10-day exposure. Clinical parameters were evaluated at week 7 and 13. No test substance related mortality. A test substance-related statistically significant increase was observed in the striated teeth at 125 mg/kg/day in both males and females. No test substance related changes in body weight or body weight gain, food intake or food efficiency in any of the dose groups neither for females nor males were reported. There were no reported exposure-related effects on the neurobehavioral tests performed. The rate of hepatic β-oxidation was measured after 10 and 90 days of exposure and in the 3-month recovery groups. In males an increase in β-oxidation was observed in the high-dose group (ap. 160% during exposure and 130% after recovery). In females an increase in β-oxidation was observed in the high-dose group (130% and 152% following 10 days and 90 days exposure, respectively), but returned to control level in the recovery group. A statistically significant increase in hepatic ß-oxidation was also observed in females exposed to 25 mg/kg/day at the 90-day time-point. The test substance was considered a mild inducer of hepatic peroxisomal β-oxidation in rats. A test-substance related statistically significant increase in liver weight was reported in females from 25 mg/kg bw/day and in males and females from 125 mg/kg bw/day. Liver weights returned to normal in the recovery groups. Increased liver weight was associated with microscopic	Ladics et al., 2008

			hepatocellular hypertrophy at 125 mg/kg bw/day in males only. This effect was considered to be a physiological adaptive response to the metabolism of 8:2 FTOH and not adverse. Following 90 days exposure, a test-substance-related adverse increase in the incidence of focal hepatic necrosis was reported in males at 25 mg/kg bw/day (5/10 animals) and at 125 mg/kg bw/day (3/10 animals) with an average severity of lesion of 1,0 in both groups. No necrosis was observed in control animals. The necrosis was accompanied by a minimal inflammatory response. After 3 months of recovery the incidence of hepatocelluar necrosis in male rats was increased at 125 mg/kg bw/day (7/10 animals) compared to controls (3/10 animals). A test-substance related statistically significant increase in kidney weight was reported in males and females from 25 mg/kg bw/day during exposure, but not in the recovery groups. Moreover, microscopic renal tubular hypertrophy was observed from 25 mg/kg bw/day in males only. However, no correlative microscopic or clinical pathological evidence of renal toxicity were present. Therefore, the kidney weight changes and microscopic hypertrophy were not considered adverse findings. A test-substance-related adverse increase in the incidence and severity of chronic progressive nephropathy was reported in females at 125 mg/kg bw/day. After 3 months of recovery the incidence and severity of chronic progressive nephropathy increased at 125 mg/kg bw/day in females. The incidence and/or degree of thyroid gland lesions (altered colloid) were increased in males of all dose groups. Altered thyroid colloids occurs spontaneously in Sprague-Dawley rats and increases with age. The test substance related increase in thyroid lesions was not considered adverse as there were no other microscopic alterations observed. Other treatment related effects at 25 mg/kg/d was reduced red cell mass, reticulocytosis, increased serum cholesterol, increased alkaline phosphatase, decreased trigycerid, increased protein, albumin and calcium levels, increased urine volume and decreased urine ketone concentration. The plasma fluorine levels increased in the dosing period in the highest dose-groups (largest	

			increase in females), but three months post treatment the plasma fluorine levels were similar to controls. Urine fluorine was increased in a dose-dependent manner. After 3 months recovery, total urine fluorine was approximately 3 times greater than controls in males and slightly greater in the females of the high-dose groups, indicating continued metabolism of the test substance. The enamel organ ameloblast cells were degenerated and disorganised in rats of the high-dose group. These lesions were still present in some animals of the recovery group and were considered an adverse effect caused by fluoride toxicosis. Under the conditions of the study, the NOAEL for 8:2 FTOH for males was 5 mg/kg bw/day based on the incidence of hepatic necrosis from 25 mg/kg bw/day. The NOAEL for females was 25 mg/kg bw/day based on the incidence and severity of chronic progressive nephropathy at 125 mg/kg bw/day.	
Sprague-Dawley rats	0, 25, 100, 250 mg/kg bw/day of a test substance of fluoroalkylet anol (FTOH mixture CAS no. 65530-60-1, 95 % purity, containing 27% 8:2 FTOH)	90 days	All animals were treated daily by gavage and the test substance was solved in aqueous methylcellulose. No test substance related mortality or neurotoxicity was reported. Body weights and /or nutritional parameters were significantly reduced at 100 and 250 mg/kg bw/day, and these effects were reversible. Broken and absent teeth were observed in rats dosed with 250 mg/kg bw/day, and microscopic tooth lesions occurred at 100 and 250 mg/kg bw/day and persisted with decreased severity throughout recovery. Decreased red cell mass parameters occurred at 90 days in the 250 mg/kg bw/day group, but red cell counts were normal thereafter during recovery. Statistically significant increases in liver weight parameters were present in males and females administered 25 (males only), 100, or 250 mg/kg bw/day for 90 days. The increased liver weights correlated with microscopic hepatocellular hypertrophy in the two highest dose groups. Hepatic β-oxidation was increased in a dose-dependent manner and persisted through 1 month of recovery at 250 mg/kg bw/day. Increased kidney weights were observed at 25 (females only), 100 and 250 mg/kg bw/day. These elevated weights persisted in the high dose group after recovery and correlated with microscopic tubular hypertrophy (males only). Thyroid follicular hypertrophy was present at 100 and 250 mg/kg bw/day but was not present after	Ladics et al., 2005

			recovery. Total fluorine in whole blood increased continuously with dosing and achieved steady state in approximately 42 days. Both plasma and urine fluoride levels were elevated in a dose-dependent manner.	
Sprague-Dawley rats (S-D rats).	Oral by gavage 0, 25, 100 and 250 mg/kg/day as a suspension in 0,5 % aqueous methylcellulose of a mixture of FTOHs (27% 8:2 FTOH).	Daily exposure. Animals were exposed 74 days prior to cohabitation and during mating, gestation and lactation.	The P1 generation was evaluated for several parameters like body weights, feed consumption, clinical signs, gross pathology, sperm parameters, oestrous cycle and reproductive performance. A statistically significant decrease in bodyweight at the 250 mg/kg /d group during and after the cohabitation period, but no concomitant reduction in bodyweight gain in P1 males during that period. No test-substance related clinical signs of toxicity were observed in the P1 or F1 generation rats. There was an increase in tooth clipping required in males administered 250 mg/kg/d, consistent with dental problems in the sub-chronic toxicity study of Ladics et al.(2005). There were no test substance related effects on oestrous cycle parameters, sperm morphology and motility or epididymal sperm counts in the P1 generation. At 100 and 250 mg/kg/d there was a statistical significant increase in testicular spermatid numbers in the P1 male rats. This was not considered test-substance related because the means for these groups were within the historical control range for previous studies. The increase appeared to be due to slightly lower than usual mean weight in the control groups. There were no toxicologically significant pathology findings in the P1, F1 pups or F1 generation adult rats. The NOAEL in the P1 generation for systemic toxicity was 100 mg/kg/day based on a statistically significant reduction in body weights, and increase in tooth clipping required in males at 250 mg/kg bw/day.	Mylchreest et al., 2005 a

4.7.1.2 Repeated dose toxicity: inhalation

No data available.

4.7.1.3 Repeated dose toxicity: dermal

No data available.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

No data available.

4.7.1.6 Other relevant information

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4.7.1.7 Summary and discussion of repeated dose toxicity

Oral: In a 90 day oral repeated dose toxicity study (Ladics et al. 2008; key study) a test-substance related increase in the incidence of focal hepatic necrosis was reported in males from 25 mg/kg bw/day. The necrosis was accompanied by a minimal inflammatory response. After a recovery period of 3 months the incidence of hepatocellular necrosis increased in the 125 mg/kg bw/day dose group in males. In females a test-substance-related adverse increase in the incidence and severity of chronic progressive nephropathy was reported at 125 mg/kg bw/day. After a 3 month recovery period the incidence and severity of chronic progressive nephropathy was increased at 125 mg/kg bw/day in females.

The liver appears to be the most sensitive target organ for 8:2 FTOH toxicity based on the available studies. This finding is supported by studies reporting hepatotoxic effects of PFOA, the major metabolite of 8:2 FTOH in male rats. However, the adversity of the liver toxicity observed in the study by Ladics (2008) is found not to be severe enough for the classification as repeated dose toxicity.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

See above 4.7.1.7

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

According to the DSD criteria multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity are effects that support classification for repeated dose toxicity. The liver toxicity observed at 25 and 125 mg/kg bw in male rats are not considered adverse according to the DSD criteria.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

In the opinion of the Norwegian CA the studies provided on 8:2 FTOH are sufficient to reach a conclusion on the potential classification for repeated dose toxicity. A classification for repeated dose toxicity is not warranted.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

See above 4.7.1.7

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

According to the CLP classification guidance (Annex 1: 3.9.2.7.3, point *e*) "multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity" are effects that support classification for specific target organ toxicity. The liver toxicity observed at 25 and 125 mg/kg bw in male rats are not considered adverse according to the CLP criteria.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

In the opinion of the Norwegian CA the studies provided on 8:2 FTOH are sufficient to reach a conclusion on the potential classification for repeated dose toxicity. A classification for STOT RE is not warranted.

4.9 Germ cell mutagenicity (Mutagenicity)

Tables of relevant repeated dose toxicity studies are included below.

4.9.1 Non-human information

4.9.1.1 In vitro data

Test	Species	Conc. (µg/plate)	Metabolic activ.	Observations and Remarks	Ref.
OECD - 409	S. typhimurium TA 98, TA 100, TA 1535, TA 1537 and E.coli WP2uvrA	0, 33.3, 100, 333, 1000, 3330 and 5000	± S9	Purified 8:2 Alcohol (Haskell Number 24691) was tested in triplicate in a Salmonella e-coli reverse mutation assay with DMSO as vehicle. No toxicity was observed at any dose levels with and without S9. The study did not fulfil the criteria for positive response concerning mean number of revertants/plate. The positive control for TA 1537 without S9 was not fulfilled. The other positive controls were according to the recommendation. Conclusion: negative for mutation in vitro.	Covance study 22900-0-409OECD

4.9.1.2 In vivo data

Test	Species	Conc. (mg/kg)	Metabolic activ.	Observations and Remarks	Ref.
OECD – 454 Range-finding study	Male Crl:CD®(SD) IGS BR rats	2000		Limit dose *in vivo* rat micronucleus assay by oral gavage in 3 males and 3 females with 0.5 % methylcellulose as vehicle. Conclusion: No lethality or clinical toxicity was reported.	Covance Study: 22900-0-454OECD
OECD – 454	Male Crl:CD®(SD) IGS BR rats	0, 500, 1000 and 2000		In vivo rat micronucleus assay by oral gavage in 6 males/dose/harvest time points with 0.5 % methylcellulose as vehicle. Cyclophosphamide (60 mg/kg bw) was used as a positive control and analysed at 24 h post treatment. Bone marrow was harvested from 5/6 rats at 24 h and 48 h after treatment. There were no lethality or sign of clinical toxicity post dosing and no detection of cytotoxicity up to 2000 mg/kg bw. No statistically significant increase in MNPCE at any dose levels. Conclusion: The effect of 8:2 FTOH is considered negative in rat micronucleus assay under the current test conditions up to limit dose.	Covance Study: 22900-0-454OECD

4.9.2 Human information

No data available

4.9.3 Other relevant information

No data available

4.9.4 Summary and discussion of mutagenicity

Based on the two reports available on genotoxicity of 8:2 FTOH there seems to be no concern for genotoxicity. This is also supported with the findings from two other genotoxicity studies on a mix of several FTOH's (27% 8:2 FTOH) which both were negative (Gudi R and Brown C

DuPont-5580 (Gudi R and Brown C) and Wagner WO and Klug ML DuPont-5579(Wagner VO and Klug ML)).

4.9.5 Comparison with criteria

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4.9.6 Conclusions on classification and labelling

No classification for mutagenicity is proposed.

4.10 Carcinogenicity

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

No data available

4.10.1.2 Carcinogenicity: inhalation

No data available

4.10.1.3 Carcinogenicity: dermal

No data available

4.10.2 Human information

No data available

4.10.3 Other relevant information

8:2 FTOH is metabolised to PFOA and data for this is presented in chapter 5.1, Toxicokinetics. Two carcinogenicity studies following oral exposure to APFO (Cas. No. 3825-26-1) shows induction of liver adenomas, Leydig cell adenomas, and pancreatic acinar cell tumours in male Sprague-Dawley rats. Consequently, in the current classification proposals for APFO and PFOA submitted to ECHA april 2011, these substances are proposed classified according to the DSD criteria with Carc. Cat. 3; R40, and according to CLP criteria as Carc 2; H351.

4.10.4 Summary and discussion of carcinogenicity

The *in vitro* and *in vivo* mutagenicity studies were negative. No carcinogenicity studies were available for 8:2 FTOH. PFOA is proposed classified for carcinogenicity (CLP: Carc. 2, H351) and a possible conversion of 8:2 FTOH to PFOA in humans thus requires consideration. Since a threshold is considered to exist for a potential carcinogenic effect for PFOA and that possibly relatively low level of PFOA is biotransformed from 8:2 FTOH in humans, a classification for carcinogenicity is not further considered for 8:2 FTOH.

4.10.5 Comparison with criteria

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4.10.6 Conclusions on classification and labelling

The Norwegian CA considers the data insufficient for an evaluation of the carcinogenicity of 8:2 FTOH. Following a weight-of-evidence approach PFOA is proposed classified for carcinogenesis (CLP: Carc. 2, H351) and a possible conversion of 8:2 FTOH to PFOA in humans thus requires consideration. Since a threshold is considered to exist for a potential carcinogenic effect for PFOA and that possibly relatively low level of PFOA is biotransformed from 8:2 FTOH in humans, a classification for carcinogenicity is not further considered for 8:2 FTOH.

4.11 Toxicity for reproduction

Tables of relevant repeated dose toxicity studies are included below.

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Species	Route	Dose	Exposure time (h/day)	Number of generations exposed	Observations and Remarks	Ref.
Sprague-Dawley rats 20/sex/group	Oral gavage	0, 25, 100, 250 mg/kg bw/day of the mixture of FTOHs (27% 8:2 FTOH).	Daily for a period of 74 days before cohabitation during mating, gestation and lactation.	One generation	Three weeks prior to cohabitation with males vaginal smears were collected daily from all P1 females to evaluate the oestrous cycle until copulation was confirmed. Sperm parameters for the first 10 of the 20 P1 males in each exposure group were evaluated. The right cauda epididymis was weighed and sperm were evaluated for motility and morphology. The left cauda epididymis and testis were frozen for later quantification. At postnatal day (PND) 0, 4, 7, 14 and 21 live pups were counted by sex and weighed. They were also examined for abnormal behaviour and appearance, any dead missing or abnormal pups were recorded. F1 generation male and female rats were monitored daily for vaginal opening (beginning PND 21) or perputial separation (beginning PND 35) until criterion was achieved, and body weight was recorded at the day of achievement. **Results:** *Systemic toxicity*; No test-substance related mortality occurred in the P1 and F1 generation rats. However, one female rat died in each of the 100 and 250 mg/kg/d groups. This seems not to be related to the test	Mylchreest et al., 2005a

					substance. A significant decrease in bodyweight in the 250 mg/kg/d group during and after cohabitation period (10 % reduction), but no concomitant reduction in bodyweight gain in P1 males during that period. No test-substance related clinical signs of toxicity were observed in the P1 or F1 generation rats. There was an increase in tooth clipping required in males administered 250 mg/kg/d, consistent with dental problems in the sub-chronic toxicity study of Ladics et al.(2005). The NOAEL for systemic toxicity was 100 mg/kg bw/day based on body weight reduction. *Reproductive toxicity:* There were no test-substance related effects on oestrous cycle parameters, sperm morphology, motility or epididymal sperm counts in the P1 generation. At 100 and 250 mg/kg/day there was a statistical significant increase in testicular spermatid numbers in the P1 male rats. This was not considered test-substance related because the means for these groups were within the historical control range for previous studies. The increase appeared to be due to slightly lower than usual mean weight in the control groups. Litter size at birth was reduced from 100 mg/kg bw/day (14.7, 13.4, 12.4 and 12.5 in the 0, 25, 100 and 250 mg/kg bw/day dose group, respectively), and number of live pups per litter on day 0 (14.6, 13.2, 11.3 and 12.1 in the 0, 25, 100 and 250 mg/kg bw/day dose group, respectively) and on lactation day 4 (14.6, 12.9, 10.8, 11.8 in the 0, 25, 100 and 250 mg/kg bw/day dose group, respectively). At 250 mg/kg bw/day a statistically significant reduction in pup weights on PND 4, 7, 14, and 21 was reported, that was 74-78 % of	

					control values on PND 21 in males and females. There were no toxicologically significant pathological findings in the F1 pups or P1 or F1 generation adult rats. NOAEL for the FTOH mixture containing 27 % 8:2 FTOH reproductive toxicity was 25 mg/kg/day based on litter size reduction.	

4.11.1.2 Human information

No data available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Species	**Route**	***Dose mg/kg/day ppm **Conc. (mg/l)**	**Exposure time (h/day)**	**Exposure period: - number of generations or - number of days during pregnancy**	**Observations and Remarks**	**Ref.**
Sprague-Dawley rats (Crl:CD®(SD)IGS BR rats), 22/group	Oral by gavage	0, 50 ,200 or 500 mg/kg/day of 8:2 FTOH.	daily	Gestation days 6-20.	In the developmental toxicity study groups of time-mated female rats were given 0, 50, 200 or 500 mg/kg/day 8:2 FTOH by oral gavage from gestation day 6-20. At day 21 all surviving dams were sacrificed. The abdominal and thoracic viscera were examined and the uterus from each rat were removed and dissected for examination of the uterine content. The doses were chosen based on a repeated dose range finding study (DuPont-6357). *Maternal toxicity:* Four dams in the highest dose group were sacrificed in extremis on gestation day (gd) 10, 11, 12	Mylchreest et al., 2005b

					or 18, and one dam was found dead on gd 16. Clinical observations in the highest dose group included increased occurrence of alopecia, diarrhoea, hunched-over posture, weakness and fur-staining. There were no clinical observations at 50 or 200 mg/kg bw/day. There were no test substance-related effects on maternal body weight or weight gain at either 50 or 200 mg/kg. At 500 mg/kg bw/day the mean maternal weight gain over the course of the study (gd 6-21) was 25% lower compared to the control group, and the mean final maternal body weight was statistically significant reduced (around 10 %) compared to control group. A statistically significant reduction in food consumption was reported at 500 mg/kg bw/day, and occasionally at 200 mg/kg bw/day, however, the reduction was not considered as adverse toxicity. In the highest dose group necropsy observations included pale kidneys in one dam, however, the other dams appeared normal. The NOAEL for maternal toxicity was 200 mg/kg bw/day. *Developmental toxicity*: No effects were reported on the number of corpora lutea, implantation sites, litter size, resorptions, foetal weight, and sex ratio. No malformations were reported. An exposure-related statistically significant increase in skeletal variations was reported from 200 mg/kg bw/day. At 200 mg/kg bw/day these include increased incidence of delayed skull bone ossification and at 500 mg/kg bw/day increases in the occurrence of delayed pelvic bone ossification (in foetuses (and litters): 1(1), 0(0), 0(0),	

					9(5), in controls, 50, 200, 500 mg/kg/day) and wavy ribs (in foetuses (and litters): 0(0), 0(0), 0(0), 5(2), in controls, 50, 200, 500 mg/kg/day) were recorded. The incidence of delayed skull ossification (in foetuses (and litters): 22(8), 31(12), 45(16), 55(12), in controls, 50, 200, 500 mg/kg/day) was significantly different from the concurrent control, but within the range of the historical control data. Moreover the effects were observed in the absence of a test-substance related effect on foetal body weight. The NOAEL for developmental toxicity in rats was 50 mg/kg bw /day.	
Sprague-Dawley rats (S-D rats). 20/sex/group	Oral by gavage	0, 25, 100 or 250 mg/kg/day of FTOH mixture (27% 8:2 FTOH).	daily	One generation. For a period of 74 days prior to cohabitation, during mating, gestation and lactation.	Time-mated female S-D rats were given 0, 25, 100 or 250 mg/kg/day of a commercial mixture of different FTOH (27% 8:2 FTOH). Body weight, feed consumption, clinical signs, gross pathology, sperm parameters, oestrous cycle and reproductive performance were evaluated for the P1 generation. The F1 offspring were evaluated during the lactation period for growth and survival and given a gross pathology examination at weaning. A subset of the offspring were retained and evaluated for changes in body weights, feed consumption, clinical signs, and age at onset of vaginal opening and pre-pubertal separation. Gross pathology was performed on postnatal day 60. **Results:** *Systemic toxicity*; No test-substance related mortality occurred in the P1 and F1 generation rats. However, one female rat died in each of the 100 and 250 mg/kg/day groups. This seems not to be related to the test substance. Body weight and feed consumption parameters	Mylchreest et al., 2005a.

					were similar across groups during gestation and lactation in P1 females. In males, a significant decrease in body weight at the 250 mg/kg/day group during and after cohabitation period (10 % reduction) was observed, but no concomitant reduction in bodyweight gain in the P1 males during that period. No test-substance related clinical signs of toxicity were observed in the P1 or F1 generation rats. There was an increase in tooth clipping required in males administered 250 mg/kg/day, consistent with dental problems in the subchronic toxicity study of Ladics et al. (2003). The NOAEL for systemic toxicity was 100 mg/kg bw/day based on decreased body weight in P1 males. *Reproductive toxicity:* Litter sizes at birth were reduced at doses from 100 mg/kg bw/day (14.7, 13.4, 12.4 and 12.5 in the 0, 25, 100 and 250 mg/kg bw/day dose group, respectively), and number of live pups per litter on day 0 (14.6, 13.2, 11.3 and 12.1 in the 0, 25, 100 and 250 mg/kg bw/day dose group, respectively) and on lactation day 4 (14.6, 12.9, 10.8, 11.8 in the 0, 25, 100 and 250 mg/kg bw/day dose group, respectively). At 250 mg/kg bw/day a statistically significant reduction in pup weights on pnd 4, 7, 14, and 21 was reported, that was 74-78 % of control values on pnd 21 in males and females. There were no toxicologically significant pathology findings in the F1 pups or F1 generation adult rats. The NOAEL for developmental toxicity for the FTOH mixture containing 27 % 8:2 FTOH was 25 mg/kg/day.	

Sprague-Dawley rats (S-D rats). 20/rats/group	Oral by gavage	0, 50, 200 or 500 mg/kg/day of FTOH mixture (27% 8:2 FTOH).	daily	Gestation days 6-20	In the developmental toxicity study groups of time-mated female S-D rats were given a commercial mixture of FTOH (27% 8:2 FTOH). On day 21 of gestation dams were sacrificed and the thoracic and abdominal viscera were examined. The uterine contents were removed and examined, and foetuses were evaluated for alterations. Increased foetal skeletal alterations were recorded at 200 and 500 mg/kg/day. There was a significant increase in delayed skull bone ossification at 200 mg/kg/day. This significant increase was within the relevant historical control range. Maternal body weights were reduced (5%) at 500 mg/kg/day. There were no maternal or developmental toxicity at the lower doses. Based on these findings the NOAEL for developmental toxicity of the mixture of FTOHs containing 27 % 8:2 FTOH was 50 mg/kg/day.	Mylchreest et al., 2005a.

4.11.2.2 Human information

No data available

4.11.3 Other relevant information

8:2 FTOH is metabolised to PFOA. Classification of PFOA is based on read-across, agreed in RAC, to APFO since most of the studies are performed with APFO. Animal experiments with APFO has shown increased postnatal pup mortality, decreased pup body weight, full litter resorption and delayed sexual maturation in the absence of marked maternal toxicity.

The classification for PFOA was agreed in the former TC C&L group in October 2006 for reproductive toxicity with Repr. Cat.2 R61 according to DSD criteria (classification according to CLP: Repr. 1B, H360D).

Summary of the findings for developmental toxicity that lead to the classification proposal for APFO/PFOA, from CLH-dossier submitted by Norway

In an oral 2-generation study (York, 2002; Butenhoff et al., 2004) in rats in the 30 mg/kg/day dose group one pup died on Lactation Day (LD) 1. Additionally, on LD 6 and 8 significant increases in the number of pups found dead were reported at 3 and 30 mg/kg/day. Pup body weight on a per litter basis was significantly reduced up to lactation day 15 in the 30 mg/kg/day dose group (LD 1; 5.5 vs 6.3 in controls, LD 8; 11.9 vs. 13.3 in controls, and LD

15; 22.9 vs. 25.0 in controls). Furthermore, significant delays in sexual maturation (the average of preputial separation in males and vaginal patency in females) were reported at 30 mg/kg/day (52.2 days of age vs.48.5 days of age in controls in males, and 36.6 days of age vs. 34.9 days of age in female). When the body weights were co varied with the time to sexual maturation, the time to sexual maturation in both males and females showed still a dose-related delay that was statistically significant at $p \leq 0.05$. These effects were reported in the absence of maternal toxicity. However, in rat developmental toxicity studies following oral or inhalation exposure to APFO minimal effects were reported in the offspring.

In a mouse developmental toxicity study (Lau et al., 2006) early pregnancy loss, severely compromised postnatal survival, delays in general growth and development as well as sex-specific alterations in pubertal maturation were reported.

In the developmental toxicity study in mice by Wolf et al., 2007 the observations suggested that the postnatal developmental toxicity of APFO in mice were mainly due to gestational exposure and that exposure earlier in gestation produces stronger responses.

In the developmental toxicity studies in mice by White et al., 2007, 2009 a window of mammary gland sensitivity in late fetal and early neonatal life was reported, and the effects were reported to be persistent. This was confirmed in two additional strains of mice in a study by Yang et al., 2009.

In the study by Abbott et al., 2007 it was shown that several of the developmental effects in mice may be influenced by PPARα (post-natal lethality, delayed eye opening and deficits in postnatal weight gain) although other mechanisms may contribute. In contrast, early pregnancy loss appeared to be independent of PPARα expression. PPARα agonists induce both peroxisome proliferation and increased expression of PPARα target genes. While some of these effects are shared by the rodent and human PPARα receptor, the hepatic proliferative response and anti-apoptotic activity of PPARα activation associated with induction of liver tumours are only seen in rodents. Although several studies suggests that PPARα play an important role in APFO induced developmental toxicity it is not known whether the human PPARα will mediate a similar response. Thus, at present PPARα mediated developmental effects cannot be regarded as irrelevant for humans. Furthermore, some of the reproductive toxicity effects observed, full litter resorption and effects on mammary gland development, are present also in PPARα KO animals.

The developmental toxicity reported in mice had a different profile compared to the developmental toxicity reported in rats. The different findings in rats and mice are likely due to the different pharmacokinetics of APFO in rats and mice. Renal elimination is high in rat females leading to a significantly lower serum concentration of PFOA in pregnant rats than in pregnant mice. In the study by Lau et al., 2006 the serum levels of APFO was measured in adult rats and mice receiving daily oral gave of APFO. In rats given 10 mg/kg bw/day for 20 days the serum levels of APFO were 111 µg/ml in males and 0.69 µg/ml in females, and in mice given 20 mg/kg bw/day for 17 days the serum levels were 199 µg/ml in males and 171 µg/ml in females. Furthermore, in pregnant rats, a plasma concentration of 79-80 µg/ml was reached after 2 hours following oral exposure to 30 mg/kg bw/day (Hinderliter et al., 2005) and declined by 98% after 22 hours (Kemper and Jepson, 2003). In contrast, in the study by Lau et al., 2006 a dose-dependent accumulation of APFO was noted in pregnant mice at term.

In conclusion: Based on the increased postnatal pup mortality, decreased pup body weight and delayed sexual maturation observed in several mice studies, as well as in the rat 2-generation study, in the absence of marked maternal toxicity, a classification of APFO for developmental effects according to Directive 67/548/EEC with Repr. Cat. 2; R61 is proposed. Developmental toxicity was thoroughly discussed in the former TC C&L group and the group concluded on a classification of APFO for developmental toxicity in Repr. Cat. 2; R61. According to CLP criteria APFO is proposed classified as Repr. 1B, H360D.

4.11.4 Summary and discussion of reproductive toxicity

Fertility

In a one-generation study (Mylchreest et al,. 2005a) in rats exposed to a mixture of FTOHs with 27 % 8:2 FTOH no effects were reported on sperm parameters, oestrus cycle and reproductive performance in rats up to 250 mg/kg bw/day.

Development

In a developmental toxicity study (Mylchreest et al., 2005b) with exposure to 8:2 FTOH from gestation day 6-20 no malformations were reported. However, increases in skeletal variations were reported from 200 mg/kg bw/day. At 200 mg/kg bw/day these include an increase in the incidence of delayed skull bone ossification and at 500 mg/kg bw/day increases in the occurrence of delayed pelvic bone ossification and wavy ribs were recorded. Severe maternal toxicity was reported at 500 mg/kg bw/day and included test-substance related mortality and a 25% reduction in mean weight gain from gestation day 6-21. There were no test substance-related effects on maternal body weight or weight gain at either 50 or 200 mg/kg. At 200 mg/kg/day the incidence of delayed skull ossification was significantly different from the concurrent control, but within the range of the historical control data. Moreover the effects were observed in the absence of a test-substance related effect on foetal body weight. The appearance of skeletal variations without concomitant decreased foetal body weight and in the absence of maternal toxicity are findings that warrant attention (Carney and Kimmel, 2007).

A one-generation study and a developmental toxicity study were performed with a commercial mixture of FTOH which contained 27 % 8:2 FTOH (Mylchreest et al., 2005a). In the one-generation study litter size at birth and the number of live pups per litter on day 0 and postnatal day (PND) 4 were reduced with the latter starting at 100 mg/kg bw/day. At 250 mg/kg bw/day a statistically significant reduction in pup weights on PND 4, 7, 14, and 21 were reported, that was 74-78 % of control values on PND 21. These effects were reported in absence of maternal toxicity.

Similar to what was observed in Mylchreest et al., 2005b, increased foetal skeletal alterations were reported in the developmental toxicity study using a fluortelomer-mixture (27 % 8:2 FTOH). There were increased incidences of foetal skeletal alterations (delayed skull and pelvic bone ossification and wavy ribs) accompanied by a slight decrease in mean foetal weight. There was a significant increase in delayed skull bone ossification at 200 mg/kg/day, along with 3 % reduced maternal body weight gain. This significant increase was within the relevant historical control range. At 500 mg/kg/day there was a 5 % reduction in maternal body weight.

4.11.5 Comparison with criteria

The findings of the developmental studies 8:2 FTOH are not considered of sufficient adversity to warrant classification, even if referred studies report qualitatively similar findings. As part of a weight-of-evidence approach, we consider the rat as a poor model to reveal potential developmental effects of 8:2 FTOH and its metabolites, especially the female rat, due to ADME considerations (Chapter 4.1 Toxicokinetics). One major urine metabolite following exposure to 8:2 FTOH is PFOA. A toxicokinetic study (Fasano et al. 2009) has shown that following 8:2 FTOH exposure of rats, the blood level of PFOA is very low in females compared to males. The low level of PFOA in female rats exposed to 8:2 FTOH is proposed to be partly due to the rapid elimination of PFOA in female rats. The serum levels of PFOA (789±41 ng/mL) in pregnant female mice exposed to one single dose of 30 mg/kg bw 8:2 FTOH by gavage (Henderson and Smith, 2007) were significantly higher than the serum level of PFOA (C_{max} of 102 ng/mL) in female rats exposed daily to 5 mg/kg bw for 45 days (Fasano et al., 2009). The toxicokinetic data suggests the female mouse as a more appropriate model than female rats for assessing

developmental effects. Moreover, the evident developmental effects of PFOA in mice support this suggestion, however in presence of increased maternal liver weight (Lau et al., 2006). Moreover, the elimination rate of PFOA is markedly shorter in mice (days vs hours) than in humans (years) (Chapter 4.1 Toxicokinetics). This difference in PFOA elimination kinetics makes the evaluation of the reproductive toxicity of 8:2 FTOH based on data from rats of limited value. Studies with 8:2 FTOH in mice show that PFOA is present in blood of offspring and that exposure may occur via lactation (Henderson and Smith 2007, Chapter 4.1 Toxicokinetics and 4.7 Repeated Dose Toxicity). A low, but significant biotransformation of 8:2 FTOH to PFOA has been shown *in vitro* in human hepatocytes (Chapter 4.1 Toxicokinetics). A low biotransformation of 8:2 FTOH to PFOA may, due to the long half-life of PFOA in humans (2-4 years) and the bioaccumulation, contribute to the total body burden of PFOA in humans exposed to 8:2 FTOH. Moreover, the findings in the developmental studies (Mylchreest et al., 2005 a and b) suggest the occurrence of similar developmental effects on offspring as following exposure to PFOA.

4.11.6 Conclusions on classification and labelling

Using a weight-of–evidence approach based on 1) ADME consideration of female rats exposed to FTOH, and their low levels of serum metabolites combined with 2) supportive information concerning developmental effects of one major serum metabolite (PFOA), 3) the long half-life of PFOA in humans (2-4 years) and bioaccumulation and 4) coherent developmental effects following exposure to 8:2 FTOH and PFOA, we propose a classification for 8:2 FTOH equal to PFOA.

The classification for PFOA was agreed in the former TC C&L group in October 2006 for reproductive toxicity with Repr. Cat.2 R61 according to DSD criteria (classification according to CLP: Repr. 1B, H360D).

For more information on the weight-of–evidence approach on 8:2 FTOH, see Annex I.

No classification for fertility is proposed.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

FTOH is metabolised into PFOA in all mammalian species studied, and as PFOA has recently been proposed by the RAC to be classified for reproductive toxicity (Cat. 1B; CLP), the Dossier submitter (DS) proposes to classify FTOH based on the formation of a metabolite (i.e. PFOA) which is a reproductive toxicant.

According to the Dossier submitter, it is clear that there are species differences in the metabolism of FTOH which makes direct species comparisons difficult. The *in vivo* metabolism of FTOH is faster in mice than in rats, but PFOA is a major metabolite in both species. The half-life of PFOA in both species is in the order of hours to weeks, with the shortest half-life in female rats. *In vitro* experiments have shown formation of PFOA from FTOH in mouse, rat, and human hepatocytes, with the rate of PFOA formation possibly being 5-fold and 12-fold lower in human hepatocytes than in rat and mouse hepatocytes, respectively.

However, although the rate of PFOA formation is slow in humans, the very long half-life of PFOA in humans (3.8 years) will contribute to bioaccumulation of PFOA in humans, and thus to risks for reproductive effects of PFOA.

The notion that metabolism of 8:2 FTOH to PFOA is a relevant mechanism for reproductive toxicity of 8:2 FTOH is supported by FTOH having similar, albeit less severe, effects as PFOA on rat reproduction. A one–generation study on a mixture of FTOHs

(27% 8:2 FTOH) in rats showed a dose-dependent decrease in litter size and number of live pups per litter at postnatal day (PND) 0 and 4. The effects were statistically significant from the dose level of 100 mg/kg bw/d (litter size -16%, number of live pups per litter -23% and -26% at PND 0 and 4, respectively), in the absence of any signs of maternal toxicity.

Comments received during public consultation

Comments were received from 6 Member states and 3 industry organisations. Among the Member States, two supported the proposal, two disagreed with the proposal, and two didn't express a clear position. Three comments focused on the need for a more detailed evaluation of the kinetics, and two comments suggested considering whether also classification for effects via lactation would be warranted. The three industry organisations disagreed with the proposal, based on e.g. that PFOA is a minor rather than major metabolite of 8:2 FTOH, that the read across criteria of the guidance documents is not followed, and that there is no evidence that PFOA actually accumulates with age in humans.

Assessment and comparison with the classification criteria

There are two developmental toxicity studies and one one-generation study available for FTOH, although one study concerns 8:2 FTOH and the two other studies concern a mixture of FTOHs containing 27% 8:2 FTOH. The exact composition of other FTOHs in the mixture is not known. However, based on the similar toxicological effects of 8:2 FTOH and the FTOH mixture, both with regard to reproductive toxicity and repeated dose toxicity, it is assumed that all these studies can be used to inform about the toxicity of 8:2 FTOH.

The 8:2 FTOH rat developmental toxicity study showed severe maternal toxicity at 500 mg/kg bw/d, including 23% maternal mortality, and effects at that dose level (delayed bone ossification) are therefore not relevant. At 200 mg/kg bw/d, a statistically significant increase in skeletal variations was noted, which is not considered adverse in the context of classification.

In the rat developmental toxicity study on the FTOH mixture, increased foetal skeletal alterations were observed at 500 mg/kg bw/d in the presence of slightly decreased male fetal weight and a 5% reduction in maternal body weight. Overall, these effects are not sufficiently adverse to warrant classification.

The one-generation study in rats of the FTOH mixture (containing 27% 8:2 FTOH), showed a roughly dose-dependent decrease in litter size (14.7, 13.4, 12.4, and 12.5) and number of live pups per litter at birth (14.6, 13.2, 11.3 and 12.1) and at lactation day 4 (14.6, 12.9, 10.8, 11.8) in the 0, 25, 100 and 250 mg/kg bw/day dose group, respectively. The effects were statistically significant from the dose level of 100 mg/kg bw/d (litter size -16%, number of live pups per litter -23% and -26% at PND 0 and 4, respectively). It is, however, noted that the effects at 250 mg/kg bw/d are not more severe than at 100 mg/kg bw/d, raising some questions about the dose dependency. The overall good consistency between the other dose groups still speaks in favour of a substance-related effect on development. Furthermore, FTOH is rather rapidly metabolised, and if the toxicity is related to the metabolites, it is possible that saturation of metabolism probably will occur at high dose levels. At 250 mg/kg bw/day, a statistically significant reduction in pup weights on PND 4, 7, 14, and 21 was reported, that was 74-78 % of control values on PND 21 in males and females. After clarifications from the Dossier submitter, the RAC concludes that there were no signs of maternal toxicity at 100 and 250 mg/kg bw/d.

The decreased litter size (-16%) and impaired early survival of the pups that occurred in the absence of any maternal toxicity provide some evidence of developmental toxicity and constitute a borderline case for classification in category 2.

The CLP criteria state that a substance should be placed in Category 2 as a 'Suspected human reproductive toxicant' when the data provide;

"*…some evidence from humans or experimental animals, possibly supplemented with other information…..on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.*

Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects".

The arguments for classification are that a decreased litter size and impaired pup survival (in the absence of maternal toxicity) are very serious effects, whereas arguments against are that the dose-response could be more convincing and that the critical study is conducted on a FTOH mixture (containing 27% 8:2 FTOH) and not on 8:2 FTOH itself. Overall, the RAC is of the opinion that the evidence for reproductive toxicity of this FTOH-mixture is possibly border-line sufficient for classification, but does not think that a study on a commercial FTOH mixture (containing 27% 8:2 FTOH) can be used as the basis for classifying 8:2 FTOH.

In addition, there is a study in mice with an unusual study design that has been considered by the RAC. Pregnant animals were dosed once by gavage with 30 mg/kg bw/d 8:2 FTOH on gestation day (GD) 8. On the day of birth, pup mortality was slightly, but statistically significantly increased, with 31% of the dams having at least 1 (mean 1.4) non-viable pup versus 0% in controls (average litter size 13±2). The study may indicate toxicological effects by 8:2 FTOH, but because of the design and the slight effects, the results are not sufficiently robust for classification.

Regarding the metabolism of FTOH into PFOA, the RAC notes the large species differences with respect to metabolism and excretion of these substances hampering comparisons. Information on the *in vivo* metabolism of FTOH to PFOA in humans is not available. *In vitro* studies on hepatocytes from different species indicate that humans are slower in the formation of PFOA from 8:2 FTOH than rodents. On the other hand, PFOA has a half-life in humans in the orders of years, whereas the half-life in rodents is in the order of hours-weeks, showing that the potential for building up high concentrations of PFOA (once formed) in the body is very high in humans as compared to in rodents. The question is whether the formation of PFOA is sufficiently high to warrant a classification based on the developmental toxicity of PFOA (see the RAC opinion on classification of PFOA at http://echa.europa.eu/documents/10162/e7f15a22-ba28-4ad6-918a-6280392fa5ae).

The CLH dossier describes two reports where the metabolism of 8:2 FTOH has been studied in hepatocytes. Martin *et al.* (2005) showed that 1.4% of the available 8:2 FTOH (18 µM) was transformed in 4 hours into PFOA in rat hepatocytes (kept in open flasks). Nabb *et al.* (2007) showed that 0.24% of the available 8:2 FTOH (20 µM) was transformed in 2 hours into PFOA in rat hepatocytes (kept in closed vials). It is noted that 18-20 µM are rather high concentrations, and that the use of lower concentrations potentially could have resulted in a higher percentage of formed PFOA. However, Nabb *et al.* (2007) state that this concentration is below metabolic saturation, but no data is presented. These studies indicate that the formation of PFOA in rat hepatocytes may be in the order of 1%.

Nabb *et al*. (2007) have also compared the metabolism of 8:2 FTOH between rat, mouse and human hepatocytes. It should be noted that rodent hepatocytes were freshly prepared from young animals, whereas the human hepatocytes were purchased frozen and obtained from only three rather old men (54, 68, and 80 years of age). It is also noted that when comparing metabolism at 30 minutes and 2 hours, rats and mice had formed much more PFOA after 2 hours than after 30 minutes (2.4 and 5.6-fold, respectively), whereas the human hepatocytes had only formed slightly more PFOA (1.3-fold). However, cell viability was > 85% in all preparations.

The metabolic rates in rat and human hepatocytes were compared by Nabb *et al.* (2007) in different ways, indicating a 5-fold higher capacity in rat than in human hepatocytes based on metabolism expressed as pmol formed PFOA/min/10^6 cells, a 12-fold difference based on molar concentrations detected at the end of the incubation, or a 9.5-fold difference when hepatocyte metabolism had been converted into whole body capacity. When comparing human and mouse hepatocytes, the difference becomes slightly more than 2-fold higher than above, e.g., a 12-fold higher capacity in mouse than in human hepatocytes based on metabolism expressed as pmol formed PFOA/min/10^6 cells.

It is concluded by the RAC that the data is too limited as a basis for quantitative comparison. Based on the available information, it might seem that the formation of PFOA from 8:2 FTOH in rat hepatocytes is in the order of 1%, and that the corresponding rate in humans might be 5-fold lower, i.e. in the order of 0.2%. However, whereas PFOA is rather quickly excreted in rodents, the half-life in humans is in the order of years.

Read across based on “common significant metabolites” is a valid reason for classification (CLP guidance V3.0, section 1.4.3), but there is no quantitative guidance for how much hazardous metabolites that need to be formed to trigger classification. Although not comparable, it is noted that the generic concentration limits of ingredients of a mixture classified as reproductive toxicants are 0.3% (cat 1) or 3% (cat 2).

Based on comments in the public consultation, a comparison has been made between serum concentrations of PFOA in mice exposed to either 8:2 FTOH or PFOA (see supplemental information below). A 6-fold higher dose of 8:2 FTOH than of PFOA gave a PFOA-concentration 1/10 of that measured in the PFOA-exposed mice after a single administration, possibly indicating that a 60-fold higher dose of 8:2 FTOH than of PFOA has to be administered to mice to give similar serum concentrations of PFOA.

When extrapolating this information to humans, it has to be considered that

- human formation of PFOA from 8:2 FTOH seems to be slower than in mice,
- but that the half-life of PFOA in humans (years) is much longer than in mice (weeks).

Based on the hepatocyte studies, and assuming that the hepatocyte experiments are relevant as indicators for the *in vivo* formation of PFOA from FTOH, the human metabolism may be 1/12 of the metabolism in mice, whereas the half-life in humans may be 50-fold longer than in mice.

Although PFOA is likely to be formed *in vivo* from 8:2 FTOH, the amount formed is too small to warrant classification. Thus, in mice a 60-fold higher dose of 8:2 FTOH than of PFOA has to be administered to give similar serum concentrations of PFOA after single administrations. When extrapolating to humans, the RAC believes that the very slow rate of metabolism to PFOA in humans is more important than a long half-life of PFOA in humans and that accordingly, one cannot assume that 8:2 FTOH will exert any toxicity in humans via formation of PFOA. The proposal to classify 8:2 FTOH for reproductive toxicity with Repr. 1B, H360D, is thus not supported by the RAC. There are indications of

developmental toxicity from the commercial FTOH containing 27% 8:2 FTOH, but as it is not known which components that are responsible for the effects, this data has little impact on the classification of 8:2 FTOH. Overall, the RAC is of the opinion that the available data does not permit classification of 8:2 FTOH for reproductive toxicity.

Supplemental information - In depth analyses by the RAC

There is a general agreement that because of the very fast excretion of PFOA in rats, and especially in female rats, the rat is not the ideal model for studying formation of PFOA from FTOH or effects of PFOA. The mouse may be a better model with respect to a slower excretion of PFOA, although still much faster than the human excretion of PFOA. It was proposed in the public consultation to focus on mouse data, and to use mouse serum concentrations of PFOA in mice exposed to PFOA and 8:2 FTOH, respectively, to assess the possible importance of the PFOA-metabolite in mice exposed to 8:2 FTOH. The study by Henderson and Smith (2007) on 8:2 FTOH and the study by Fenton *et al*. (2009) on PFOA are relatively similar, in that pregnant mice have been given single doses of these substances by gavage and serum concentrations of PFOA have been analysed 24 hours later. The dosing was performed on GD 8 and 17, respectively, but this difference is not likely to affect the kinetics too much. More importantly, the dose of 8:2 FTOH was 6 times higher than the dose of PFOA (30 and 5 mg/kg bw/d, respectively), which has to be considered in this analysis. Serum concentrations of PFOA were 789 ng/ml and approximately 8000 ng/ml (estimated from figure 2 in Henderson and Smith (2007)), respectively. A 6-fold higher dose of 8:2 FTOH than of PFOA gave a PFOA-concentration 1/10 of that measured in the PFOA-exposed mice. If assuming linear kinetics, and assuming similar relationships after single and repeated exposure, the data indicate that in the order of a 60-fold higher dose of 8:2 FTOH than of PFOA has to be administered to mice to give similar serum concentrations of PFOA. This comparison is very uncertain, but if using the comparison and a LOAEL of 5 mg/kg bw/d for PFOA, doses in the order of 300 mg/kg bw/d of 8:2 FTOH would be needed to cause reproductive toxicity. However, it is not known if and how adult mice would tolerate this dose of FTOH.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

No data available.

4.12.1.2 Immunotoxicity

No data available.

4.12.1.3 Specific investigations: other studies

No data available.

4.12.1.4 Human information

No data available.

4.12.2 Summary and discussion

No data were available on other effects following exposure to 8:2 FTOH.

4.12.3 Comparison with criteria

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4.12.4 Conclusions on classification and labelling

No conclusion can be drawn on classification of 8:2 FTOH for other effects.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not considered in this dossier.

6 OTHER INFORMATION

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Annexes

Annex I: Justification for a weight-of-evidence approach for considering effects of the metabolite perfluorooctanic acid (PFOA) of 8:2 fluorotelomer alcohol (8:2 FTOH)

Due to insufficient data for 8:2 FTOH for carcinogenicity (no data available) and reproductive toxicity (rat studies exists, but are not informative), it is relevant to consider effects of the major serum and liver metabolite PFOA (Cas No 335-67-1), formed following exposure to 8:2 FTOH. PFOA is proposed classified for carcinogenicity and developmental effects (CLP: Carc. 2, H351; Repr. 1B, H360D) and a possible conversion of 8:2 FTOH to PFOA in humans thus requires consideration. Since a threshold is considered to exist for a potential carcinogenic effect for PFOA, and that possibly relatively low level of PFOA is metabolised from 8:2 FTOH in humans, a classification for carcinogenicity is not further considered. Only a classification for reproductive toxicity for 8:2 FTOH is proposed.

Below is a presentation and discussion of the information related to biotransformation of 8:2 FTOH to PFOA to be used in assessment of developmental effects. In addition, a presentation and evaluation of human data related to PFOA accumulation is included.

There are marked differences in ADME of both 8:2 FTOH and PFOA between species, and also between sexes (rats) (se table in 4.1.3). Furthermore, there are very few data on the toxicokinetic properties of 8:2 FTOH in humans, and an evaluation have to be largely based on animal data. Since there is so little information on the conversion of 8:2 FTOH to PFOA in humans, a conservative approach is suggested.

With respect to toxicokinetics, mice seem to have a more efficient conversion of 8:2 FTOH to PFOA than rats. The serum levels of PFOA (789±41 ng/mL) in pregnant female mice exposed to one single dose of 30 mg/kg bw 8:2 FTOH by gavage (Henderson and Smith, 2007) were significantly higher than the serum level of PFOA (C_{max} of 102 ng/mL) in female rats exposed daily to 5 mg/kg bw for 45 days (Fasano et al., 2009). ADME of 8:2 FTOH in rats indicated major sex differences, which were not evident in mice. This is equivalent to observations following exposure to PFOA. In mice exposed to PFOA, more pronounced developmental effects were observed in the offspring compared to similar studies in rats (York et al., 2002; Butenhoff et al., 2004; Lau et al., 2006). It is well documented that PFOA is a peroxisome proliferator (Ikeda et al., 1985; Pastoor et al., 1987; Sohlenius et al., 1992) and that this mechanism contribute to the observed developmental effects (Abbott et al., 2007). However, even in peroxisome proliferators-activated receptor alpha (PPARα) KO mice, effects on the offspring were observed (Abbott et al., 2007). This indicates that not only PPARα, but also other mechanisms are involved. Furthermore, in a recent study (Abbott et al., 2010) PPAR*α* was shown to be highly expressed in the human fetal liver, making an interaction between PFOA and PPARα in the fetal and newborn liver highly likely. Taken together these findings indicate that rat is not an optimal model for examining developmental effects of 8:2 FTOH and that a mice model would have been preferred. In addition, the mechanisms of PPARα-associated developmental toxicity of PFOA are unclear and the human relevance can not be disregarded.

Reports of elevated PFOA concentrations in humans following specific exposure, either via the environment (e.g contaminated drinking water) or occupationally, show that PFOA may accumulate in humans. In two recent studies from Sweden and Norway significantly elevated PFOA levels have been reported in professional ski waxers, in comparison to general populations (Freberg et al. 2010, Nilsson et al. 2010a). In the Swedish study, blood samples were collected before the skiing season, i.e., pre-season, the next collection was at four FIS World Cup competitions in cross country skiing, and the final collection was after an unexposed 5-month post-season period (Nilsson et al. 2010a). The PFOA levels in three technicians with “low” initial levels of PFOA (<10.0 ng/mL in pre-season blood) increased from before season to post-season by 254, 134, and 120%, whereas five technicians with “high” initial levels (>100 ng/mL in preseason sample) seemed to be at steady state. In the Norwegian study, serum samples from 13 professional male ski waxers were collected (Freberg et al. 2010). The

median concentration of PFOA was 50 ng/mL after season (range; 20-174 ng/mL), which is approximately 25 times higher than the background level. A statistically significant positive association between the numbers of years as a ski waxer and concentration of PFOA in serum was observed, which suggest an accumulation.

For people living in the vicinity of a fluoropolymer production facility in Ohio, a median serum PFOA concentration of 354 ng/mL has been reported (Emmett et al. 2006). From the dependence of serum levels on the person's use of water, it was concluded that drinking water was the major route of exposure. In the same study group, markedly higher levels of PFOA were associated with working at the chemical plant that was the source of the contamination (Steenland et al. 2009). Workers who no longer worked at the plant had much higher levels than non-workers, but lower levels than those who continued working there.

In Germany, PFC contaminated material had been applied on a large agricultural area leading to the contamination of drinking water sources. Plasma PFOA levels were around 24 ng/mL in adult residents from the contaminated area which was 4.4 (males) and 8.3 (females) times higher than PFOA levels from a control region (Wilhelm et al. 2008).

Very high serum concentrations have been reported in fluorochemical production workers with mean concentrations of PFOA in the range of 500 to 7,000 ng/mL depending on the type of job (Fromme et al. 2009). The highest serum level reported for PFOA was 114,100 ng/mL in 1995.

In three publications addressing human blood levels of PFOA with life time, no correlation between PFOA concentration and age was reported (Calafat et al., 2007; Olsen 2003; 2004). However, in the US NHANES study Calafat and co-workers (2007) found higher levels of PFOA in males at age 26 and 39 (fertile age), but not at age 55, compared to females. Similar finding has also been observed in a Japanese study (Harada et al., 2004). In a study by Thomsen and co-workers relatively high levels of PFOA was found in breast milk. After breast-feeding for a year, the concentration of PFOA in the breast milk was reduced with more than 90%. This demonstrates a significant transfer of PFOA to breast-fed children and a significantly reduced PFOA level in the mothers (Thomsen et al., 2010). A highly reduced PFOA level in breast-feeding women may at least partly explain the lower levels of PFOA in females compared to males at fertile age (26 and 39 year) shown in the NHANES study. Also, PFOA in diet is an important exposure source. It has been shown that people eating more shrimps have statistically significant higher levels of PFOA than people eating less (Haug et al., 2010). Lifestyle factors like ski-waxing, prolonged use of protective coating of fabrics, carpets and paper may also be important indoor environmental exposure sources. In a previous study, levels of PFOA in dust samples was highly correlated to serum levels in humans and the study indicated that inhalation of PFOA in the indoor environment may be a significant contributing source to total PFOA exposure (Haug et al., 2011). As a result of different activities and age of fabrics and furniture, exposure via indoor environment may also vary between age groups. Taken together, breastfeeding, differences in diet, life style and indoor environment are important exposure factors not addressed in the studies by Calafat (2007) and Olsen (2003;2004) and are factors that most likely will hide the measurable accumulation of PFOA with age. This is further supported by two Norwegian studies using multiple linear regression analyses to adjust for different contributing factors. In the Norwegian Fish and Game Study (n=175) levels of PFOA in serum from men and women increase statistically significant with age (Haug et al., 2010). Also in a study with 41 women in the age of 25-45 years a statistically significant increase in the PFOA levels with age was found (Haug et al., 2011). These two studies strongly indicate that PFOA levels increase with age, but that breast feeding, diet and indoor environment are important factors for PFOA exposure that need to be addressed in the evaluation of human exposure and accumulation of PFOA.

In conclusion; Based on low serum levels of PFOA in pregnant female rats compared to mice after 8:2 FTOH administration, in addition to less pronounced developmental effects found in studies with PFOA, rat seems not to be an appropriate model for examining developmental effects of 8:2 FTOH and its metabolites, and a mice model would have been more suitable. Although the biotransformation of 8:2 FTOH to PFOA in humans seems to be low, the half-life

of PFOA in humans is very long ($T_{1/2}$ of 3.8 years; Olsen et al., 2007), and humans have a significantly slower elimination rate than rodents (from hours to days in rats and mice respectively). Based on human studies, PFOA accumulates with time and exposure. Taken together, a classification based on PFOA should be considered for developmental effects of 8:2 FTOH.

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