Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol

EC Number: 211-477-1
CAS Number: 647-42-7

CLH-O-0000007052-84-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

26 November 2021

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

International Chemical Identification:

3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol

EC Number: **211-477-1**

CAS Number: **647-42-7**

Index Number: **n.a.**

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON MEDETOMIDINE
3,3,4,4,5,5,6,6,7,7,8,8,8-TRIDECAFLUOROOCTAN-1-OL

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON MEDETOMIDINE
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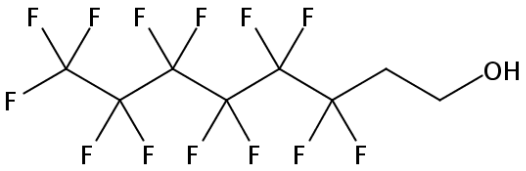
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol
Other names (usual name, trade name, abbreviation)	6:2 FTOH
EC number (if available and appropriate)	211-477-1
EC name (if available and appropriate)	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol
CAS number (if available)	647-42-7
Molecular formula	C8H5F13O
Structural formula	
SMILES notation (if available)	OCCC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F
Molecular weight or molecular weight range	364.1 g mol^{-1}

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
See table 1			

Table 3: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
-				

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 4: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	-										
Dossier submitters proposal	tbd	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol	211-477-1	647-42-7	STOT RE 2 Aquatic Chronic 2	H373 (skeletal system) H411	GHS08 GHS09 Wng	H373 (skeletal system) H411	-		
Resulting Annex VI entry if agreed by RAC and COM					STOT RE 2 Aquatic Chronic 2	H373 (skeletal system) H411	GHS08 GHS09 Wng	H373 (skeletal system) H411	-		

Table 5: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)		
Oxidising gases		
Gases under pressure		
Flammable liquids		
Flammable solids		
Self-reactive substances		
Pyrophoric liquids		
Pyrophoric solids		
Self-heating substances		
Substances which in contact with water emit flammable gases		
Oxidising liquids		
Oxidising solids		
Organic peroxides		
Corrosive to metals		
Acute toxicity via oral route		
Acute toxicity via dermal route		
Acute toxicity via inhalation route		
Skin corrosion/irritation		
Serious eye damage/eye irritation		
Respiratory sensitisation		
Skin sensitisation		
Germ cell mutagenicity		
Carcinogenicity		
Reproductive toxicity		
Specific target organ toxicity-single exposure		
Specific target organ toxicity-repeated exposure	Harmonised classification proposed	**Yes**
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Harmonised classification proposed	**Yes**
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There is no information available if this compound had been previously discussed by the TC C&L.

RAC general comment

3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol, hereinafter referred to with its abbreviation, **6:2 FTOH**, is registered under REACH for intermediate use only.

Structural formula of 6:2 FTOH

6:2 FTOH is a liquid (at 20 °C and 101.3 kPa) with a boiling point of 88 – 95 °C (at 28 – 30 mmHg), water solubility of 18.8 mg/L (at 22.5 °C) and Log K_{OW} of 4.54 (data from the REACH registration dossier).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Requirement for harmonised classification by other legislation or process.

Further detail on need of action at Community level
The German CA investigated the possibility to identify 6:2-FTOH as substance of very high concern. A prerequisite to fulfil the criteria for toxicity is the harmonised classification and labelling as STOT RE 2.

Disagreement by DS with current self-classification
Notified classification and labelling are inconsistent and contradictory as seen below (as of 09.09.2020):
Acute Tox. 4 (H302) = 92 of 166
STOT RE 2 (H373) = 36 of 166
STOT SE 3 (H335) = 68 of 166
STOT RE 1 (H372) = 1 of 166
Skin Irrit. 2 (H315) = 68 of 166
Eye Irrit. 2 (H319) = 68 of 166
Aquatic Chronic 2 (H411) = 23 of 166
Not classified = 6 of 166

5 IDENTIFIED USES

According to the registration dossier the compound is used as intermediate.

6 DATA SOURCES

The following search terms were used:
3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol, 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluoro-1-octanol, 1,1,2,2-Tetrahydroperfluoro-1-octanol, 1H,1H,2H,2H-Perfluorooctanol, 1H,1H,2H,2H-Perfluorooctan-1-ol, 1-Octanol, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-, 6:2 fluorotelomer alcohol, 6:2 FTOH, 6-2-fluorotelomer alcohol.

The following data sources were used: Pubmed, Web of Science, Scopus, Embase, SpringerLink, Wiley online Library, Taylor & Francis.

7 PHYSICOCHEMICAL PROPERTIES

Table 6: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20 °C and 101,3 kPa	liquid	REACH registration dossier	Visual observation
Melting/freezing point	-33.09 °C at atmospheric pressure	REACH registration dossier	According to OECD Guideline 102 Differential scanning calorimetry
Boiling point	88-95 °C at 28-30 mmHg	Day, Richard I.; GB 994607 1965 CAPLUS, cited from Scifinder	
Relative density	1.6782 g/cm^2 at 25 °C	Day, Richard I.; GB 994607 1965 CAPLUS, cited from Scifinder	
Vapour pressure	18 Pa at 25 °C (Gas-phase NMR) 44 Pa at 25 °C (Scott Method) 108 Pa at 35 °C (Gas Saturation Method)	REACH registration dossier	
Water solubility	18.8 mg/L at 22.5 °C	REACH registration dossier	Equivalent or Similar to OECD Guideline 105 flask method
Partition coefficient n-octanol/water	Log Pow: 4.54	REACH registration dossier	Equivalent or Similar to OECD Guideline 107 shake-flask method to: flask method

8 EVALUATION OF PHYSICAL HAZARDS

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.2 Skin corrosion/irritation
This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.3 Serious eye damage/eye irritation
This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.4 Respiratory sensitisation
This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.5 Skin sensitisation
This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.6 Germ cell mutagenicity
This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.7 Carcinogenicity
This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.8 Reproductive toxicity
This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.9 Specific target organ toxicity-single exposure
This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

10.10 Specific target organ toxicity-repeated exposure

Table 7: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
"28-day Repeated Dose Toxicity Study in Mammalian Species" according to Notification No 1121002 of the Pharmaceutical and Food Safety Bureau, MHLW, No 2 of the Manufacturing Industries Bureau, METI & No 031121002 of the Environmental Health Departement, MOE. Equivalent to OECD TG 407 Reliability 2 Rats, Crl:CD(SD) at 5 weeks of age, males and females, Five animals/sex/dose No data on fluoride concentrations in plasma, urine, dentin or bone matrix. No histopathology data on the trabecular bone (only decalcified bone medullary cavity examined).	Oral study, 5, 25 and 125 mg/kg body w/day and a vehicle control group. The substance was dissolved in olive oil (vehicle) and applied daily by gavage. 28 days of treatment. Additional animals were used for two recovery groups (vehicle and 125 mg/kg body w/day). The recovery period was 14 days. In compliance with GLP. The purity of 6:2 FTOH was 99.8 %.	Decreased locomotor activity, decreased respiration rate and (on day 7 only) incomplete eye opening in the males in the 125 mg/kg group was observed during the dosing period. Discoloration of the incisors was observed in two of five male animals in the 25 mg/kg bw/d group and in ten of ten male animals in the 125 mg/kg bw/d group. Mottled teeth were noted in one of five male animals in the 25 mg/kg bw/d group and eight of ten male animals in the 125 mg/kg bw/d group. In the recovery period discoloration of the incisors and mottled teeth were observed in five of five males in the 125 mg/kg bw/d recovery group. A delamination of the low incisor tip surface was observed in four of five males in the 125 mg/kg bw/d recovery group. At the termination of the dosing period, relative liver weight was significantly increased in the 125 mg/kg bw/d group (males). At the termination of the recovery period, relative testis weights were significantly increased in the 125 mg/kg bw/d group. Decreased iron pigments of the ameloblasts at maturation stage was observed in the incisor of one male rat of the 125 mg/kg bw/d group at the termination of dosage and in one male rat of the 125 mg/kg bw/d recovery group. All five male rats of the 125 mg/kg bw/d group showed periportal hypertrophy of the hepatocytes in the liver. Discoloration of the incisors was observed in three of five female animals in the 25 mg/kg bw/d group and in ten of ten female animals in the 125 mg/kg bw/d group. Mottled teeth were observed in six of ten animals in the 125 mg/kg bw/d group. In the recovery period discoloration of the incisors and mottled teeth and delamination of the low incisor tip surface were observed in five of five females in the 125 mg/kg bw/d recovery group. At the termination of the dosing period, relative liver weight was increased in the 25 mg/kg bw/d group. Absolute and relative liver weights were increased in the 125 mg/kg bw/d group (females). At the termination of the recovery period, the relative liver and ovary weights were significantly increased in the 125 mg/kg bw/d group. Increased ALT and ALP activities were seen in male and female animals and increased total cholesterol in the female animals of the 125 mg/kg bw/d group. On necropsy enlargement of the liver in females and blackish discoloration of the glandular stomach were observed in males from 25 mg/kg and in females at 125 mg/kg bw/d. Submucosal edema in the glandular stomach in males and females, haemorrhage and necrosis of the fundic mucosa of the glandular stomach,	(Hita Laboratory, 2007) (study report)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		decreased goblet cells in the colon and periportal hypertrophy of the hepatocytes in males and diffuse liver cell hypertrophy in (all five) females were observed in the 125 mg/bw group. Decreased iron pigments of the ameloblasts at maturation stage was observed in the incisor of two female rat of the 125 mg/kg bw/d group at the termination of dosage and in one female rat of the 125 mg/kg bw/d recovery group. An irregular alignment of the ameloblasts at maturation stage was observed in the incisor of three female rats in the 125 mg/kg bw/d recovery group. Decreased iron pigments of the ameloblasts at maturation stage was observed in the incisor of two female rat of the 125 mg/kg bw/d group at the termination of dosage and in one female rat of the 125 mg/kg bw/d recovery group. An irregular alignment of the ameloblasts at maturation stage was observed in the incisor of three female rats in the 125 mg/kg bw/d recovery group. In the recovery group, in addition to discoloured incisors, mottled teeth and decreased iron pigments of the ameloblasts at maturation stage observed during and the end of the dosing period, delamination of the low incisor tip surface, cell infiltration of the gingiva and irregular alignment of ameloblasts at maturation stage were newly observed. Changes in the stomach, intestinal tract and liver had disappeared or improved.	
90-day study According to US EPA OPPTS 870.3100 (1998), OECD TG 408 Reliability 2 Rats (Crl:CD(SD)) were approximately 7 to 8 weeks old at study start. 10 animals/sex/dose group Fluoride concentrations were determined in plasma and urine. No histopathology data on the	Oral study, 5, 25, 125 and 250 mg/kg bw/d and a vehicle control group. The substance was diluted in NANOpure water. All animals were dosed once daily by gavage at a dose volume of 5 mL/kg bw. All animals were treated for 90 days. Additional animals were assigned to recovery groups of 1 month (10 animals/sex/dose) and three month (5 animals/sex/dose). In compliance with GLP. The purity of 6:2	Substance-related mortality was observed at 125 mg/kg bw/d (1/25 females at day 62) and 250 mg/kg bw/d (6/25 males and 13/25 females from days 22 to 84), with the majority of the deaths attributed to kidney degeneration and necrosis. Following 90 days of dosing, effects on organ weights were present in the testes, liver and kidney of males and in livers and kidneys of females. No effects on organ weights were observed at 5 mg/kg/day in males or females. Relative testes weight was increased in males at all test doses. Relative liver and kidney weight parameters were increased in male rats dosed with 25, 125, and 250 mg/kg/day and in female rats dosed with 125 mg/kg/day and in the single female rat remaining at 250 mg/kg/day. Following a one-month recovery, male and female rats at the highest dose had increased liver weights, whereas no increases over control rats were observed following 3 months of recovery. In addition, thyroid weights in female rats were increased in the 250 mg/kg/day group at the 1-month recovery time point and in the 25, 125, and 250 mg/kg/day groups at the 3-month recovery time point. Dental effects included whitened teeth and increased incidence in missing/broken/misaligned incisors in the 125 mg/kg bw/d and 250 mg/kg bw/d group. Histopathological investigations showed an effect on the ameloblastic epithelium of the teeth in male rats at 250 mg/kg bw/d. These findings were present at the 1-month recovery sacrifice (250 mg/kg bw/d both sexes),	(Charles River Laboratories Preclinical Services, 2012) (study report) Cited in (Serex et al., 2014) (publication)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
trabecular bone (only decalcified bone medullary cavity examined)	FTOH was 99.7 %.	but had resolved by the 3-month recovery sacrifice. Plasma fluoride concentrations increased from 0.1 mg/L in control animals (both sexes) and 5 mg/kg bw/d group (both sexes) and 25 mg/kg bw/d group (females only) to 0.2 mg/L in 25 mg/kg bw/d group (males only). At 125 mg/kg bw/d the plasma fluoride concentration yielded 0.7 mg/L in males and 0.6 mg/L in females. At 250 mg/kg bw/d the plasma fluoride concentration yielded 0.9 mg/L in males (mean of 9 animals) and to single value of 1.1 mg/L in the only surviving female rat. Urine fluoride was increased in all treated male and female groups, dose-relationship at 5 and 25 mg/kg/d was less clear for females. Liver effects of minimal severity were observed at the end of dosing in females at 25 mg/kg/day and above and in male rats at 125 mg/kg/day and above. These effects included single-cell necrosis, vacuolization, oval/biliary hyperplasia, hepatocellular hypertrophy and periportal inflammation. In males, none of the effects were noted at the 1-month recovery sacrifice. In females, most of these effects were not present at the 1-month recovery sacrifice, and by 3 months only a few rats (125 and 250 mg/kg/day females) had biliary hyperplasia. At 3 months recovery, all doses (5, 25 125 and 250 mg/kg bw/d) were investigated by histopathology.	
Subacute Inhalation Toxicity: 28 d study According to OECD TG 412 Reliability 2 Rat, Crl:CD (SD) 8 weeks old at study start 20/ sex/group (control; high dose), 10 males and females used for recovery group, 10/sex/group (low-dose; mid-dose)	Inhalation study (vapour, whole body), 0, 1, 10, 100 ppm The substance was formulated in a vehicle of corn oil 6 hours/day, 5 day/week, 23 exposures over a 4-week period In compliance with GLP. The purity of 6:2 FTOH was 99.94 %.	No effect on body weight, food consumption, clinical signs of toxicity 100 ppm (1.49 mg/l): decreased motor activity in males during 4th week of exposure (rerversible during recovery period); increased absolute liver weights; clinical chemistry: increased mean serum bilirubin levels, increased ALT in females; microscopic findings: increased lamination of dentin of the incisor teeth and incomplete decalcification of enamel of the incisors and the bone trabeculae in tibia and femur, but no histopathological effects observed	(DuPont Haskell Global Centers for Health & Environmental Sciences, 2011) (study report)
According to OECD TG 415. Reliability 2 Sprague-Dawley rats	Oral study, 5, 25, 125 and 250 mg/kg bw/d and a vehicle control group. The substance was formulated in a	In the parental generation rats some unscheduled deaths occurred at 125 and 250 mg/kg bw/d. At 250 mg/kg bw/d, three male rats and 13 female rats were found dead or were humanely euthanized due to adverse clinical signs during the dosage period. For the males, deaths occurred between test days 76 and 81. For females, the deaths occurred throughout	(Charles River Laboratories, 2008) (study report)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
(Crl:CD(SD)), were approximately 7 weeks old at study start. 20 animals/sex/dose group No data on fluoride concentrations in plasma, urine, dentin or bone matrix. No histopathology data on the trabecular bone (only decalcified bone medullary cavity examined).	vehicle of 0.5 % aqueous methylcellulose. All animals were dosed once daily by gavage at a dose volume of 5 mL/kg bw. Male and female rats were dosed for at least 70 days prior to mating, and throughout the cohabitation period (ca 2 weeks), gestation (females only), and lactation (females only). Offspring from the first generation were not directly dosed. In compliance with GLP. The purity of 6:2 FTOH was 99.7 %.	all phases of the dosing period, beginning on test day 23. During premating dosing four females died, during gestation period five females died and finally, during lactation period four females died. In the 125 mg/kg bw/d group, three male rats were found dead. Deaths occurred between test days 76 and 86. Test substance-related effects on the teeth were the most common clinical signs observed in parental male and female rats, and the incidence were statistically significantly higher at 125 and 250 mg/kg bw/d. The most common findings were increased incidences of whitened teeth, missing teeth, misaligned or broken teeth, and overgrown incisors. These effects were observed throughout the dosing periods for males, typically with the first occurrences late in the premating period. The effects on the teeth in females were more frequently observed in gestating and lactating dams, most likely due to the increased duration of test substance administration. The pups of the first generation were investigated at day 22 post-partum. No macroscopic effects were reported on the teeth of the offspring.	Cited in (O'Connor et al., 2014) (publication)
According to OECD TG 415 and US EPA, OPPTS 870.3550. Reliability 2 Mice, Crl:CD1(ICR). Male mice were 50 days old at study start. Female mice were 75 days old at study start. 15 animals/sex/dose group Liver, nose/teeth, ovaries, uterus, vagina, and mammary gland were examined microscopically	Oral study, 1, 5, 25 and 100 mg/kg bw/d and a vehicle control group. The substance was formulated in a vehicle of 0.1 % Tween-80 in 0.5 % aqueous methylcellulose. All animals were dosed once daily by gavage at a dose volume of 5 mL/kg bw. Male mice were dosed for 70 days prior to mating and throughout the cohabitation period (ca 2 weeks). Female Mice were dosed for 14 days prior to mating, and	One male and two female mice at 100 mg/kg bw/d were found dead or humanely euthanized during the premating of gestation periods. During the lactation period, females administered 100 mg/kg bw/d exhibited statistically significantly lower body weight gain, food consumption, and food efficiency during the intervals (lactation day) LD 0-7 and LD 7-14, compared with controls, resulting in statistically significantly lower body weight on LD 7 and LD 14. There were no effects in females at dose levels ≤ 25 mg/kg bw/d. Organ weight effects were observed in liver and kidney of males and females at 100 mg/kg bw/d with statistically significantly increased relative organ weights. In males the relative testes weight was statistically significantly increased at 100 mg/kg bw/d and in females the relative uterus weight was statistically significantly increased at 100 mg/kg bw/d. Microscopic findings indicative of toxic effects on the liver were present in males and females at 100 mg/kg bw/d and in low incidences in females at 25 mg/kg bw/d. These changes were generally more severe in females and included hepatocellular hypertrophy, oval cell hyperplasia, single cell necrosis of hepatocytes, and cystic degeneration (females only). Minimal microscopic hepatocellular hypertrophy was also present in males at 5 and 25 mg/kg bw/d and in females at	(DuPont Haskell Global Centers for Health & Environmental Sciences, 2013) (study report) Cited in (Mukerji et al., 2015) (publication)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
in all dose levels of parental animals.	throughout the cohabitation period, gestation, and lactation. In compliance with GLP. The purity of 6:2 FTOH was 99.7 %.	5 mg/kg bw/d. Substance-related changes in the incisor teeth, consistent with fluoride exposure, were present in males and females at 100 mg/kg bw/d. These changes included degeneration and atrophy of ameloblastic epithelium, accentuation of the normal laminar pattern of dentin and an increase in observed incomplete decalcification of enamel and/or dentin. Degeneration and atrophy of ameloblasts was characterized by segmental disorganization and attenuation of ameloblastic epithelium of the incisor teeth. Lamination of dentin was characterized by the presence of concentric basophilic rings within the dentin of these teeth. There were no adverse changes in the teeth of males or females at dose levels ≤ 25 mg/kg bw/d. Incomplete decalcification of nasal bone was observed in some animals at the 100 mg/kg bw/d dose level.	
Combined Repeated Dose Toxicity Study with Reproduction/ Developmental Screening Test According to OECD TG 422 Reliability 2 Rat, Crl:CD (SD) 10 weeks old at study start 15/ sex/group (control; high dose), 5 animals used for recovery group, 10/sex/group (low-dose; mid-dose)	Oral study, 0, 25, 75, 225 mg/kg bw The substance was formulated in a vehicle of corn oil Rats were dosed for 14 days prior to mating; males until day prior to euthanasia; females through LD3 In compliance with GLP. The purity of 6:2 FTOH was 98.52 %.	225 mg/kg bw: mortalities (1/15 males, 11/15 females) related to tubular lesions in kidneys, necrosis of adrenal cortex, bone marrow depletion; clinical findings include emaciation, hypoactivity, unkempt appearance, body cool to touch and labored respiration; serum chemistry parameters: in females increased urea nitrogen, creatinine, potassium bilirubin, AST and ALT, total protein and globulin, decrease in sodium and chloride and increased albumin in males and females; higher liver weight and hepatic centrilobular hypertrophy in males; tubular degeneration, dilatation and vacuolation in kidneys in males and females (associated with pale kidneys and higher kidney weight), changes in adrenal cortex and bone marrow Reproductive performance and gestation length unaffected; dystocia in 1 female; high pup mortality and lower pup weights observed but litter data confounded by high dam mortality 75 mg/kg bw: effects on body weight and body weight gain	(WIL Research Laboratories, 2005)
Prenatal Developmental Toxicity Study According to OECD TG 414 Reliability 2 Rat, Crl:CD (SD) 67 days old at study start 22 time-mated	Oral study, 0, 5, 25, 125, 250 mg/kg bw The substance was formulated in a vehicle of 0.5 % aqueous methylcellulose. All animals were dosed once daily by gavage at a dose volume of 5 mL/kg	Dams: no mortalities observed, at 250 mg/kg bw slight increase in incidence of stained or wet fur reduced mean daily food consumption and final mean body weight (10 % lower than control); no effects on reproductive outcome; no test substance-related maternal gross post-mortem observations reported Fetal alterations at 125 and 250 mg/kg bw: incomplete ossification of skull bones and wavy and/or thickened ribs (not statistically significant), at 250 mg/kg bw incidence of delayed pelvic bone ossification increased Dams examined grossly for external and internal alterations	(DuPont Haskell Global Centers for Health and Environmental Sciences, 2008) (study report) Cited in (O'Connor et

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
females/group	bw. Daily gavage GD 6-20 In compliance with GLP. The purity of 6:2 FTOH was 99.7 %.		al., 2014) (publication)

Table 8: Summary table of human data on STOT RE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
There are no human data available.				

Table 9: Summary table of other studies relevant for STOT RE.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
There are no other studies available with relevance for STOT RE				

10.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Four repeated dose oral toxicity studies in rats (three studies) and mice (one study) showed substance-related effects on the teeth of the animals. All studies were performed in accordance with the appropriate guidelines. In the subacute study (Hita Laboratory, 2007) discoloration of the incisors, mottled teeth and delamination of the low incisor tip surface were observed in male and female rats treated with 125 mg/kg bw/d. Even if the discoloration of the incisors may be judged as less severe, mottled teeth and delamination of the low incisor tip surface have serious consequences for the stability of the teeth as shown in the studies with a longer exposure. In this 28-day study discoloration (2/5 males, 3/5 females) and mottled teeth (1/5 male) were already seen at 25 mg/kg bw/d. No recovery of teeth effects was seen at the end of the 1-month or 3-month recovery period (data available for the high dose only).

The one generation reproductive toxicity study in mice had two exposure scenarios (Mukerji et al., 2015): Male mice were exposed for 70 day prior to mating and throughout the cohabitation period of 14 day yielding a total exposure of 84 day. Female mice were exposed 14 day prior to mating, throughout the cohabitation period of 14 day, throughout gestation (18 days) and throughout lactation (21 days) yielding a total exposure of 67 days. Effects on the teeth were observed in males and females of the 100 mg/kg bw/d group. These effects included degeneration and atrophy of ameloblastic epithelium. Since this epithelium forms the enamel of the tooth, a degeneration and atrophy is judged as severe effect.

The one generation reproductive toxicity study in rats showed severe effects such as missing or broken teeth at a dosage of 125 mg/kg bw/d and higher (O'Connor et al., 2014).

Dose-related increase of mortalities with delayed occurrence were seen at 125 mg/kg bw/d (day 62) and at 250 mg/kg bw/d that together with mortalities (for males, death occurred between test days 76 and 81, for

females, the deaths occurred throughout all phases of the dosing period) seen in the one-generation study supports the need for classification.

Furthermore, a subacute inhalation study (DuPont Haskell Global Centers for Health & Environmental Sciences, 2011) showed effects on teeth such as increased lamination of dentin and incomplete decalcification of enamel of the incisors at 100 ppm (1.49 mg/l) when the whole body of rats was exposed to vapour for 6 hours/day. However, these effects did not include histopathological changes of teeth. As effects are less severe than effects observed in the oral studies, they are considered as supportive evidence.

In contrast, in a combined repeated dose toxicity study with reproduction/developmental screening test and in a prenatal developmental toxicity study no effects on teeth were reported (DuPont Haskell Global Centers for Health and Environmental Sciences, 2008; WIL Research Laboratories, 2005). However, it is not clear from the results reported if teeth were analysed in detail.

Table 10: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days [if adequate, otherwise please delete]

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
(Hita Laboratory, 2007)	25	28 days	8	STOT RE 1 (≤ 10 mg/kg bw/d guidance value)
(Serex et al., 2014)	125	90 days	125	None (≤ 100 mg/kg bw/d guidance value) (Note: Elevated urine fluoride concentration ≥ 25 mg/kg bw/d in male rats, increased plasma fluoride, ≥ 25 mg/kg bw/d)
(O'Connor et al., 2014)	125	At least 84 days	117	None (≤ 100 mg/kg bw/d guidance value)
(Mukerji et al., 2015)	100	At least 84 days in males At least 67 days in females	93.3 74.4	STOT RE 2 (≤ 100 mg/kg bw/d guidance value) STOT RE 2 (≤ 100 mg/kg bw/d guidance value)

The subchronic oral toxicity study in rats showed severe effects such as missing or broken teeth at a dosage of 125 mg/kg bw/d and higher (Serex et al., 2014). Measurements of fluoride in the plasma showed a treatment related increase from 0.1 mg/L to 0.2 mg/l in male rats at 25 mg/kg bw/d and to 0.6 (females) mg/L and 0.7 (males) mg/L in the 125 mg/kg bw/d group. Increased urine fluoride concentrations were seen in all treated male and female groups (with less clear dose-relationship for the female groups). The fluoride release from 6:2 FTOH is the most likely mode of action of the damage to the teeth.

A review emphasized the suitability of small rodents as a model for the study of human dental fluorosis (Bronckers et al., 2009).

The enamel is a target of the fluoride action. The molecular mechanism underlying enamel fluorosis is still unknown (Lyaruu et al., 2014). Enamel formation by ameloblasts is a 2-step-event. First, secretory ameloblasts synthesize and secrete a matrix rich in amelogenins, in which long thin crystal ribbons are formed. In the maturation stage, most of the matrix is removed and the crystals expand. Maturation ameloblasts have ion-transporting, resorptive, and degrading functions and cyclically transform into 1 of 2 morphologically distinct cell types; ruffle-ended or smooth-ended ameloblasts (Lyaruu et al., 2014).

Fluoride interacts with the function of ameloblasts at all stages of the development of ameloblasts. Fluoride reduces degradation of matrix proteins by lowering the output of proteases by the ameloblasts. Fluoride acts directly on protease activity in the extracellular matrix and inhibits matrix degeneration. Fluoride changes the adsorption characteristics, surface area, or surface properties of enamel crystals to which matrix proteins adhere. Fluoride reduces calcium in the enamel fluid required for protease activity. Fluoride impairs endocytosis and intracellular degradation of matrix by modulating ameloblasts. Fluoride increases apoptosis or stimulates some of the maturation ameloblasts to migrate from the ameloblastic layer (Bronckers et al., 2009).

The improper mineralization that occurs with enamel fluorosis is thought to be due to inhibition of the matrix proteinases responsible for removing amelogenin fragments. The delay in removal impairs crystal growth and makes the enamel more porous. The degree of porosity of such teeth results in a diminished physical strength of the enamel, and parts of the superficial enamel may break away (National Research Council, 2006).

EFSA used the effects on dental fluorosis to define Tolerable Upper Intake Level for fluoride for children up to the age of eight years. EFSA also review the effects on bones (skeletal fluorosis). The observed teeth effects at increased plasma and urine levels of fluoride appears similar to chronic fluorosis in humans where chronic high intake of fluoride may lead to dental fluorosis and skeletal fluorosis that is likely to result in increased fracture rates in the population. Ninety-nine percent of the total fluoride content of the body is concentrated in calcified tissue, bone and teeth (EFSA NDA Panel, 2013).

Therefore, bones and teeth are important targets of fluoride toxicity. The mechanism is described as follows. Because of similarities in size and charge, fluoride readily replaces OH- in the crystal lattice structure by an exchange and adsorption reaction. When the content of fluoride in bone reaches 2500 ppm, major pathological changes begin to occur. Mottled osteons appear, characterised by hypomineralisation, enlarged peripheral osteocyte lacunae, tangled canaliculi and increased numbers of pheripheral osterocytes with loss of osteocytes in the remainder of the osteon. Excessive fluoridation reduces the biomechanical properties of the bone, increases resorption and remodelling activities (which is observed as enlargement of the marrow cavity), converts the inner cortex to cancellous bone, and accelerates the development of resorption cavities in the outer cortical laminar zone (Woodard et al., 2002). As pointed out above, these effects on the bones may result in increased fracture rates in the population.

The studies available did not present information on the undecalcified bone matrix due to fact that such investigations need special staining procedures and were not conducted in any of the studies available. The evidence on chronic fluorosis-related mottled/broken teeth should be taken as a surrogate for systemic fluorosis assuming that bone fluorosis (that could not be detected without appropriate methodology) was also present.

10.10.2 Comparison with the CLP criteria

Four repeated dose toxicity studies in rats (three studies) and mice (one study) showed consistently substance-related adverse effects on the teeth of the animals. The extrapolated effective doses of two of these studies were within the guidance value range of STOT RE 1 or STOT RE 2 (see Table 10). In the subacute (Hita Laboratory, 2007) mottled teeth and delamination of the low incisor tip surface were observed in male and female rats treated from doses of 25 mg/kg bw/d onwards. Mottled teeth and delamination of the low incisor tip surface have serious consequences for the stability of the teeth as shown in the studies with a longer exposure and fulfil the criteria of point 3.9.2.7.3 d) “significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination”.

The one generation reproductive toxicity study in mice had two exposure scenarios (Mukerji et al., 2015): Effects on the teeth were observed in males and females of the 100 mg/kg bw/d group. These effects included degeneration and atrophy of ameloblastic epithelium. Since this epithelium forms the enamel of the tooth, a degeneration and atrophy is judged as severe effect und fulfils the criteria of point 3.9.2.7.3 d) “significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination”.

Two other studies showed serious effects such as missing or broken teeth but these effects were observed at doses beyond the guidance values of 100 mg/kg bw/d. However, these effects confirm the severity of the effects observed at doses below 100 mg/kg bw/d. The effective dose levels of the studies (O'Connor et al., 2014; Serex et al., 2014) were formally slightly above the guidance value, however indications on (urine/plasma) fluorosis were seen starting at low doses of 25 mg/kg bw/d ((Serex et al., 2014), no such data in (O'Connor et al., 2014)). Delayed mortalities at ≥ 125 mg/kg bw/d (O'Connor et al., 2014) were considered as findings supporting the classification proposal.

STOT RE 2 is preferred on a weight of evidence that takes into consideration the available data.

10.10.3 Conclusion on classification and labelling for STOT RE

Two studies showed serious effects on teeth and fulfil the criteria of point 3.9.2.7.3 d) "significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination". The effects were mottled teeth and delamination of the low incisor tip surface in one study with an extrapolated effective dose of 8 mg/kg bw/d and degeneration and atrophy of ameloblastic epithelium in the second study with an extrapolated effective dose of 74 mg/kg bw/d. These values are either within the range for guidance values for STOT RE 2 ($10 \leq C \leq 100$ mg/kg bw/d) or even in the range for guidance values for STOT RE 1 ($C \leq 10$ mg/kg bw/d) after oral exposure. By weight of evidence taking into account all available studies which consistently indicated treatment-related chronic fluorosis-related tooth abnormalities it is proposed that 6:2 FTOH should be classified and labelled as STOT RE 2, H371 (May cause damage to organs (skeletal system) through prolonged or repeated exposure).

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) presented two 28-day studies in rats (an oral and an inhalation study), one oral 90-day study in rats, two oral one-generation studies (one each in rats and in mice), one oral combined repeated-dose and reproductive toxicity screening study in rats, and one oral prenatal developmental toxicity study in rats for the evaluation of STOT RE for 6:2 FTOH.

The DS proposed to classify 6:2 FTOH for STOT RE 2 based on effects observed in the 28-day study in rats (mottled teeth and delamination of the lower incisor tip surface at 25 mg/kg bw/d supporting STOT RE 1 [equivalent guidance value ≤ 30 mg/kg bw/d]) and in the one-generation study in mice (degeneration and atrophy of ameloblastic epithelium at 100 mg/kg bw/d, supporting STOT RE 2 [equivalent guidance value ≤ 134 mg/kg bw/d based on 67 days of exposure to females in the study]).

The DS proposed to specify skeletal system as the target organ with the following justification: "*The studies available did not present information on the undecalcified bone matrix due to fact that such investigations need special staining procedures and were not conducted in any of the studies available. The evidence on chronic fluorosis-related mottled/broken teeth should be taken as a surrogate for systemic fluorosis assuming that bone fluorosis (that could not be detected without appropriate methodology) was also present.*"

Comments received during consultation

Two MSCAs and a company submitted comments during the consultation. The comment by the

company was not relevant to STOT RE. One MSCA requested further explanation for the choice of category 2 as, in their view, the effects in the 28-day study point to category 1 and the discordance in results between 28 and 90-day studies should be discussed. The DS responded that, as opposed to only one 28-day study pointing to category 1, there are also three studies with longer duration that either support category 2 or no classification. The DS also noted that the effects on teeth were identified as gross pathological findings and in only some studies the teeth were microscopically examined on decalcified H&E stained paraffin sections. The DS further added that, in order to fully assess the detailed structures of mineralised and cellular components of teeth and the bone matrix, specific embedding techniques on decalcified and non-decalcified samples at different localisations would be needed and these are not included in the standard protocols of the OECD test guidelines.

The other MSCA supported the proposed STOT RE 2 classification. However, they considered that specifying teeth as the target organ could be appropriate. The MSCA reasoned that effects on bone may have occurred in the animals, but any such effect had not been substantiated in the studies (due to lack of evaluation of bone in the studies, except in the mice study). The MSCA further claimed that whether the increased plasma fluoride levels and dental fluorosis, together with the effect on nasal bones in mice, are sufficient as surrogate for effects overall on bone was a borderline case. The MSCA also commented that, based on the liver effects observed in most of the studies, a classification as STOT RE 2 should also be considered for liver toxicity. The DS agreed to adding liver as the second target organ. The DS also agreed that specifying teeth instead of skeletal system as the primary target organ is an option. In the DS's view fluorosis should be considered as a systemic adverse effect affecting the whole skeletal system (bones and teeth) and mottled teeth are considered as an indicator for the systemic disorder of bone metabolism. Therefore, they preferred specifying skeletal system as the primary target organ.

Assessment and comparison with the classification criteria

In the CLH report, the DS identified skeletal system as the target organ for STOT RE and in the RCOM agreed to liver also being a target organ.

In the 28-day oral study (Hita Laboratory, 2007; unpublished), equivalent to OECD TG 407 and in compliance with GLP, 6:2 FTOH was administered by gavage to 5 rats/sex/dose at 5, 25 and 125 mg/kg bw/d. There were also two (vehicle and high dose) 14-day recovery groups with 5 animals/sex.

There were no mortalities in the study. Body weight and food consumption data were not presented in the CLH report. Clinical observations included decreased locomotor activity, decreased respiration rate and (on day 7 only) incomplete eye opening in the males in the 125 mg/kg bw/d group during the dosing period.

5 mg/kg bw/d group: No effects.

25 mg/kg bw/d group: Discoloration of the incisors (2 M, 3 F), mottled teeth (1 M, 0 F), increase in relative liver weight (F only) and enlargement of liver (F only) were observed.

125 mg/kg bw/d group: Discoloration of the incisors (5 M, 5 F), mottled teeth (3 M, 1 F) and decreased iron pigments of the ameloblasts at maturation stage in the incisors (1 M, 2 F) were observed. These effects persisted in the recovery group. In addition, delamination of the lower incisors tip surface (4 M, 5 F), an irregular alignment of the

ameloblasts at maturation stage in the incisors (3 F) and cell infiltration of the gingiva were newly observed in the recovery group.

Liver effects at 125 mg/kg bw/d group included: significant increase in relative weight (M), increased absolute and relative weights (F; the increased relative weight remained statistically significant in the recovery group), increased ALT and ALP activities (M, F), increased total cholesterol (F only), enlargement of liver (M, F), periportal/diffuse liver cell hypertrophy (5 M, 5 F) were observed at the end of dosing period but disappeared/improved in the recovery group.

Support for STOT RE classification: The equivalent guidance values for 28-day oral study are ≤ 30 mg/kg bw/d for Cat. 1 and ≤ 300 mg/kg bw/d for Cat. 2. RAC considers the dental effects starting at 25 mg/kg bw/d support Cat. 1. The severity of these effects considerably increased in the next dose level and the result was also supported by histopathological findings. RAC considers the liver toxicity as adaptive responses that were reversed at the end of recovery period and thus support no classification.

In the 28-day inhalation study (Dupont, 2011; unpublished), according to OECD TG 412 and in compliance with GLP, 6:2 FTOH was administered via whole-body exposure to 10 rats/sex/dose at 1, 10 and 100 ppm for 6 h/d (5 days/week). There were two (control and high dose) 1-month recovery groups with 10 animals/sex/dose.

There were no treatment-related mortalities or effects on body weights and food consumption. Clinical observations were limited to decreased locomotor activity in males during the 4th week of exposure period in the high dose group (100 ppm corresponding to 1.49 mg/L) that was reversed during recovery period.

In the high dose group, there was increased lamination of dentin of the incisors and incomplete decalcification of enamel of the incisors, and the bone trabeculae in tibia and femur that remained during the recovery period.

Liver effects observed in the high dose group were increased absolute and relative liver weight, increased mean serum bilirubin and only in females, increased ALT. All these effects were reversed during the recovery period.

Support for STOT RE classification: The equivalent guidance value for 28-day inhalation study is > 0.6 and ≤ 3 mg/L/6h/d for Cat. 2. Although the details on the effects are limited in this study, RAC considers the dental and bone effects at 1.49 mg/L supports Cat. 2. Since the liver effects in this study were reversible and with no correlated histopathological findings, RAC considers these to support no classification.

In the 90-day oral study (Charles River Laboratories, 2012; unpublished and Serex *et al.*, 2014), according to OECD TG 408 and in compliance with GLP, 6:2 FTOH was administered by gavage to 10 rats/sex/dose at 5, 25, 125 and 250 mg/kg bw/d. There were also recovery groups of 1-month (control and high dose; 10 animals/sex/dose) and 3-months (all dose levels; 5 animals/sex/dose). Fluoride concentrations were determined in plasma and urine.

Treatment-related mortality mostly attributed to kidney degeneration and necrosis was observed in the 125 mg/kg bw/d (1/25 F at day 62) and 250 mg/kg bw/d groups (6/25 M and 13/25 F from day 22 to 84). Clinical observations were also limited to 125 mg/kg bw/d and 250 mg/kg bw/d groups. The mean body weight changes were about 9% (M) and 3% (F) of the control values and were not dose dependent.

Whitened teeth and increased incidence in missing/broken/misaligned incisors were observed in

the 125 and 250 mg/kg bw/d groups. Effects on ameloblastic epithelium of the teeth were observed in the 250 mg/kg bw/d group (M only, but there was only 1 F left in this group) which were also seen after 1-month recovery period but resolved by 3-months.

There were dose-related statistically significant increases in absolute and relative liver weights at ≥ 25 mg/kg bw/d groups in males or females. Histopathology of liver revealed minimal severity effects in males at ≥ 125 mg/kg bw/d groups and in females at ≥ 25 mg/kg bw/d groups. These effects included single-cell necrosis, vacuolization, oval/biliary hyperplasia, hepatocellular hypertrophy and periportal inflammation. No quantitative details on the effects were reported in the Serex *et al.* (2014) publication. In males, none of the effects were noted at the 1-month recovery sacrifice. In females, most of these effects were not present at the 1-month recovery sacrifice, and by 3 months only a few females in the 125 and 250 mg/kg bw/d groups had biliary hyperplasia.

***Table**: Absolute and relative liver weights in the 90-day oral study (adapted from Serex et al., 2014)*

Dose in mg/kg bw/d (no. of animals)		Control (10/sex)	5 (10/sex)	25 (10/sex)	125 (10M, 9F)	250 (8M, 1F)
Males	Weight (g)	15.94 ± 1.90	16.09 ± 1.90	16.62 ± 2.02	19.09 ± 1.89*,b	22.84 ± 2.39*,a,b
	Ratio (%)	2.95 ± 0.26	3.04 ± 0.15	3.26 ± 0.16*,b	3.94 ± 0.17*,b	4.61 ± 2.39*,c
Females	Weight (g)	8.44 ± 0.70	8.67 ± 0.92	9.47 ± 0.90*	12.50 ± 1.06*	14.62c
	Ratio (%)	3.20 ± 0.23	3.13 ± 0.19	3.43 ± 0.31	4.45 ± 0.30*,a,b	5.58*,c

a: Parameter no longer statistically significantly different from control rats 1 month after cessation of dosing.
b: Parameter no longer statistically significantly different from control rats 3 months after cessation of dosing.
c: Parameter statistically significantly higher than control at 1 but not 3 months after cessation of dosing.
* statistically significant

There was a dose-related statistically significant increase in urine fluoride at ≥ 25 mg/kg bw/d groups in males or females. There was a dose-related significant increase in plasma fluoride in males (≥ 25 mg/kg bw/d groups) and females (≥ 125 mg/kg bw/d groups). Plasma fluoride was partially reversible after approximately 1 month of recovery and completely reversible after 3 months of recovery.

***Table**: Plasma and urine fluoride levels in the 90-day oral study (adapted from Serex et al., 2014)*

Dose in mg/kg bw/d (no. of animals)		Control (10/sex)	5 (10/sex)	25 (10/sex)	125 (10M, 9F)	250 (8M, 1F)
Plasma fluoride (µg/mL)	Males	0.1 ± 0	0.1 ± 0	0.2 ± 0*	0.7 ± 0.2*	0.9 ± 0.2*a
	Females	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.6 ± 0.2*	1.1a,b
Urine fluoride (µg)	Males	11.3 ± 3.1	106 ± 39	482 ± 146*	1890 ± 407*	3602 ± 670*
	Females	6.0 ± 1.9	39 ± 13	18 ± 64*	1206 ± 288*	2921

a: 0.2 µg/mL plasma fluoride after 1 month recovery; b: 0.1 mg/µL or not detected after 3 month recovery;
* statistically significant

Support for STOT RE classification: The guidance value for 90-day oral study is > 10 and ≤ 100 mg/kg bw/d for Cat. 2. Although there were no dental effects in this study at 25 mg/kg bw/d,

the effects (missing/broken/misaligned incisors) at the next dose level of 125 mg/kg bw/d were very severe. Also, the plasma and urinary fluoride levels were dose-dependently increased with reaching statistical significance already at 25 mg/kg bw/d. Therefore, RAC considers the dental effects in this study to support Cat. 2. In females, the increased liver weight was correlated with histopathological findings at ≥ 25 mg/kg bw/d. However, due to lack of quantitative details on the liver effects, RAC considers the liver effects in this study not sufficient for classification.

In the one-generation oral study in rats (Charles River Laboratories, 2008; unpublished and O'Connor *et al.*, 2014), according to OECD TG 415 and in compliance with GLP, 6:2 FTOH was administered via gavage to 20 animals/sex/dose at 5, 25, 125 and 250 mg/kg bw/d. The males were exposed for about 84 days and females for about 126 days in total.

Treatment-related mortalities were observed in the 125 mg/kg bw/d group (3 M) and in the 250 mg/kg bw/d group (3 M, and 13 F; of which 4 during premating, 5 during gestation and 4 during lactation period). In the 250 mg/kg bw/d group, the mean body weight gain during premating period was -14% (M) and -10% (F) compared to controls.

The following dental effects with statistically significant increase in incidences were observed in parental males and females at 125 and 250 mg/kg bw/d groups: whitened teeth, missing teeth, misaligned or broken teeth and overgrown incisors. These effects were mostly observed during late premating period for males and during gestation and lactation periods for females. No further details for e.g., on the number of animals affected in each group were presented in the CLH report. No macroscopic dental effects were observed in the pups (on PND 22).

Parameters relevant to liver toxicity were not examined in the study.

Support for STOT RE classification: The equivalent guidance value for 84 days oral exposure (to males) is ≤ 107 mg/kg bw/d for Cat. 2. At 125 mg/kg bw/d there were severe dental effects (missing/misaligned/broken teeth) in males. While noting that the guidance values are not meant to be strict demarcation values, RAC considers the severity of dental effects observed in this study to support Cat. 2.

In the one-generation oral study in mice (DuPont, 2013; unpublished and Mukerji *et al.*, 2015), according to OECD TG 415 and in compliance with GLP, 6:2 FTOH was administered via gavage to 15 animals/sex/dose at 1, 5, 25 and 100 mg/kg bw/d. The males were exposed for about 84 days and females for about 67 days in total.

Treatment-related mortalities (1 M, 2 F) were observed in the 100 mg/kg bw/d group during premating or gestation periods. In the 100 mg/kg bw/d group, the final mean body weight was -5% in males and there was no effect on females during the premating period compared to controls.

In the 100 mg/kg bw/d group the following effects on the incisors were observed: degeneration and atrophy of ameloblasts characterized by segmental disorganization and attenuation of ameloblastic epithelium of the incisors; lamination of dentin characterized by the presence of concentric basophilic rings within the dentin of these teeth; incomplete decalcification of enamel and dentin characterized by an increase in the observed presence of basophilic, mineralized debris in the enamel space of the incisor between the dentin and the gingiva. Furthermore, consistent with fluoride exposure, an incomplete decalcification of nasal bones in some animals was observed in the 100 mg/kg bw/d group. The numbers of animals showing effects on the incisors and nasal bones were not presented in the CLH report.

In the 100 mg/kg bw/d group, the absolute liver weights were increased by 6% (M) and 13%

(F) and the relative liver weights (statistically significant) were increased by 13% (M) and 24% (F) compared to controls. The changes in liver weights were correlated with histopathological findings (see table below) including the adverse single cell necrosis in 12 of 15 animals of both sexes.

***Table**: Histopathological findings of liver in the one-generation oral study in mice (Table 6 from Mukerji et al., 2015)*

Dose (mg/kg/day): no. examined	Male					Female				
	0(15)	1(15)	5(15)	25(15)	100(15)	0(15)	1(15)	5(15)	25(15)	100(15)
Hypertrophy, hepatocellular	0	0	9	10	15	0	0	13	12	13
Mitotic figures, increased	0	0	0	0	5	0	1	0	1	10
Oval cell hyperplasia	0	0	0	0	15	0	0	0	2	12
Cystic degeneration	0	0	0	0	0	0	0	1	1	7
Single cell necrosis	0	0	0	0	12	2	1	1	1	12
Infiltrate, mononuclear (oval cell associated)	0	0	0	0	15	0	0	0	0	10
Pigment, increased	0	0	0	0	15	0	0	0	0	0

Support for STOT RE classification: The equivalent guidance value for 67 days oral exposure (to females) is ≤ 134 mg/kg bw/d for Cat. 2. RAC considers the dental effects, and bone effects (incomplete decalcification of nasal bones) observed in this study at 100 mg/kg bw/d to support Cat. 2. Although histopathological findings were observed in the liver, no information on severity of these effects is available. Thus, RAC proposes no classification for liver effects.

In the oral combined repeated-dose and reproductive toxicity screening study (WIL Research Laboratories, 2005; unpublished), according to OECD TG 422 and in compliance with GLP, 6:2 FTOH was administered via gavage to 10 rats/sex/dose at 25, 75 and 225 mg/kg bw/d. Additional 5 rats/sex/dose were allocated to recovery groups (control and high dose). The males in the study were exposed to at least 32 days and the females to at least 39 days.

Treatment-related mortalities (1/15 M and 11/15 F) were observed in the 225 mg/kg bw/d group. Higher liver weight and hepatic centrilobular hypertrophy in males was observed in the 225 mg/kg bw/d group. No further data on the liver effects was reported in the CLH report.

Teeth were not examined in the study.

Support for STOT RE classification: The equivalent guidance value for oral exposure of around 30 days is > 30 and ≤ 300 mg/kg bw/d , and for 39 days is > 23 and ≤ 231 mg/kg bw/d for Cat. 2. RAC considers the liver effects reported in this study as not severe enough for classification.

In the oral prenatal developmental toxicity (DuPont, 2008; unpublished and O'Connor *et al.*, 2014), according to OECD TG 414 and in compliance with GLP, 6:2 FTOH was administered via gavage from GD 6 – 20 at 5, 25, 125 and 250 mg/kg bw/d. No treatment-related mortalities were observed. Teeth, bone, and liver parameters were not examined.

Overall, significant effects on teeth were consistently observed in the studies at levels supporting STOT RE 2 (except one 28-day oral study supporting STOT RE 1 but the severe dental effects were only observed at levels supporting STOT RE 2 in this study and in the other studies). The effects on bone (incomplete decalcification in nasal bone, tibia and femur suggesting significant morphological changes) in the only two studies that examined it also supports STOT RE 2. In line with the DS's view, RAC considers the evidence on chronic fluorosis-related mottled/broken teeth can be taken as an indicator of skeletal fluorosis characterised by metabolic bone disorder. However, RAC does not agree with the DS proposal to specify 'skeletal system' as the target organ as it considers that the more severe effects on teeth should be clearly communicated, which is not the case if the broader term 'skeletal

system' is used. Therefore, RAC proposes to specify 'teeth' and 'bones' as target organs. Liver toxicity manifested as, in particular the adverse single cell necrosis, was also observed in two sub-chronic exposure studies at levels supporting STOT RE 2. However, in the absence of quantitative details on these effects, RAC proposes no classification for liver effects.

RAC notes that also mortalities were observed at dose levels below (Mukerji *et al.*, 2015) or close to (Serex *et al.,* 2014, O'Connor *et al.*, 2014 and WIL, 2015) the guidance value for Cat. 2.

In conclusion, RAC proposes STOT RE 2; H373 (teeth, bones) for 6:2 FTOH.

10.11 Aspiration hazard

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 11: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
OECD TG 301 D	5 % biodegradation (test material analysis) after 28 days; average of three replicates	Reliability 4 (secondary literature); poor documentation in registration dossier (Registrant: reliability 2)	Registration dossier (Kurume Laboratory, 2010)
OECD TG 301 B	21 % CO_2 evolution after 28 days (replicate 1) 0 % CO_2 evolution after 28 days (replicate 2)	Reliability 3; not valid (difference of replicates > 20 %) (registrant: reliability 1)	Registration dossier (Dr. U. Noack-Laboratorium, 2000)
Soil (flow through system)	DT50= 1.3 days (primary degradation) Transformation products: 5:3 polyfluorinated acid, perfluorohexanoic acid, perfluoropentanoic acid, perfluorobutanoic acid 5:2 sFTOH	Reliability 2	(Liu et al., 2010a).
Soil (closed system)	DT50= 1.6 days (primary degradation) Transformation products: 5:3 polyfluorinated acid, perfluorohexanoic acid, perfluoropentanoic acid, perfluorobutanoic acid, 4:3 polyfluorinated acid, 5:2 sFTOH	Reliability 2 (registrant: reliability 2)	(Liu et al., 2010b)
River sediment system	DT50= 1.8 days (primary degradation)	Reliability 2 (registrant: reliability 2)	(Zhao et al., 2013)

Method	Results	Remarks	Reference
	Transformation products: 5:3 polyfluorinated acid, perfluorohexanoic acid, perfluoropentanoic acid, perfluorobutanoic acid, 4:3 polyfluorinated acid, 5:2 sFTOH		

11.1.1 Ready biodegradability

The ready biodegradability of 6:2 FTOH was evaluated in a closed bottle test (OECD TG 301 D). The initial concentration of 6:2 FTOH used in this study was 100 mg/L (test material). Activated sludge was used as inoculum. After 28 days, an average biodegradation of 5 % (average of three replicates) was determined. Further details on inoculum as well as test conditions were not given in the registration dossier. Upon request, the study report could not be made available to the dossier submitter.

Furthermore, a test according to OECD TG 301 B (CO_2 Evolution Test) was performed. 40 mg/L of the test material was used as initial concentration. Domestic activated sludge was used as inoculum. Further details on the study are not published. After 28 days, 21 % CO_2 evolution and 0 % CO2 evolution were observed in two replicates. The difference of the biodegradation values of the two replicates is > 20 %, therefore, the study should be considered as not valid. In addition, the test method according to OECD TG 301 B is not suitable for volatile test substances.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

No data available.

11.1.4 Other convincing scientific evidence

No data available.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

Not relevant for C & L.

11.1.4.2 Inherent and enhanced ready biodegradability tests

The biodegradability of 6:2 FTOH (2.8 µg/ml and 20 µg/ml) was investigated with a mixed aerobic bacterial culture developed from activated sludge from an industrial wastewater treatment plant (Registration dossier, (Liu et al., 2010b)). The sludge was previously exposed to fluorinated chemicals. The concentration of 6:2 FTOH decreased to 1.6-2.8 % after 7 days (primary biodegradation). Metabolite concentrations reached steady state after 14-28 days (metabolites: 6:2 fluorotelomer unsaturated acid, 5:2 secondary alcohol (5:2 sFTOH), 6:2 fluorotelomer saturated acid; 5:3 polyfluorinated acid, perfluorohexanoic acid, perfluorobutanoic acid and perfluoropentanoic acid). Adsorption of the transient metabolites to rubber septa cannot be excluded. Due to the adaption of the inoculum the study should not be used for classification and labelling purposes.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

The aerobic biodegradation of 6:2 FTOH was performed in a flow through soil incubation system (Liu et al., 2010a). After 1.3 days, 50 % of ^{14}C labelled 6:2 FTOH disappeared from soil, because of microbial degradation and volatilisation. The overall mass balance during the 84-day incubation averaged 77 % and 87 % for the live and sterile treatments, respectively. 16 % [^{14}C] 5:2 sFTOH, 14 % [^{14}C] 6:2 FTOH and 6 % [^{14}C] CO_2 were measured in the airflow after 84 days. In soil, the following stable transformation products were detected after 84 days: 5:3 polyfluorinated acid (12 %), perfluorohexanoic acid (4.5 %), perfluoropentanoic acid (4.2 %), and perfluorobutanoic acid (0.8 %). In soil-bound residues, the major transformation product was 5:3 polyfluorinated acid, which may not be available for further biodegradation in soil. In a further study, the authors investigated the aerobic biodegradation of 6:2 FTOH (without ^{14}C-labelling) in soil (closed system) (Liu et al., 2010b). 6:2 FTOH primary degradation half-life was 1.6 days. After the rapid decline of 6:2 FTOH the concentration leveled-off after 28 days. The overall mass balance in aerobic soil was ~67 % after 180 days (e.g. due to irreversible bond to soil). After 180 days the following substances were accounted: 30 % perfluoropentanoic acid, 8.1 % perfluorohexanoic acid, 1.8 % perfluorobutanoic acid, 15 % 5:3 polyfluorinated acid, 1 % 4:3 polyfluorinated acid, 3 % 6:2 FTOH, and 7.1 % 5:2 sFTOH.

In an aerobic river sediment system similar biotransformation products as in soil were detected (Zhao et al., 2013). The recovery of 6:2 FTOH and quantifiable transformation products ranged 71-88 mol% of initially applied 6:2 FTOH. The lower mass balance compared to sterile control (86-98 mol%) could be explained by formation of bound residues. The 6:2 FTOH primary degradation half-life in sediment system was estimated to be 1.8 days. After the initially rapid decrease, the 6:2 FTOH concentration was relatively constant after day 28. After 100 days 22.4 mol% 5:3 polyfluorinated acid, 20.2 mol% 5:2 sFTOH, 10.4 mol% perfluoropentanoic acid, 8.4 mol% perfluorohexanoic acid, 1.5 mol% perfluorobutanoic acid and 2.7 mol% 4:3 polyfluorinated acid were detected.

11.1.4.4 Photochemical degradation

Ellis and co-workers studied the kinetics of the reactions of Cl atoms and OH radicals with a series of fluorotelomer alcohols with differing chain lengths (4:2; 6:2, 8:2 FTOH) in 700 Torr of N2 or air, diluent at 296 +/- 2K. Interestingly, the length of the perfluorinated carbon chain residue had no discernible impact on the reactivity of the molecules. The authors conclude an atmospheric life-time of the FTOHs of 20 days by reaction with OH radicals (Ellis et al., 2003).

The photooxidation of 6:2 FTOH was investigated at the surface of TiO_2, SiO_2, Fe_2O_3, Mauritanian sand, and Icelandic volcanic ash (Styler et al., 2013). At all surfaces the photooxidation resulted in the production of surface-sorbed perfluoroalkyl carboxylic acids (PFCAs) like perfluorohexanoic acid, perfluorobutanoic acid and perfluoropentanoic acid. These results provide evidence that the heterogeneous photooxidation of FTOHs at metal-rich atmospheric surface may provide a significant loss mechanism for FTOHs and also act as a source of aerosol-phase PFCAs close to source regions. The long-range transport of these aerosols is a possible source of PFCAs to remote areas.

11.1.5 Conclusion on rapid degradation

The available information on ready biodegradability of 6:2 FTOH does no demonstrate that the substance is readily biodegradable.

A rapid primary degradation was observed for 6:2 FTOH in aquatic sediment and soil (half-life < 16 days). Nevertheless, based on the ECHA Guidance on the Application of the CLP criteria (Annex II), these data can only be used if it can be demonstrated that the degradation products do not fulfill the criteria for classification as hazardous to the aquatic environment. A hazard to the aquatic environment cannot be excluded as not for all transformation products sufficient data are available.

Since there is no clear evidence of rapid degradation (only primary degradation), 6:2 FTOH should be considered as not rapidly degradable according to CLP-criteria.

11.2 Environmental fate and other relevant information

The adsorption coefficient was investigated according to OECD TG 106 (analysis by LC/MS/MS). Three soils with organic carbon content of 0.52 to 8.18 and six initial test concentrations between 0.3 and 3.0 mg/L were used. A log Koc of 2.43 was determined at 22.5 °C using Freundlich sorption model (Liu and Lee, 2007).

11.3 Bioaccumulation

Table 12: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
OECD TG 107	Log Pow = 4.54	Reliability 2 (registrant: reliability 2)	Registration dossier (Carmosini and Lee, 2008)
OECD TG 305	BCF ≤ 36 (1 µg/L) BCF = 46 (10 µg/L)	Reliability 2 (registrant: reliability 2)	Registration dossier (Kurume Laboratory, 2002)
OECD TG 305	BCF = 24 - 99 (1 µg/L) BCF = 8.4 - 58 (10 µg/L)	Reliability 2 (registrant: reliability 2)	Registration dossier (Kurume Laboratory, 2007)

11.3.1 Estimated bioaccumulation

No data available.

11.3.2 Measured partition coefficient and bioaccumulation test data

A log Kow of 4.54 was determined according to OECD Guideline 107 (temperature and pH not provided in registration dossier). A concentration of 1740 mg/L was made with the test substance and purified octanol. The test substance/octanol solution was equilibrated with varying volumes of high purity water in 9 mL glass centrifuge tubes for 24 hours and one week. After equilibration, three to five aliquots were taken from both the aqueous and octanol phases of each tube, diluted with methanol and analysed by LC/MS/MS (Registration dossier Carmonsini and Lee, 2008).

A fish bioconcentration test according to OECD TG 305 was conducted for 28 days on *Cyprinus carpio* with two exposure levels (1 and 10 µg/L nominal; 0.835 and 9.11 µg/L measured) of the test substance (registration dossier Kurume, 2002). The test temperature ranged from 24.6 to 25.9°C and the pH from 7.6 to 7.9. The lipid content was 2.9 5 % at the start of the exposure and 2.26 % at the end of the exposure. After 28 days whole body w.w. BCF values of ≤ 36 (exposure level 1 µg/L) and 46 (exposure level 10 µg/L) were determined (steady state).

A further bioconcentration test according to OECD TG 305 (deviation: no post-exposure (depuration) phase) was conducted for 28 days on Cyprinus carpio with two exposure levels (1 and 10 µg/L nominal) of the test substance (registration dossier Kurume, 2007). After 28 days whole body w.w. BCF values of 24 - 99 (exposure level 1 µg/L) and 8.4 - 58 (exposure level 10 µg/L) were determined.

The determined BCF-values are below the CLP trigger value of 500. However, the derived Log Kow value meets the CLP trigger value for indication of bioaccumulation (Log Kow ≥4). Following the CLP regulation (section 4.1.2.8.1), the available, reliable experimental BCF determined in fish is taken in preference to the Log Kow. Therefore, 6:2 FTOH has a low potential for bioaccumulation in the aquatic environment.

11.4 Acute aquatic hazard

Table 13: Summary of relevant information on acute aquatic toxicity

Method	Species	Results[1]	Remarks	Reference
OECD 203	*Pimephales promelas*	**96h-LC_{50}= 4.84 mg/L (mean measured)**	Reliability 1 (registrant: reliability 1)	Anonymous 1, 2007

OECD 203	*Oncorhynchus mykiss*	96h-LC_{50}= 9 mg/L (nominal)	Reliability 4 (registrant: reliability 1), not enough details	Anonymous 2, 2005
OECD 203	*Oryzias latipes*	96h-LC_{50}= 5.78 mg/L (mean measured)	Reliability 4 (registrant: reliability 2), not enough details	Anonymous 3, 2007
OECD 202	*Daphnia magna*	**48h-EC_{50}= 7.84 mg/L (mean measured)**	Reliability 1 (registrant: reliability 1)	(ECHA Registration dossier: DuPont Haskell Global Centers for Health & Environmental Sciences, 2007)
OECD 202	*Daphnia magna*	48h-EC_{50}= 8.3 mg/L (nominal)	Reliability 4 (registrant: reliability 1), not enough details	(ECHA Registration dossier: Safepharm Laboratories Ltd., UK, 2005)
OECD 202	*Daphnia magna*	48h-EC_{50}= 8.2 mg/L (mean measured)	Reliability 4 (registrant: reliability 2), not enough details	(ECHA Registration dossier: Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan, 2007)
OECD 201	*Pseudokirchneriella subcapitata*	**72h-E_rC_{50}= 14.8 mg/L (measured)**	Reliability 1 (registrant: reliability 1)	(ECHA Registration dossier: DuPont Haskell Global Centers for Health & Environmental Sciences, 2007)
OECD 201	*Desmodesmus subspicatus*	72h-E_rC_{50}= 7.8 mg/L (measured)	Reliability 4 (registrant: reliability 1), not enough details	(ECHA Registration dossier: Safepharm Laboratories Ltd., UK, 2005)
OECD 201	*Pseudokirchneriella subcapitata*	72h-E_rC_{50}> 5.19 mg/L (measured)	Reliability 4 (registrant: reliability 2), not enough details	(ECHA Registration dossier: Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan, 2007)

[1] Test material: CAS 647-42-7; EC 211-477-1

11.4.1 Acute (short-term) toxicity to fish

Three studies for short-term toxicity to fish are available.

An 96-hour acute toxicity test with fathead minnow, *Pimephales promelas*, according to OECD TG 203 was conducted in a static test-type. The test concentrations were analytically monitored by LC/MS analysis (nominal test concentrations: 0, 1.0, 2.0, 4.0, 8.0, and 16.0 mg/L; mean measured: 0, 0.751, 1.61, 3.12, 7.52 and 16.4 mg/L). For the test 5 organisms per replicate and 2 replicates were used. The photoperiod was 16 hours light per day (light intensity: 126-710 lux). The test temperature ranged from 21.4 to 21.6 °C, the pH from 7.0 to 7.3 and the dissolved oxygen concentration from 5.4 to 8.5 mg/L. No control mortality or behavioural abnormalities occurred. The fish in the control had a standard length of 2.2 to 2.8 cm and a wet weight, blotted dry, of 0.140 to 0.304 g. The validity criteria were fulfilled. The test resulted in an 96h-LC_{50} of 4.84 mg/L (mean measured concentrations) and an 96h-LC_{100} of 7.52 mg/L (mean measured). At 3.12 mg/L (mean measured) and below no mortality occurred.

The second 96-hour acute toxicity test was conducted with *Oncorhynchus mykiss* according to OECD TG 203 in a semi-static test-type. The test concentrations were analytically monitored. The test concentrations

ranged from 1.3 to 13 mg/L (nominal). The validity criteria were fulfilled according to the registrant. The resulting 96h-LC_{50} was 9 mg/L (nominal). The reliability was difficult to access because of too little details provided in the registration dossier.

The third 96-hour acute toxicity study was conducted with ricefish, *Oryzias latipes*, according to OECD TG 203 in a semi-static test-type. The test concentrations were analytically monitored and ranged from 2 to 10 mg/L (measured). The validity criteria were fulfilled according to the registrant. The test resulted in an 96h-LC_{50} of 5.78 mg/L (mean measured concentrations) and an 96h-NOEC of 3.06 mg/L (mean measured).

The second and the third study were evaluated with Reliability score of 4 because in the registration dossier not enough details were provided to score them better.

11.4.2 Acute (short-term) toxicity to aquatic invertebrates

Three studies for short-term toxicity to aquatic invertebrates are available.

The first acute toxicity test with *Daphnia manga* was conducted according to OECD TG 202 under GLP with analytical monitoring (LC/MS/MS) in a static test-type. The test temperature ranged from 20.1 to 20.4 °C and the pH from 7.2 to 7.6. Nominal test concentrations were 0, 0.625, 1.25, 2.50, 5.00, and 10.0 mg/L and the mean measured concentrations were 0, 0.600, 1.23, 2.39, 4.90, and 9.29 mg/L. For the test system, 10 organisms were used per test vessel and two replicates per concentration. The photoperiod was 16 hours light per day (495-534 lux). The resulting 48h-EC_{50} (endpoint: immobility) was 7.84 mg/L and the 48h-NOEC was 2.39 mg/L based on mean measured concentrations. Lethargy was observed in surviving daphnids in the 2.39, 4.90, and 9.29 mg/L mean measured concentrations at the end of the study. The validity criteria were fulfilled.

The second acute toxicity test with *Daphnia manga* was conducted according to OECD TG 202 under GLP with analytical monitoring under static test conditions. The test concentrations ranged from 0.14 to 14 mg/L (nominal). The resulting 48h-EC_{50} (endpoint: immobility) was 8.3 mg/L and the 48h-NOEC was 2.5 mg/L based on nominal concentrations. According to the registrant, the validity criteria were fulfilled.

The third acute toxicity test with *Daphnia manga* was conducted according to OECD TG 202 with analytical monitoring in a static test-type. The test concentrations ranged from 1.30 to 15.5 mg/L (nominal). The resulting 48h-EC_{50} (endpoint: immobility) was 8.2 mg/L and the 48h-NOEC was 1.33 mg/L based on mean measured concentrations. According to the registrant, the validity criteria were fulfilled.

The second and the third study were evaluated with Reliability score of 4 because in the registration dossier not enough details were provided to score them better.

11.4.3 Acute (short-term) toxicity to algae or other aquatic plants

Three studies concerning the toxicity to aquatic algae and cyanobacteria are available.

The first toxicity study with *Pseudokirchneriella subcapitata* was conducted according to OECD TG 201 under GLP with analytical monitoring (LC/MS) in a static test-type. The test temperature ranged from 23.8 to 24.0 °C and the pH value from 7.97 to 9.97. Nominal test concentrations were 0.200, 0.640, 2.00, 6.60, and 21.0 mg/L and the mean measured concentrations were 0.154, 0.623, 2.22, 7.10, and 23.5 mg/L. Four replicates were used per concentration (3 for the test and 1 for analytical sampling). The photoperiod was 24 hours light per day (6670 to 6980 lux). The resulting 72h-E_rC_{50} was 14.8 mg/L (measured). The NOE_rC was 2.22 mg/L (measured). All validity criteria (cell counts increased in the blank control by at least a factor of 16 in 72 hours and the coefficient of variation of average specific growth rates during the whole test period (0-72 hr) in the blank control replicates did not exceed 7%) were fulfilled.

The second toxicity study with *Desmodesmus subspicatus* was conducted according to OECD TG 201 under GLP with analytical monitoring in a static test-type. Mean measured concentrations ranged from 1.13 to 13 mg/L (1.3, 2.3, 3.1, 6.7 and 13). The resulting 72h-E_rC_{50} was 7.8 mg/L (measured). The NOE_rC was 1.3 mg/L (measured). All validity criteria were fulfilled according to the registrant.

The third toxicity study with *Pseudokirchneriella subcapitata* was conducted according to OECD TG 201 with analytical monitoring in a static test-type. Mean measured concentrations ranged from 0.0966 to 9.45 mg/L. The resulting 72h-E_rC_{50} was greater than 5.19 mg/L (mean measured). The NOE_rC was 1.47 mg/L (measured). All validity criteria were fulfilled according to the registrant.

11.4.4 Acute (short-term) toxicity to other aquatic organisms

No data available.

11.5 Long-term aquatic hazard

Table 14: Summary of relevant information on chronic aquatic toxicity

Method	Species	Results[1]	Remarks	Reference
OECD 305	*Cyprinus carpio*	no abnormality in behaviour or appearance was noted	Test not suitable to evaluate long-term fish toxicity	Anonymous 5, 2007
OECD 211 EPA 797.1330	*Daphnia magna*	**21d-NOEC= 2.16 mg/L (mean measured**)	Reliability 1	(ECHA Registration dossier: DuPont Haskell Global Centers for Health & Environmental Sciences, 2007)
OECD 201	*Pseudokirchneriella subcapitata*	**72h-NOE_rC= 2.22 mg/L (measured)**	Reliability 1	(ECHA Registration dossier: DuPont Haskell Global Centers for Health & Environmental Sciences, 2007)
OECD 201	*Desmodesmus subspicatus*	72h-NOE_rC= 1.3 mg/L (measured)	Reliability 4 (registrant 1), not enough details	(ECHA Registration dossier: Safepharm Laboratories Ltd., UK, 2005)
OECD 201	*Pseudokirchneriella subcapitata*	72h-NOE_rC= 1.47 mg/L (measured)	Reliability 4 (registrant 2), not enough details	(ECHA Registration dossier: Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan, 2007)

[1] Test material: CAS 647-42-7; EC 211-477-1

11.5.1 Chronic toxicity to fish

There is one long-term toxicity test to fish documented in the registration dossier. As the test is a bioaccumulation study not covering the sensitive life stages of the organism, this test is not suitable to assess the chronic toxicity to fish.

11.5.2 Chronic toxicity to aquatic invertebrates

One long-term toxicity test to the aquatic invertebrate Daphnia magna is available conducted according to OECD TG 211 under GLP with analytical monitoring (LC/MS/MS) in a semi-static test-type. The test temperature ranged from 20.6 to 21.6 °C, the pH value from 7.6 to 8.1 and the dissolved oxygen concentration from 5.4 to 9.0 µg/L. The nominal test concentrations amounted to 0, 0.65, 1.3, 2.5, 5, and 10 mg/L and the mean measured concentrations amounted to 0, 0.557, 1.11, 2.16, 4.46, and 8.57 mg/L. Two organisms per test vessel were used and the control was composed of 5 replicates. The number of replicates for the test concentrations was not described in the RSS but it would be likely that this replicate number was also used for the test concentrations. The photoperiod was 16 hours light per day (17-40 lux). The test resulted in a NOEC of 2.16 mg/L (based on mean measured concentrations) (basis for the effect: adult survival, total live young per female, total immobile young per surviving female and length and weight of surviving females at day 21). As 90 % of the adults in the control test solution survived at the end of the test and the sum of live young produced per surviving female adult in 21 days was 112 (and therefore ≥ 60) all validity criteria were fulfilled.

11.5.3 Chronic toxicity to algae or other aquatic plants

Three studies concerning the toxicity to aquatic algae and cyanobacteria are available. For study details please see section 11.4.3.

The first toxicity study with *Pseudokirchneriella subcapitata* resulted in a NOE_rC of 2.22 mg/L (measured).

The second toxicity study with *Desmodesmus subspicatus* resulted in a NOE_rC of 1.3 mg/L (measured).

The third toxicity study with *Pseudokirchneriella subcapitata* resulted in a NOE_rC of 1.47 mg/L (measured).

11.5.4 Chronic toxicity to other aquatic organisms

No data available.

11.6 Comparison with the CLP criteria

11.6.1 Acute aquatic hazard

The lowest valid EC50/LC50 for classification is 4.84 mg/L for the fish Pimephales promelas. Therefore, no Aquatic Acute classification is necessary.

Table 15: Comparison with criteria for acute aquatic hazards

	Criteria for acute environmental hazards	**6:2 FTOH**	**Conclusion**
Acute Aquatic Toxicity	Cat. 1: $LC_{50}/EC_{50}/ErC_{50} \leq 1$ mg/L	Fish: 96h-LC_{50}= 4.84 mg/L (m) (*Pimephales promelas*) Invertebrates: 48h-LC_{50}= 7.84 mg/L (m) (*Daphnia magna*) Algae: 72h-ErC_{50}= 14.8 mg/L (m) (*Pseudokirch. subcapitata*)	**No classification necessary**

11.6.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

For aquatic invertebrates and algae long-term toxicity studies are available. The most sensitive NOEC was obtained from the test with Daphnia magna, being 2.16 mg/L (based on measured concentration). The test with algae resulted in a NOEC in the same order of magnitude.

Table 16: Comparison with criteria for long-term aquatic hazards

	Criteria for environmental hazards	**6:2 FTOH**	**Conclusion**
Rapid Degradation	Half-life hydrolysis < 16 days Readily biodegradable in a 28-day test for ready biodegradability (> 70 % DOC removal or > 60 % theoretical oxygen demand, theoretical carbon dioxide) Primary degradation: half-life < 16 days (if degradation products do not fulfil criteria for classification as hazardous to the aquatic environment)	no data available not readily biodegradable half-life < 16 days (soil, aquatic sediment), but hazard to the aquatic environment cannot be excluded for all transformation products	**Not rapidly degradable**
Bioaccumulation	BCF ≥ 500	BCF ≤ 36 - 99	**Not bioaccumulative (low potential for**

			bioconcentration in the aquatic environment)
Aquatic Toxicity	Not rapidly degradable substances: Cat. 1: NOEC ≤ 0.1 mg/L Cat. 2: NOEC ≤ 1 mg/L (based on Table 4.1.0 (b) (i) of the CLP Regulation)	Fish: No appropriate long-term toxicity tests are available. Invertebrates: 21d NOEC= 2.16 mg/L (m) (*Daphnia magna*) Algae: 72h-NOE_rC= 2.22 mg/L (n.a.) (*Pseudokirchneriella subcaptiata*)	**Aquatic Chronic 2** *(based on fish-LC_{50})*
	<u>Surrogate approach in absence of appropriate chronic toxicity reference data</u> (based on Table 4.1.0 (b) (iii) of the CLP Regulation)<u>:</u> Not rapidly degradable substances and/or bioaccumulative substances: Cat. 1: E/LC_{50} ≤ 1 mg/L Cat. 2: E/LC_{50} > 1 to ≤ 10 mg/L Cat. 3: E/LC_{50} > 10 to ≤ 100 mg/L	Fish: 96h-LC_{50}= 4.84 mg/L (m) (*Pimephales promelas*)	

11.7 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

6:2 FTOH is not rapidly degradable and has a low potential for bioconcentration in the aquatic environment. Chronic toxicity data on aquatic invertebrates and algae do not require an aquatic chronic classification according to Table 4.1.0 (b) (i). As chronic data is available for aquatic invertebrates and algae, the surrogate approach based on Table 4.1.0 (b) (iii) is not applicable. For chronic fish toxicity assessment not appropriate toxicity reference data is available and therefore the surrogate approach is used. The most protective valid acute LC_{50} is 4.84 mg/L (measured concentration) for *Pimephales promelas*. According to Figure 4.1.1 of the CLP Regulation the aquatic chronic classification is based on the most stringent outcome of the two assessments according to Table 4.1.0 (b) (i) and (iii). This results in a classification of 6:2 FTOH as Aquatic Chronic 2, H411 (based on Table 4.1.0 (b) (iii) of the CLP Regulation.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

There is no current entry in Annex VI of the CLP Regulation for the substance 6:2 FTOH.

The DS proposal was to classify the substance as Aquatic Chronic 2, H411, the substance being not rapidly degradable, based on the surrogate approach and the lowest EC_{50} obtained with *Pimephales promelas* (4.84 mg/L, mean measured (mm)) due to no chronic aquatic toxicity data available for the most acutely sensitive species (fish).

The physico-chemical characteristics show that 6:2 FTOH has moderate water solubility (18.8 mg/L at 22.5 °C) and vapour pressure of 18 Pa at 25 °C, indicating moderate to high volatility.

Degradation

There are several aquatic degradation / transformation products of 6:2 FTOH. In an aerobic river sediment system 5:3 acid (F(CF2)5CH2CH2COOH, 22.4 mol%), perfluoropentanoic acid (PFPeA, 10.4 mol%), perfluorohexanoic acid (PFHxA, 8.4 mol%), perfluorobutanoic acid (PFBA, 1.5 mol%), 6:2 fluorotelomer saturated acid (6:2 FTCA, <1 mol%), 6:2 fluorotelomer unsaturated acid (6:2 FTUCA, <1 mol%), 4:3 acid (F(CF2)4CH2CH2COOH, 2.7 mol%), 5:2 ketone [F(CF2)5C(O)CH3, 1.5 mol%), and 5:2 sFTOH (F(CF2)5CH(OH)CH3, 20.2 mol%) were detected after 100 days (Anonymous, 2013). In a mixed aerobic bacterial culture developed from activated sludge, 6:2 FTOH degraded at day 28 into 6:2 FTUCA (25%) and 5:2 sFTOH (17%) as the two dominant metabolites with 6:2 FTCA (5.7%), 5-3 acid (5.5%), PFHxA (5.1%), and PFBA and PFPeA each less than 0.5% yield. (Anonymous, 2013)

A summary of the relevant information on rapid degradability is provided in Table 11 of the CLH report.

Abiotic degradation

No data is provided on the hydrolysis of the substance.

Biodegradation

Rapid biodegradation

No reliable/ valid studies are presented on ready biodegradability for 6:2 FTOH. Available data of questionable reliability according to OECD TG 301D indicated low degradation (5% after 28 days, Anonymous, 2010; Anonymous, 2000).

The recovery of 6:2 FTOH and quantifiable transformation products of 71-88 mol% of initially applied 6:2 FTOH in an aerobic river sediment system. The primary DT_{50} in sediment system was estimated to be 1.8 days. After the initial rapid decrease, the 6:2 FTOH concentration was relatively constant after day 28 (Anonymous, 2013).

The concentration of 6:2 FTOH decreased to 1.6-2.8% after 7 days (primary biodegradation) in a study with mixed aerobic bacterial culture developed from activated sludge (Anonymous, 2010). However, due to the adaption of the inoculum the study is not considered valid for classification purposes.

The DS concluded that since there is no clear evidence of rapid degradation (only primary degradation) and a lack of available data on the fate and hazardous properties of the degradation products, 6:2 FTOH does not fulfil the criteria to be considered as rapidly degradable in the aquatic environment, according to the CLP criteria.

Bioaccumulation

A summary of the available information on bioaccumulation is provided in Table 12 of the CLH report.

A Log K_{OW} of 4.54 has been determined according to OECD TG 107 (Anonymous, 2008).

Two OECD TG 305 fish bioconcentration test results have been provided showing whole body wet weight based BCF values of ≤ 36 (exposure level 1 µg/L) and 46 (exposure level 10 µg/L)

at steady state and 24 - 99 (exposure level 1 µg/L) and 8.4 - 58 (exposure level 10 µg/L) with *Cyprinus carpio* after 28 days of exposure respectively (Anonymous, 2002; Anonymous, 2007).

The DS concludes that 6:2 FTOH is considered to have low potential for bioaccumulation in the aquatic environment due to the higher preference given in the CLP Regulation to experimental data (BCF values below the threshold value of 500) over Log K_{OW} (meeting the CLP trigger value of Log $K_{OW} \geq 4$).

Aquatic toxicity

Aquatic acute toxicity

A summary of the relevant information on aquatic acute toxicity is presented in Table 13 of the CLH report.

Aquatic acute toxicity studies are presented for all three trophic levels: fish, invertebrates, algae and other aquatic plants.

One valid OECD TG 203 acute fish toxicity study is available with *Pimephales promelas* in a static test design presenting an LC_{50} of 4.84 mg/L (mm) after 96 h of exposure (Anonymous 1, 2007).

For invertebrates, one reliable acute study has been given. The *Daphnia magna* OECD TG 202 immobilisation test provided an EC_{50} of 7.84 mg/L (mm) after 48 h exposure (Anonymous, 2007).

One reliable OECD TG 201 study is available with *Pseudokirchneriella subcapitata* showing a 72 h E_rC_{50} value of 14.8 mg/L (mm) (Anonymous, 2007).

The studies provided with a questionable reliability due to lack of detailed information on the test design were all in similar range for all trophic levels. According to the provided valid studies, fish are found to be the most sensitive species. The lowest EC_{50} is obtained with *Pimephales promelas* (4.84 mg/L (mm)) not meeting the CLP classification criteria for aquatic acute hazards so the **DS proposed not to classify 6:2 FTOH for acute hazards based on the L(E)C_{50} ≥ 1 mg/L in CLP Table 4.1.0 (a).**

Aquatic chronic toxicity

Valid data for aquatic chronic toxicity are only presented for two trophic levels: invertebrates and algae. No long-term fish test was available at the time of the CLH dossier submission.

The OECD TG 211 reproduction test (semi-static, 21 d) with *Daphnia magna* resulted in a NOEC of 2.16 mg/L (mm) based on adult survival, total live young per female, total immobile young per surviving female and length and weight of surviving females.

The OECD TG 201 study with *Pseudokirchneriella subcapitata* resulted in a NOE_rC of 2.22 mg/L (mm). The other two studies presented for algae and aquatic plants were not considered to meet the validity criteria and were in the same order of magnitude. (Anonymous, 2007)

Since no chronic aquatic toxicity data was available for fish but fish species are acutely the most sensitive endpoint, the DS considered based on a surrogate approach and for a not rapidly degradable substance that 6:2 FTOH **fulfils the criteria for classification as Aquatic Chronic Category 2, H411 based on the L(E)C_{50}> 1 and ≤ 10 mg/L in CLP Table 4.1.0 (b)(iii).**

Comments received during consultation

One company(importer) supported the classification proposal and submitted information on an ongoing OECD TG 234 study still being, at that time, conducted.

Three MSs supported the proposed classification and the classification approach based on the NOECs, the substance being not rapidly degradable and the use of the surrogate approach for the trophic level where no adequate NOEC is available (fish species).

The actual validity of the studies provided was questioned by one MS as no full study reports seemed to be available for assessment. The MS also reported a non-valid invertebrate study indicating a NOEC lower than the NOEC values from the valid invertebrate and algae studies but considered this to be of low relevance as the values would not change the classification proposal. The DS noted that detailed test results were requested from industry but were not provided. The proposed chronic classification would be the same as the proposed one, as all the study results provided are in the same range. The DS additionally noted that the results from the studies with a reliability of 3 and 4 were not used in the derivation of the classification proposal.

One MS suggested to use QSAR modelling to support biodegradation and bioconcentration data. The DS considered models of only limited relevance for per- and polyfluorinated substances. The same MS also noted the test concentrations might be lower than the recommended in OECD TG 305 and it was unclear if the standard BCF calculation had been adapted for growth of fish and normalised on a 5% of lipid content. The DS informed the MS on lipid normalised BCF values and noted that no information on growth was available, as well as on the lipid content from another study.

One MS requested additional information on the transformation products to support the conclusion on the substance being not rapidly degradable and noted that the substance's hazard profile could be also driven by potentially more hazardous transformation products. The MS also asked for the outcome of an additional, ongoing OECD TG 234 study to be taken into account. The DS provided the additional information on the transformation products supporting the presented outcome of the classification proposal and showing that the transformation products are also considered as not rapidly degradable and do not pose a greater hazard to aquatic environment based on the available data.

The DS did not have the full study report of the additional OECD TG 234 study available at the time of the submission and left the results to be interpreted by RAC.

Comments received during the ad hoc consultation

Industry eventually provided the report of the OECD TG 234 study with fish and an ad hoc consultation was held. For confidentiality reasons only an extended robust study summary from the REACH Registration dossier was placed under the consultation. The full study report was made available for RAC for evaluation.

Three MSs supported the use of OECD TG 234 study results indicating a classification of Aquatic Chronic 1, H410 and an M-factor of 1.

One MS noted the uncertainties in the validity criteria of this study that may not allow reaching the highest reliability score and that the lack of another chronic study on vertebrates (which

may be the most sensitive species as seen in acute studies) may have led to a lower NOEC and, thus, a higher M-factor. However, the MS considered the reliability of the study being acceptable and sufficient for classification purposes.

One MS also noted the lack of details in the study summary provided and questioned meeting the OECD TG 234 validity criteria due to, for example, variation in the measured concentrations. The same MS mentioned that the NOEC for hatching success based on geometric mean measured concentration (0.0231 mg/L) should be used for the classification, due to problems maintaining and achieving the nominal concentrations in the test system.

Two MSs noted the overall study lowest NOEC value of 0.0137 mg/L (mm). One of them suggested that the lowest chronic fish toxicity endpoint relevant for the purpose of hazard classification is the 122-d NOEC of 0.0231 mg/L (mm) for hatching success. It was also mentioned the EC_x values would be useful to determine the chronic classification outcome. The MS also suggested to take into account the review of the Evaluating MS for the parallel REACH Substance Evaluation process, once available.

One MS commented on the self-classification of the substance 6:2 FTMA which seems to be based on the lowest NOEC value of 0.0162 mg/L from the new OECD TG 234 study with 6:2 FTOH and was unsure of the calculation and the validity of this value.

Animporter took note of the OECD TG 234 study results and proposed to classify 6:2 FTOH as Aquatic Chronic 1, H410 with an M-factor of 1, based on a lowest NOEC value of 0.0137 mg/L.

Additional key elements

Analyses

The Fish Sexual Development Test was conducted in accordance with the OECD TG 234 and under GLP. The test protocol was followed, control and solvent control groups were also included. *Oryzias latipes* were exposed to a range of concentrations of the test substance dissolved in water under semi-static test conditions with daily renewal of the test media. The test was conducted at nominal test concentrations of 0.030, 0.096, 0.30, 0.96 and 3.0 mg/L. Nominal concentrations were generally not achieved and were not maintained within ±20% of the mean measured values over the 24-hour exposure period due to the volatile nature of the test substance and the requirement to aerate the vessels to maintain the health of the fish. The geometric mean measured concentrations were calculated to be 0.00933, 0.0137, 0.0231, 0.0537 and 0.0953 mg/L. Given the variability in measured concentrations throughout the test, the results have been calculated based on both nominal and mean measured concentrations. A validated analytical method was available with a detection limit of 0.005 mg/L. An overall mean recovery of 95% and a coefficient of variation of 5.8% has been reported fitting well within the validity criteria.

The dissolved oxygen concentration should be at least 60% (air saturation value) for the duration of the test to meet the validity criteria. The oxygen concentration was observed to be below 60% ASV in several instances. Thereafter, the aeration was increased and the oxygen concentration returned to >60% ASV. However, this was considered not to affect the integrity of the test given that no adverse effects were observed at the time.

The water temperature did differ by more than 1.5ºC between test chambers during the test but was maintained at 25 ± 2ºC.

A statistically significant reduction in survival was observed for the solvent control when

compared to the control group. However, the effect was only a 9% reduction and therefore the biological relevance of this is questionable, based on the allowed control mortality in chronic aquatic toxicity testing. All statistical analyses of effects were limited to the solvent control group, instead of pooled controls, to take into account the mortality in the solvent control group.

No abnormal behaviour or appearance was observed during the test. At the highest nominal concentration, some of the fish were observed to be lethargic and at the bottom of the vessel. However, these effects did not persist.

A statistically significant effect was observed on hatching success at nominal concentrations of 0.96 mg/L and above. Hatching success in the control treatments was >80% (actual: control, 87%, solvent control, 94%). In addition, statistically significant effects were observed on survival and growth (male and female body length and weight) at the highest nominal test concentration of 3.0 mg/L. Post-hatch survival of fish larvae in the control treatments was ≥70% (actual: control, 92%, solvent control, 84%). The weight and length of the control fish was >150 mg and >20 mm, respectively, at the end of the test. Sex ratio (% males or females) in the control treatments (based on phenotypic evaluation) was 30 – 70%.

Whilst no statistically significant increase or decrease in vitellogenin (VTG) concentration in female fish was observed, statistically significant effects were observed for male secondary sex characteristics and male VTG concentration. A significant decrease in the number of anal fin papillae was observed at the 3.0 mg/L test concentration whilst a significant increase in VTG concentration was observed at the 0.30 mg/L test concentration and above.

***Table**: A summary of the NOEC and LOEC values for the various endpoints*

Response	Nominal concentration (mg/L)		Geometric Mean Measured Concentration (mg/L)	
	NOEC	LOEC	NOEC	LOEC
Hatching success	0.30	0.96	0.0231	0.0537
Final survival	0.96	3.0	0.0537	0.0953
Female growth (lengths and weights)	0.96	3.0	0.0537	0.0953
Male growth (lengths and weights)	0.96	3.0	0.0537	0.0953
Genetic sex ratio (% males)	≥ 3.0	> 3.0	≥ 0.0953	> 0.0953
Phenotypic sex ratio (% males)	≥ 3.0	> 3.0	≥ 0.0953	> 0.0953
VTG concentration (female)	≥ 3.0	> 3.0	≥ 0.0953	> 0.0953
VTG concentration (male)	0.096	0.30	0.0137	0.0231
Secondary sex characteristics	0.96	3.0	0.0537	0.0953

Assessment and comparison with the classification criteria

Comparison with the criteria

The CLH report did not include information on the hydrolysis of 6:2 FTOH and behaviour in the water-sediment system, as well as ready biodegradation.

According to the available data presented by the DS on primary degradation of the substance, the lack of sufficient information on the hazardous properties of the aquatic degradation / transformation products and considering the criteria on rapid degradability defined in Section

4.1.2.9.3 of the CLP Regulation indicating that *primary biodegradation does not normally suffice in the assessment of rapid degradability unless it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment*, RAC agrees to consider 6:2 FTOH as **not rapidly degradable** for classification purpose.

RAC notes that no data have been provided as part of the CLH dossier showing toxicity of the degradation products and limited information has been provided as part of the consultation round. RAC also notes that a known longer-term degradation product is PFHxA with an available restriction proposal and RAC opinion (https://www.echa.europa.eu/documents/10162/8fa51c6a-70e4-1a20-5170-d34e58771a5a). RAC considered PFHxA as a very persistent substance in the environmental compartments. According to the degradation studies provided in the CLH dossier, in the mixed aerobic bacterial culture the metabolites' concentrations reached steady-state after 14–28 days and at day 28 concentration of PFHxA was 5.1% (Anonymous, 2010b). The study in aerobic river sediment system does not specify the time of the steady state of the transformation products but after 100 days 8.4 mol% PFHxA was detected (Anonymous, 2013).

Regarding bioaccumulation, RAC notes the available experimental BCF and Log K_{OW} data and considers the information sufficient to come to conclusion on the bioaccumulation potential of the substance in the aquatic compartment. According to the Section 4.1.2.8 of the CLP Regulation, experimentally determined BCF values provide a better measure and shall be used in preference to partition coefficients, if available. Therefore, RAC agrees with the DS and concludes that 6:2 FTOH has **no potential for bioaccumulation** based on the available information on the BCF in fish (in the range of 24 - 99 (1 µg/L) and 8.4 - 58 (10 µg/L)) that are well below the cut-off value of 500.

RAC agrees with the DS that the most sensitive fish species LC_{50} value was that for *Pimephales promelas* (equal to 4.84 mg/L). Based on this study, but also all the other acute data that can be considered scientifically robust and reliable to be used for classification purposes, RAC concludes that **no classification for aquatic acute hazards** is warranted for 6:2 FTOH.

RAC took into account the results of the additional OECD TG 234 fish study provided with *Oryzias latipes* as the test species and considers the study results valid for classification purposes despite minor discrepancies from the validity criteria. The most sensitive parameter (VTG concentration in males) has not been chosen due to VTG being only a mechanistic parameter and a measure for determining endocrine effects not the adverse effects for fish e.g. growth, survival or hatching success etc. RAC refers to previous similar conclusions on Triadimenol (ISO); α-tert-butyl-β-(4-chlorophenoxy)-1H-1,2,4-triazole-1-ethanol (EC: 259-537-6, CAS: 55219-65-3).

RAC notes that the outcome of the proposed classification would not change even if a lower available NOEC value, for example one based on the male VTG concentration, would be used and notes that the details of the calculations based on the nominal and mean measured concentrations were not available as part of the full study report. Finally, RAC points out that preference was given to the use of EC_{10} values compared to NOECs when applying chronic classification procedure. However, no statistical analysis has been performed to determine the ECx.

Thus, the lowest chronic fish toxicity endpoint is the 122-d NOEC of 0.0231 mg/L (gm) for hatching success that leads to an Aquatic Chronic 1 classification with an M-factor of 1 for 6:2 FTOH.

Taking into account that the chronic fish study was not available for the DS at the time of the submission of the CLH report, RAC does not support the originally proposed classification of Aquatic Chronic 2, H411 based on the surrogate approach. As reliable chronic data are now available for all trophic levels, the classification is based on CLP Table 4.1.0 (b)(i). Therefore, RAC proposes to consider the lowest chronic endpoint as the 122-day NOEC for *Oryzias latipes* of 0.0231 mg/L (mm) based on hatching success, resulting in a classification of **Aquatic Chronic 1, H410** for 6:2 FTOH. According to Table 4.1.3 in CLP Regulation an **M-factor** of **1** for a **not rapidly degradable** substance is warranted between the range of 0.01 and 0.1 mg/L.

RAC notes that if additional data become available either on the biodegradation, bioaccumulation potential and the degradation products in the environment and acute or chronic toxicity of 6:2 FTOH and its metabolites, the classification could be reconsidered.

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Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification:

3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol

EC Number: **211-477-1**

CAS Number: **647-42-7**

Index Number: **n.a.**

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Version number: **2.0**

Date: **January 2021**

CONTENTS

1 ENVIRONMENTAL HAZARDS

1.1 Degradation

1.1.1 Ready biodegradability (screening studies)

[Study 1: (ECHA Registration dossier: Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan, 2010)]

Study reference:

Kurume Laboratory, Chemicals Evaluation and Research Institute (2010): Biodegradation study of 13F-EtOH by microorganisms. Report no. 15463 (report date: 2010-07-01)

Detailed study summary and results:

The ready biodegradability of 6:2 FTOH was evaluated in a closed bottle test (OECD TG 301 D). The initial concentration of 6:2 FTOH used in this study was 100 mg/L (test material). Activated sludge was used as inoculum. Further details on inoculum as well as test conditions were not given in the registration dossier. After 28 days an average biodegratation of 5% (average of three replicates) was determined.

Test type:

OECD Guideline 301 D, GLP not specified

Test substance:

• equivalent

Materials and methods:

• Details on inoculum: activated sludge (adaption not specified)

• Duration of test: 28 days

• Initial test substance concentration: 100 mg/L based on test mat.

Results:

• Degradation: 5 % (based on test material analysis) after 28 days (average of three replicates: 5%, 6% and 4%)

[Study 2: (ECHA Registration dossier: DR. U. NOACK-LABORATORIUM, 2000)]

Study reference:

DR. U. NOACK-LABORATORIUM (2000): Fluowet EA 600 Ready Biodegradability, Modified Sturm Test. Report no. AST75701 (report date 2000-11-15)

Detailed study summary and results:

A test according to OECD TG 301 B (CO2 Evolution Test) was performed. 40 mg/L of the test material was used as initital concentration. Domestic activated sludge was used as inoculum. Further details on the study

are not published. After 28 days 21% CO2 evolution and 0% CO2 evolution were observed in two replicates. The difference of the biodegratation values of the two replicates is > 20%, therefore, the study should be considered as not valid.

Test type:

OECD Guideline 301 B, GLP

Test substance:

• equivalent

Materials and methods:

• Details on inoculum: activated sludge, domestic (adaption not specified)

• Duration of test: 28 days

• Initial test substance concentration: 40 mg/L based on test mat.

Results:

• Degradation: 21 % (CO2 evolution) after 28 days; 0 % (CO2 evolution) after 28 days

1.1.2 Aquatic simulation tests

[Study 1: Zhao et al. 2013, aerobic river sediment system)]

Study reference:

Zhao L., Folsom P.W., Wolstenholme B.W., Sun H., Wang N., and Buck R.C. (2013): 6:2 fluorotelomer alcohol biotransformation in an aerobic river sediment system. Chemosphere 90 (2), 203-209. DOI: 10.1016/j.chemosphere.2012.06.035

Detailed study summary and results:

In an aerobic river sediment system similar biotransformation products as in soil and activated sludge were detected.. The recovery of 6:2 FTOH and quantifiable transformation products ranged 71-88 mol% of initially applied 6:2 FTOH. The lower mass balance compared to sterile control (86-98 mol%) could be explained by formation of bound residues. The 6:2 FTOH primary degradation half-life in sediment system was estimated to be 1.8 days. After 100 days 22.4 mol% 5:3 polyfluorinated acid, 10.4 mol% perfluoropentanoic acid, 8.4 mol% perfluorohexanoic acid, and 1.5 mol% perfluorobutanoic acid were detected.

Test type:

OECD Guideline 308, GLP not specified

Test substance:

• equivalent (>97% purity)

Materials and methods:

• Details on water/sediment sample: The river sediment was collected from Brandywine Creek, PA at

the position of Latitude 39° 51 min 34 s, longitude 75° 35 min 55 s,and 78 m above sea level. River water was collected from the same location. The collected sediment was sandy loam (67% sand, 24% silt, and 9% clay) with 5.3% organic matter or 3.1% organic carbon content. The pH of the sediment was 6.9 and the cation exchange capacity (CEC) was 86 mmol/kg sediment.

• Duration of test: 100 d

• The biotransformation was conducted in 119-mL glass serum bottles. Twenty grams wet sediment containing 9.3 g dry weight and 10.7 mL river water, 25 mL pure river water, and 5 mL mineral media were added sequentially into each test vessel. The mineral medium solution contained 85 mg/L of KH2PO4, 218 mg/L of K2HPO4, 334 mg/ L of Na2HPO4·2H2O, 5 mg/ L of NH4Cl, 36.4 mg/L of CaCl2·2H2O, 22.5 mg/ L of MgSO4·7H2O, and 0.25 mg/L of FeCl3·6H2O with a pH of 7.0. For the sterile control, autoclaved sediment and river water were used instead and triple antibiotics (kanamycin, chloramphenicol, and cycloheximide) were added to the sterile sediment to a final concentration of 200 mg/kg sediment. The sample bottles containing live and sterile sediment were crimp-sealed with butyl rubber stoppers and aluminum caps and incubated at room temperature for 5 d before dosing 6:2 FTOH to initiate the experiment. After the pre-incubation, each bottle was inverted and 10 µL of 6:2 FTOH stock solution (5000 mg/ L) made in 50% ethanol (v/v, ethanol:water = 1:1) was injected into the sediment with a 10-µL glass microsyringe. The initial 6:2 FTOH dosing concentration was determined based on the LC/MS/MS detection limit that would allow low levels (1–2% of initially applied 6:2 FTOH) of transformation products to be quantified and environmentally relevant. Some of the live sediment sample bottles after pre-incubation were only dosed with 10 µL of 50% ethanol into each bottle as live matrix control for monitoring headspace O2 content during the biotransformation and also serving as background blank for LC/MS/MS analysis. After dosing with 6:2 FTOH or 50% ethanol, the bottle was gently shaken to disperse the dosed solution throughout the sediment system.. Two C18 SPE cartridges) pre-activated with acetonitrile (CH3CN), were coupled with two 18-gauge needles, which were pushed into the headspace of each sample bottle. These two cartridges enabled air exchange between headspace and ambient air and also captured 6:2 FTOH and other volatile intermediates during biotransformation. After dosing with 6:2 FTOH or 50% ethanol and connecting with C18 cartridges, the sample bottles were kept static for 10–15 min to settle the sediment before the bottles were shaken continuously at about 35 rpm on an orbital shaker at room temperature. The 35 rpm low-velocity motion ensured aqueous phase aeration but did not disturb the settled sediment phase.

• Sampling and sample preparation: At each sampling time (days 0, 2, 7, 14, 28, 56, and 100), three live, three sterile control, and two live matrix control bottles were sacrificed for sampling and processing to analyze 6:2 FTOH and transformation products. The day 0 sample bottles were processed within 15 min after dosing with 6:2 FTOH stock solution or 50% ethanol. At each sampling time except day 0, the O2 content in the headspace of the live matrix control bottles (dosed with only 50% Ethanol) was measured using a headspace Oxygen Analyzer Model 905 to estimate the aerobic condition of the live sediment system. The C18 cartridges were disconnected from all the three live, three sterile control, and two live matrix

control bottles and each was eluted with 5 mL CH3CN for LC/MS/MS analysis. Each of the butyl rubber stoppers from the bottles was removed and transferred to a glass vial containing 5 mL CH3CN to extract 6:2 FTOH and other volatile products. The aqueous phase from each bottle was decanted to a glass bottle containing 60 mL CH3CN for extraction of 6:2 FTOH and transformation products. Forty milliliters of CH3CN was added to the remaining sediment of each bottle, which was immediately crimp-sealed with a fresh butyl rubber stop and aluminum cap for the extraction. All extractions were carried out at 50°C for 2–5 d on an orbital shaker kept at 200 rpm. The extract solution (first extract) from each of the sediment sample bottles was decanted to a glass container after centrifugation at ~1000 rpm (162g) with a Sorval GSA rotor for 15–20 min. The remaining sediment was extracted again with 40 mL CH3CN plus 40 µL of 5 MNaOH at 50 °C overnight and the extract solution (2nd extract) was decanted to a glass container after the centrifugation. All the processed sample solutions described above were filtrated through nylon filters (0.45 lmpore) and stored at ~10 °C before subject to LC/MS/MS analysis.

Results:

• primary degradation DT_{50} = 1.8d

• transformation products and transient intermediates after 100 d: 5:3 acid [F(CF2)5CH2CH2COOH] (22.4 mol%), perfluoropentanoic acid (10.4 mol%), perfluorohexanoic acid (8.4 mol%), perfluorobutanoic acid (1.5 mol%), 6:2 FTCA [F(CF2)6CH2COOH] (<1 mol%), 6:2 FTUCA [F(CF2)5CF=CHCOOH] (<1 mol%), 4:3 acid [F(CF2)4CH2CH2COOH] (2.7 mol%), 5:2 ketone [F(CF2)5C(O)CH3] (1.5 mol%), and 5:2 sFTOH [F(CF2)5CH(OH)CH3] (20.2 mol%)

• In sterile sediment control samples, only 6:2 FTOH was detected and ranged between 86 and 98 mol% of day 0 concentration over 100 d. The recovery of 6:2 FTOH and quantifiable transformation products in live samples ranged 71–88 mol% of initially applied 6:2 FTOH over 100 d. The bound residues formed between live sediment and 6:2 FTOH or 5:3 acid catalyzed by microbial enzymes may explain the slightly lower recovery in live sediment system versus sterile controls.

• partition of sedimentdosed 6:2 FTOH and formed transformation products: sterile controls: 51 mol% remained in the sediment phase, 3.4 mol% was partitioned to the aqueous phase, and 32 mol% was volatized to the headspace on day 100; live samples: 39 mol% remained in the sediment phase, 16 mol% was partitioned to the aqueous phase, and 15 mol% was volatized to the headspace on day 100.

1.1.3 Other degradability studies

[Study 1: Liu et al. 2010b; mixed aerobic bacterial culture]

Study reference:

Liu J., Wang N., Szostek B., Buck R.C., Panciroli P.K., Folsom P.W., Sulecki L.M., and Bellin C.A. (2010b): 6-2 Fluorotelomer alcohol aerobic biodegradation in soil and mixed bacterial culture. Chemosphere 78 (4), 437-444. DOI: 10.1016/j.chemosphere.2009.10.044

Detailed study summary and results:

The biodegradability of 6:2 FTOH (2.8 µg/ml and 20 µg/ml) was investigated with a mixed aerobic bacterial culture developed from activated sludge from an industrial wastewater treatment plant. The sludge was previously exposed to fluorinated chemicals. The concentration of 6:2 FTOH decreased to 1.6-2.8% after 7 days (primary biodegradation). Metabolite concentrations reached steady state after 14-28 days (metabolites: 6:2 fluorotelomer unsatured acid, 5:2 secondary alcohol (5:2 sFTOH), 6:2 fluorotelomer satured acid; 5:3 polyfluorinated acid, perfluorohexanoic acid, perfluorobutanoic acid and perfluoropentanoic acid). Adsorption of the transient metabolites to rubber septa cannot be excluded. Due to the adaption of the inoculum the study should no be used for classification and labelling purposes.

Test type:

OECD Guideline 302 A, GLP not specified

Test substance:

• equivalent (99% purity)

Materials and methods:

• Details on inoculum: mixed bacterial culture from activated sludge (industrial), activated sludge was adapted;

• Duration of test: 90 days

• Initial test substance concentration: 2.8 µg/ml based on test mat.

• The test vessels were 120-mL glass serum bottles sealed with natural rubber septa and the vessels were incubated on an orbital shaker at 150 rpm at 20–25 °C. The incubation was initiated by aseptically adding 30 mL of the mixed bacterial culture to each test vessel then dosing with 6 µL of 6:2 FTOH stock solution prepared in ethanol. Sterile treatments were prepared by autoclaving the culture and dosing with the triple antibiotics (kanamycin, chloramphenicol and cycloheximide) at 100 mg/L final concentration each to further inhibit microbial or enzymatic activities. Three treatments of duplicate vessels were prepared: (1) untreated (matrix) live culture with 6 µL ethanol; (2) 6:2 FTOH treated live culture; and (3) 6:2 FTOH treated sterile culture. At each sampling time point, a total of six test vessels were sacrificed for sample processing and extraction of 6:2 FTOH and potential metabolites. The O2 content was also measured at each time point in untreated live culture to approximate O2 content in treated live sample bottles.

• sampling and sample preparation: In mixed bacterial culture, test vessels were extracted and analysed at 0, 2, 7, 14, 28, 60, and 90 d. Prior to vessel opening, the pH was measured with a miniaturized pH probe pierced through the sample vessel rubber septa. Without breaking the seal, ~1.0 mL of the headspace gas was sampled with a gas-tight syringe and analyzed for the oxygen content with a 4900 Micro-GC Then, without breaking the seal, 5 mL of culture was drawn out with a syringe for fluoride measurement, using a

previously described method.. Finally, the entire sample vessel was extracted with 30 mL of acetonitrile injected with a syringe through the septum. The vessel was first shaken at room temperature for 1–2 h, the seals were removed and the rubber septum was pushed into the bottle to be extracted together with the culture by acetonitrile. The vessels were then recrimped with a new septum and shaken at 50 °C for 2–7 d. After extraction, 20 mL of the total acetonitrile extract was filtered through a 0.45 µm nylon filter and stored frozen (~ -20 °C) until chemical analysis by LC/MS/MS.

Results:

• Degradation: 6:2 FTOH concentration decreased rapidly and stabilized after day 7 at 1.6-2.8% of the total 6:2 FTOH mass applied at day 0 (7.7 nmol/mL). No metabolism was observed in untreated and sterilized control test systems. Metabolite concentrations reached steady-state after 14–28 d. At day 28, 6:2 fluorotelomer unsatured acid (6:2 FTUCA) (25%) and 5:2 sFTOH (17%) were the two dominant metabolites with 6:2 fluorotelomer satured acid (6:2 FTCA) (5.7%), 5-3 acid (5.5%), perfluorohexanoic acid (PFHxA, 5.1%), and perfluorobutanoic acid (PFBA) and perfluoropentanoic acid (PFPeA) each less than 0.5% yield. 6:2 FTUCA, 5:2 sFTOH and 6:2 FTCA are not terminal metabolites and they would be expected eventually to be converted to terminal metabolites such as PFPeA, PFHxA, and 5-3 acid. 6-2 FTOH degradation virtually ceased after 14–28 d.

[Study 2: Liu et al. 2010a, flow through soil incubation system]

Study reference:

Liu J., Wang N., Buck R.C., Wolstenholme B.W., Folsom P.W., Sulecki L.M., and Bellin C.A. (2010a): Aerobic biodegradation of [14C] 6:2 fluorotelomer alcohol in a flow-through soil incubation system. Chemosphere 80 (7), 716-723. DOI: 10.1016/j.chemosphere.2010.05.027

Detailed study summary and results:

The aerobic biodegradation of 6:2 FTOH was performed in a flow through soil incubation system. After 1.3 days, 50% of 14C labelled 6:2 FTOH disappeared from soil, because of microbial degradation and volatilisation. The overall mass balance during the 84-day incubation averaged 77% and 87% for the live and sterile treatments, respectively. 16% [14C] 5:2 sFTOH, 14% [14C] 6:2 FTOH and 6% [14C] CO2 were measured in the airflow after 84 days. In soil the following stable transformation products were detected after 84 days: 5:3 polyfluorinated acid (12%), perfluorohexanoic acid (4.5%), perfluoropentanoic acid (4.2%), and perfluorobutanoic acid (0.8%). In soil-bound residues, the major transformation product was 5:3 polyfluorinated acid, which may not be available for further biodegradation in soil.

Test type:

Flow through Soil simulation test

Test substance:

• equivalent (purity 99%)

Materials and methods:

• Details on soil sample: Sassafras soil

• Duration of test: 84 d

• Seven identical systems were constructed to make three replicates of live soil, three replicates of sterile soil and one soil matrix. The soil matrix sample was used for LC/MS/MS analysis of background levels of 6:2 FTOH and its potential transformation products over 84 d. The incubation was initiated by adding 200 lL working solution (972µL [1,2-14C] 6:2 FTOH test substance diluted with 2.028 mL absolute ethanol) into the soil microcosms in the 500-mL vessel (live and sterile) and manually mixed with a sterile spatula. The initial 14C dosed was experimentally determined to be 5.3 x 105 dpm (disintegrations per minute) g^{-1} soil (oven-dry mass), which corresponds to 8 nmol/g or 2.9 µg/g (including both 14C-labeled and unlabeled 6:2 FTOH). The system maintenance included keeping constant air flow rate, replacing the soda lime traps when needed, and changing the NaOH traps every four weeks.

• Sampling and sample preparation: At each designated sampling time (days 0, 1, 4, 7, 14, 29, 56, 84), a soil aliquot of ~5.0 g was removed from each vessel for three sequential extractions performed in a 20-mL glass bottle. The soil was first extracted with 15 mL acetonitrile (CH3CN) for 2–7 d at 50°C, centrifuged at 657g for 20 min and the supernatant was removed for analysis. For the second and third extractions, the soil was shaken overnight with 15 mL 90/10 (v/v) CH3CN/250 mM NaOH solution at 50 °C, neutralized with 80 µL of 5 M HCl and centrifuged at 657g for 20 min. All soil extracts were stored at below -10 °C in the dark before chemical analysis. The bottle septum was replaced after each soil sampling and extracted with 5 mL CH3CN at 50 °C for 2–7 d. On each sampling day, the C18 cartridges were removed from the soil vessel outlet and replaced with fresh cartridges. The removed cartridges were eluted with 5 mL CH3CN individually for the first 7 d, then in tandem for the later sampling days. Periodically, 0.5 mL of aqueous NaOH solution from each trap was withdrawn from each trap and counted for radioactivity.

• Analytical methods: Radioactivity was determined using a Beckman LS5000 TD liquid scintillation counter. The soil CH3CN extracts and C18 cartridge eluent were analysed using a liquid chromatography/accurate radioisotope counting (LC/ARC) system. The first soil extracts were treated with NaOH and Envicarb™ activated carbon before LC/ARC and LC/MS/ MS analysis to recover 6:2 FTOH and transformation products that were conjugated to dissolved soil components

Results:

• Half of the [1,2-14C] 6:2 FTOH disappeared from soil in 1.3 d, undergoing simultaneous microbial degradation and partitioning of volatile transformation product(s) and the 6:2 FTOH precursor into the air phase.

• The overall 14C (radioactivity) mass balance in live and sterile treatments was 77–87% over 84-d incubation. In the live test system, 36% of total 14C dosed was captured in the airflow (headspace), 25% as soil-bound residues recovered via thermal combustion, and 16% as soil extractable. After 84 d, [14C] 5:2 sFTOH [F(CF2)5CH(OH)14CH3] was the dominant transformation product with 16% molar yield and primarily detected in the airflow. The airflow also contained [1,2-14C] 6:2 FTOH and 14CO2 at 14% and

6% of total 14C dosed, respectively. The other significant stable transformation products, all detected in soil, were 5:3 acid [F(CF2)5CH2CH2COOH, 12%], PFHxA [F(CF2)5COOH, 4.5%] and PFPeA [F(CF2)4COOH, 4.2%]. Soil-bound residues as well as conjugates between fluorinated transformation products and dissolved soil components were only observed in the live test system and absent in the sterile soil, suggesting that such binding and complexation are microbially or enzymatically driven processes. At day 84, 5:3 acid is postulated to be the major transformation product in soil-bound residues, which may not be available for further biodegradation in soil environment.

[Study 3: Liu et al. 2010b; soil]

Study reference:

Liu J., Wang N., Szostek B., Buck R.C., Panciroli P.K., Folsom P.W., Sulecki L.M., and Bellin C.A. (2010b): 6-2 Fluorotelomer alcohol aerobic biodegradation in soil and mixed bacterial culture. Chemosphere 78 (4), 437-444. DOI: 10.1016/j.chemosphere.2009.10.044

Detailed study summary and results:

The authors investigated the aerobic biodegradation of 6:2 FTOH (without ^{14}C-labelling) in soil (closed system). 6:2 FTOH primary degradation half-life was 1.6 days. The overall mass balance in aerobic soil was ~67% after 180 days (e.g. due to irreversible bond to soil). After 180 days the following substances were accounted: 30 % perfluoropentanoic acid, 8.1% perfluorohexanoic acid, 1.8% perfluorobutanoic acid, 15% 5:3 polyfluorinated acid, 1 % 4:3 polyfluorinated acid, 3 % 6:2 FTOH, and 7.1% 5:2 sFTOH.

Test type:

OECD Guideline 307, GLP not specified

Test substance:

• equivalent (99% purity)

Materials and methods:

• Details on soil sample: Sassafras soil from a forested area undisturbed for at least 40 years in Newark, Delaware; The soil was a sandy loam (52% sand, 34% silt, and 14% clay) with 3.8% organic matter (OM), pH of 5.8 (1:1 soil:water) and microbial biomass carbon of 150 µg/ g dry soil

• Duration of test: 180 days

• 6:2 FTOH was initially dosed at 2.9µg/ g soil (or 8.0 nmol/ g). Parent and metabolites were measured in three compartments: volatile analytes in the headspace, volatile analytes absorbed onto the rubber septa, and study analytes in soil by exhaustive solvent extraction of the entire test vessel. 120-mL glass serum bottles were incubated statically in dark at 20–25 °C for 180 d. Approximately 10 g dry weight equivalent of sieved Sassafras soil was added to each vessel, and dosed with 10 µL of 6:-2 FTOH stock solution prepared in ethanol. For each sampling time point, four treatments of triplicate vessels were prepared: (1) untreated (matrix) live soil with 10 lL ethanol; (2) 6:2 FTOH treated live soil; (3) 6:2 FTOH treated sterile soil; and (4) sterile soil treated with selected metabolites.

• sampling and sample preparation: T*est vessels were sacrificed at each time point for extraction and sample analysis at 0, 2, 7, 14, 28 60, 90, 120, and 180 d. The O2 content was also measured at each time point in untreated live soil to approximate O2 content in treated live sample bottles. Prior to opening the soil test vessels, the headspace gas of a sample bottle was pumped out through two needles inserted through the septum using an air pump at a rate of 1.5 L/ min for 1 min. The outlet air went through two C18 cartridges in series to capture volatile fluorinated compound(s). Each cartridge was eluted with 5 mL acetonitrile and subject to chemical analysis. The rubber septum was then removed and extracted in 5 mL acetonitrile at 50 °C for 2–7 d. The soil was subjected to two sequential extractions in the test vessel sealed with a new rubber septum to enhance recovery of 6:2 FTOH and potential metabolites. For the first extraction, 20 mL acetonitrile was added to the soil of each bottle, and the vessels were shaken at 50 °C for 2–7 d and then centrifuged at 1000 rpm for 20 min to collect the supernatants (extracts). For the second extraction, 18 mL acetonitrile and 2 mL of 250 mM NaOH were added to the soil of each bottle. The final concentration of acetonitrile was 90% and NaOH was 25 mM. The vessels were shaken at 50 °C overnight. 0.5 mL of 1 M HCl was then added to each bottle to neutralize the extract and the vessels were centrifuged at 1000 rpm for 20 min to collect the neutralized supernatants.All the extracts, from C18 cartridge, septa and soils, were stored under frozen conditions (~ -20°C) and later analyzed by LC/MS/MS.*

Results:

• treated sterile control: ~87–113% 6:2 FTOH mass balance over 180 d; treated live soil samples: ~67% 6:2 FTOH mass balance after 180 days (metabolites irreversibly bound to soil and and non-extractable)

• half-life (primary degradation) = 1.6 d

• metabolites after 180 d: perfluoropentanoic acid (30%), 5:3 acid (15%), perfluorohexanoic acid (8.1%), 5-2 sFTOH (7.1%), perfluorobutanoic acid (1.8%), 4:3 acid (~1%), 5:2 sFTOH (7%)

1.2 Bioaccumulation

1.2.1 Bioaccumulation test on fish

[Study 1: (ECHA Registration dossier, Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan, 2002)]

Study reference:

Kurume Laboratory, Chemical Evaluation and Research Institute (2002): Bioconcentration study of C6-2 alcohol in carp. Report no.: 43771 (report date: 2002-01-31)

Detailed study summary and results:

A fish bioconcentration test according to OECD TG 305 was conducted for 28 days on Cyprinus carpio with two exposure levels (1 and 10 µg/L nominal; 0.835 and 9.11 µg/L measured) of the test substance (registration dossier Kurume, 2002). The test temperature ranged from 24.6 to 25.9°C and the pH from 7.6 to 7.9. The lipid content was 2.95% at the start of the exposure and 2.26% at the end of the exposure. After 28 days whole body w.w. BCF values of ≤ 36 (exposure level 1 µg/L) and 46 (exposure level 10 µg/L) were determined (steady state).

Test type:

OECD Guideline 305, GLP; derivation: no post-exposure (depuration) phase

Test substance:

• equivalent (purity 99.8 wt%)

Materials and methods:

• Sampling intervals/frequency for test organisms: The test fish of Level 1 and 2 were analysed five times in duration of exposure. Four fish were taken out at each sampling time and divided into two groups, then both were analysed individually. Because one fish was too small to take out the stored sample for the measurement of lipid content, two fish a group were employed.

The control fish were analysed before the experimental starting and after the experimental completion. Four fish were taken out at each sampling time and divided into two groups, and then each was analysed individually. In addition, two fish were taken out and three groups (two fish per group) were used for measurement of lipid contents.

• Sampling intervals/frequency for test medium samples: The test water of each level was analysed once before first analysis of test fish and at the same time as analysis of test fish thereafter. The number of each sample was one .

• Details on sampling and analysis of test organisms and test media samples: Analysis of test item in test water and test fish was performed with gas chromatography-mass spectrometry (GC-MS) analysis. If the test item was bioaccumulated in the test fish, the test item had a possibility to be metabolised in the fish body. Therefore, C5F11COOH (hereafter mentioned as "carboxylic acid") which was an estimated metabolite of the test item in the test fish was analysed at the same time. Analysis of the carboxylic acid in the test fish was performed with the liquid chromatography - tandem mass spectrometry (LC/MS/MS).

• Details on pretreatment: Extraction: Test fish were extracted (polytron on ice) with acetonitrile/isopropyl alcohol (7/3 V V) then centrifuged and filtered.

• Identification and quantification of test substance/product:

- Separation method: Test item in water and fish - Gas chromatograph-mass spectrometer Shimadzu Corporation. type QP-5000; Carboxylic acid - Liquid chromatograph-mass spectrometer Agilent type HP-1100 Micromass type Quattro Ultima

- Conditions (column, mobile phase, etc.): Test item in water and fish - INNOWAX 30 m x 0.25 mm I.D.; Carboxylic acid - L-column ODS 15 cm x 2.1 mm LD.

- Detection method: Test item in water and fish - Electron ionisation (El); Carboxylic acid – Ionisation mode Electrospray, Detection ion Negative,

Detection mode Selected reaction monitoring

• Vehicle: yes

• Test organisms: Cyprinus carpio

- Source: Sugishirna fish farm, Japan

- Age at study initiation: Yearling fish

- Length at study initiation: 6.6 - 6.8 cm

- Health status: The fish were checked visually at receipt and those showing any abnormalities were removed.

- Description of housing/holding area: 100-L glass tank

- Feeding during test: yes

- Food type: Feed for fry of carp

Proteins content ≥ 43.0 %

Lipid content ≥ 3.0 %

- Amount: Amount corresponding to about 2 % of total body weight was fed twice a day in halves.

The fish were starved for 24 hours before sampling.

- Acclimation period: flow-through system for 41 days

- Acclimation conditions (same as test or not): same

• Study design:

- Route of exposure: aqueous

- Test type:flow-through

- Water / sediment media type: natural water: freshwater

- Total exposure / uptake duration: 28d

• Test conditions:

- Hardness: 58.6 mg/L

- Test temperature: 24.6 - 25.9 °C

- pH: 7.6 - 7.9

- Dissolved oxygen: 7.0 -8.0 mg/L

- TOC: 1.6 mg/L

- Salinity: fresh water

Details on test conditions

TEST SYSTEM

- Test vessel: 100-L glass tank

- Type of flow-through: 0.02 mL/min for stock solution and 1600 mL/min for dilution water, 2304 L/day of test water, was supplied.

- Renewal rate of test solution: 2304 L/day of test water

- No. of organisms per vessel: test - 29; control - 12

- No. of vessels per concentration (replicates): 1

- No. of vessels per control / vehicle control (replicates): 1

TEST MEDIUM / WATER PARAMETERS

- Source/preparation of dilution water: Groundwater from the premises of Kurume Laboratory.

It was confirmed that the dilution water met the ministerial ordinance of the Ministry of Health and Welfare (December 21, 1992), water quality criteria for fisheries (Shadanhozin Nihon Suisansigen Hogokyokai, March 1983), OECD Guidelines for Testing of Chemicals, "Fish, Early-life Stage Toxicity Test" (Guideline 210, July 17, 1992) and environmental quality standards for water pollutants No.14 (Revised February 22, 1999, Environment Agency) or OECD Guidelines for Testing of Chemicals, " Bioconcentration: Flow-through Fish Test (Guideline 305, June 14, 1996)".

- nominal and measured concentrations:

Nominal concentrations: High exposure level (Level 1) - 10 µg/L, Low exposure level (Level 2) - 1 µg/L

Mean Measured: High exposure level (Level 1) - 9.11 µg/L, Low exposure level (Level 2) - 0.835 µg/L

Results:

• lipid content: 2.95% (start of exposure); 2.26% (end of exposure)

• BCF = 46 (whole body w.w.), high exposure level; time of plateau: 28 d; calculation basis: steady state

• BCF ≤ 36 (whole body w.w.), low exposure level; time of plateau: 28 d; calculation basis: steady state

• BCF of metabolite carboxylic acid ≤ 1.1 (whole body w.w.) at high exposure level and ≤ 12 (whole body w.w.) at low exposure level

[Study 2: (ECHA Registration dossier, Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan, 2007)]

Study reference:

Kurume Laboratory, Chemical Evaluation and Research Institute (2007): Bioconcentration study of 13F-EtOH in carp. Report no.: 44807 (report date: 2007-03-19)

Detailed study summary and results:

A bioconcentration test according to OECD TG 305 (deviation: no post-exposure (depuration) phase) was conducted for 28 days on Cyprinus carpio with two exposure levels (1 and 10 µg/L nominal) of the test substance (registration dossier Kurume, 2007). After 28 days whole body w.w. BCF values of 24 - 99 (exposure level 1 µg/L) and 8.4 - 58 (exposure level 10 µg/L) were determined.

Test type:

OECD Guideline 305, GLP not specified; derivation: no post-exposure (depuration) phase

Test substance:

• equivalent

Materials and methods:

• Test organisms: Cyprinus carpio

• Study design:

- Route of exposure: aqueous

- Test type:flow-through

- Water / sediment media type: natural water: freshwater

- Total exposure / uptake duration: 28d

• Test conditions:

- Nominal concentrations: High exposure level (Level 1) - 10 µg/L, Low exposure level (Level 2) - 1 µg/L

Results:

• BCF = 8.4-58 (whole body w.w.), high exposure level; time of plateau: 28 d; calculation basis: steady state

• BCF = 24 -99 (whole body w.w.), low exposure level; time of plateau: 28 d; calculation basis: steady state

1.3 Acute toxicity

1.3.1 Short-term toxicity to fish

[Study 1: Anonymous 1, 2007]

Study reference:

H-28078: Static-Renewal, Acute, 96-Hour Toxicity Test with Fathead Minnow, Pimephales

study report

Detailed study summary and results:

The 96-hour acute toxicity test with fathead minnow, Pimephales promelas, according to OECD TG 203 was conducted in a static test-type. The test concentrations were analytically monitored by LC/MS analysis (nominal test concentrations: 0, 1.0, 2.0, 4.0, 8.0, and 16.0 mg/L; mean measured: 0, 0.751, 1.61, 3.12, 7.52 and 16.4 mg/L). For the test 5 organisms per replicate and 2 replicates were used. The photoperiod was 16 hours light per day (light intensity: 126-710 lux). The test temperature ranged from 21.4 to 21.6°C, the pH from 7.0 to 7.3 and the dissolved oxygen concentration from 5.4 to 8.5 mg/L. No control mortality or behavioural abnormalities occurred. The fish in the control had a standard length of 2.2 to 2.8 cm and a wet weight, blotted dry, of 0.140 to 0.304 g. The validity criteria were fulfilled. The test resulted in an 96h-LC_{50} of 4.84 mg/L (mean measured concentrations) and an 96h-LC_{100} of 7.52 mg/L (mean measured). At 3.12 mg/L (mean measured) and below no mortality occurred.

Test type:

OECD Guideline 203 (Fish, Acute Toxicity Test) (no deviations; GLP, analytical monitoring: LC/MS)

Test substance:

• equivalent

• Degree of purity: 99.7%

Materials and methods:

• Test species and origin: Pimephales promelas

• Age at study initiation: >112 days

• Test conditions: Dissolved oxygen: 5.4 - 8.5 mg/L; pH: 7.0 - 7.3; Total Alkalinity: 49 mg/L as CaCO3, EDTA Hardness: 116 mg/L as CaCO3; Test temperature: 21.4 to 21.6°C; Photoperiod: 16 hours light and 8 hours darkness, which included 30 minutes of transitional light (3-125 Lux) preceding and following the 16-hour light interval. Light intensity: approximately 126-710 Lux; static test type; no vehicle

• Tested doses: 0, 1.0, 2.0, 4.0, 8.0, and 16.0 mg/L

• Test duration/total exposure duration: 96 h

• Test design: - Test vessel: 4-L Erlenmeyer flasks; - Renewal rate of test solution (frequency/flow rate): renewed at day 2 (48 hours); - No. of organisms per vessel: 5; - No. of vessels per concentration (replicates): 2; - No. of vessels per control (replicates): 2

Results:

• Observations in the controls: Mortality of control: none; Behavioural abnormalities: none observed; Observations on body length and weight: Fish in the dilution water control ranged from 2.2 to 2.8 cm in standard length and 0.140 to 0.304 g in wet weight, blotted dry, at test end.

• Observations in the test system: Mortality of mean measured test concentrations: 100% mortality at 7.52, and 16.4 mg/L; Behavioural abnormalities: none

• Monitoring of test concentrations: Measured concentrations: 0, 0.751, 1.61, 3.12, 7.52 and 16.4 mg/L

• LC50 at 96 hours: 4.84 mg/L (meas.) (95% confidence interval of 3.12 to 7.52 mg/L); NOEC at 96 hours: 3.12 mg/L (meas., mortality); LC100 at 96 hours: 7.52 mg/L (meas.)

[Study 2: (Anonymous 2, 2005)]

Study reference:

C6-2AL: ACUTE TOXICITY TO RAINBOW TROUT (Oncorhynchus mykiss) / study report

Detailed study summary and results:

The 96-hour acute toxicity test was conducted with Oncorhynchus mykiss according to OECD TG 203 in a semi-static test-type. The test concentrations were analytically monitored. The test concentrations ranged from 1.3 to 13 mg/L (nominal). The validity criteria were fulfilled according to the registrant. The resulting 96h-LC_{50} was 9 mg/L (nominal). The reliability was difficult to access because of to little details provided in the registration dossier.

Test type:

OECD Guideline 203 (Fish, Acute Toxicity Test) (no deviations; analytical monitoring)

Test substance:

• equivalent

• Degree of purity: 99.7%

Materials and methods:

• Test species and origin: Oryzias latipes

• Test conditions: semi-static test type

• Tested doses: at start of exposure 2.00 to 10.0 mg/L (measured)

• Test duration/total exposure duration: 96 h

• Test design: -

Results:

• LC50 at 96 hours: 9.0 mg/L (nominal) (95% confidence interval of 7.7 - 10 mg/L); NOEC at 96 hours: 2.3 mg/L (nominal, mortality)

[Study 3: (Anonymous 3, 2007)]

Study reference:

A 96-hour Acute Toxicity Study of 13F-EtOH with Medaka / study report

Detailed study summary and results:

The 96-hour acute toxicity study was conducted with ricefish, Oryzias latipes, according to OECD TG 203 in a semi-static test-type. The test concentrations were analytically monitored and ranged from 2 to 10 mg/L (measured). The validity criteria were fulfilled according to the registrant. The test resulted in an 96h-LC_{50} of 5.78 mg/L (mean measured concentrations) and an 96h-NOEC of 3.06 mg/L (mean measured).

Test type:

OECD Guideline 203 (Fish, Acute Toxicity Test) (no deviations; analytical monitoring)

Test substance:

• equivalent

• Degree of purity: not reported

Materials and methods:

• Test species and origin: Oryzias latipes

• Test conditions: semi-static test type

• Tested doses: 2.00 to 10.0 mg/L (measured).

• Test duration/total exposure duration: 96 h

• Test design: -

Results:

• LC50 at 96 hours: 5.78 mg/L (geom.mean meas.) (95% confidence interval of 4.92 – 6.89 mg/L); NOEC at 96 hours: 3.06 mg/L (geom.mean meas., mortality)

1.3.2 Short-term toxicity to aquatic invertebrates

[Study 1: (ECHA Registration dossier: DuPont Haskell Global Centers for Health & Environmental Sciences, 2007)]

Study reference:

H-28078: Static, Acute, 48-Hour Toxicity Test with Daphnia magna / DuPont Haskell Global Centers for Health & Environmental Sciences / study report

Detailed study summary and results:

The acute toxicity test with Daphnia manga was conducted according to OECD TG 202 under GLP with analytical monitoring (LC/MS/MS) in a static test-type. The test temperature ranged from 20.1 to 20.4°C and the pH from 7.2 to 7.6. Nominal test concentrations were 0, 0.625, 1.25, 2.50, 5.00, and 10.0 mg/L and the mean measured concentrations were 0, 0.600, 1.23, 2.39, 4.90, and 9.29 mg/L. For the test system 10 organisms were used per test vessel and two replicates per concentration. The photoperiod was 16 hours light per day (495-534 lux). The resulting 48h-EC50 (endpoint: immobility) was 7.84 mg/L and the 48h-NOEC was 2.39 mg/L based on mean measured concentrations. Lethargy was observed in surviving daphnids in the 2.39, 4.90, and 9.29 mg/L mean measured concentrations at the end of the study. The validity criteria were fulfilled.

Test type:

OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test))(no deviations, GLP, analytical monitoring: LC/MS/MS LOD= 0.007 mg/L LOQ= 0.375 mg/L, no vehicle)

Test substance:

• test material used in the study is equivalent to the substance identified in the C&L dossier.

• Degree of purity: 99.7%

• Impurities (or a note that the impurities do not affect the classification)

Materials and methods:

• Test species and origin: Daphnia magna

• Species life stage: < 24h; collected from the 3rd and 6th broods of 14- and 20-day old parent daphnids

• Test conditions:

- *Hardness: Total alkalinity (dilution water control) 47 mg/L as CaCO3; hardness (dilution water control) 137 mg/L as $CaCO_3$;*
- *Test temperature: 20.1 to 20.4°C (dilution water control;*
- *pH 7.2 to 7.6 (dilution water control); static test type*

• Test duration/total exposure duration: 48h

• Test design:

- *test concentrations 0, 0.625, 1.25, 2.50, 5.00, and 10.0 mg/L (nominal) or 0, 0.623, 1.25, 2.49, 4.99, and 9.97 mg/L (nominal, adjusted for purity);*
- *Test vessel: Glass vials (44-mL). The test chambers were covered with modified lids containing a septum during the test. The modified lids were used to ensure zero-headspace in the test chambers. A zero-headspace chamber was used to ensure the maintenance of test concentrations for the duration of the study.*
- *No. of organisms per vessel: 10*
- *No. of vessels per concentration (replicates): 2*
- *No. of vessels per control (replicates): 2*
- *Photoperiod: A photoperiod of 16 hours light and 8 hours darkness was employed, which included 30 minutes of transitional light (15-19 Lux) preceding and following the 16-hour light interval.*
- *Light intensity: 495-534*

Results:

• Observations in the test system: Behavioural abnormalities: Lethargy was observed in surviving daphnids in the 2.39 (3 of 20), 4.90 (3 of 15), and 9.29 mg/L (5 of 7) mean, measured test concentrations at the end of the study.

• Monitoring of test concentrations: 0, 0.600, 1.23, 2.39, 4.90, and 9.29 mg/L (mean measured)

• EC50 at 48 hours: 7.84 mg/L (95% confidence interval 6.75 to 9.39 mg/L); NOEC at 48 hours: 2.39 mg/L; EC100 at 48 hours: 9.29 mg/L

[Study 2: (ECHA Registration dossier: Safepharm Laboratories Ltd., UK, 2005)]

Study reference:

C6-2AL: ACUTE TOXICITY TO DAPHNIA MAGNA / Safepharm Laboratories Limited / study report

Detailed study summary and results:

The acute toxicity test with Daphnia manga was conducted according to OECD TG 202 under GLP with analytical monitoring under static test conditions. The test concentrations ranged from 0.14 to 14 mg/L (nominal). The resulting 48h-EC_{50} (endpoint: immobility) was 8.3 mg/L and the 48h-NOEC was 2.5 mg/L based on nominal concentrations. According to the registrant, the validity criteria were fulfilled.

Test type:

OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) (no deviations, GLP, analytical monitoring, no vehicle)

Test substance:

• test material used in the study is equivalent to the substance identified in the C&L dossier.

• Degree of purity: 99.8 %

Materials and methods:

• Test species and origin: Daphnia magna

• Test conditions: static test system

• Test duration/total exposure duration: 48h

• Test design: test concentrations ranged from 0.14 to 14 mg/L (nominal).

Results:

• EC50 at 48 hours: 8.3 mg/L (95% confidence interval: 7.1 – 9.8 mg/L); NOEC at 48 hours: 2.5 mg/L

[Study 3: (ECHA Registration dossier: Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan, 2007)]

Study reference:

A 48-hour Acute Immobilization Study of 13F-EtOH with Daphnia magna / Kurume Laboratory / study Report

Detailed study summary and results:

The acute toxicity test with Daphnia manga was conducted according to OECD TG 202 with analytical monitoring in a static test-type. The test concentrations ranged from 1.30 to 15.5 mg/L (nominal). The resulting 48h-EC_{50} (endpoint: immobility) was 8.2 mg/L and the 48h-NOEC was 1.33 mg/L based on mean measured concentrations. According to the registrant, the validity criteria were fulfilled.

Test type:

OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) (no deviations; GLP not specified; analytical monitoring; no vehicle)

Test substance:

• Test material used in the study is equivalent to the substance identified in the C&L dossier.

• Degree of purity: not reported

Materials and methods:

• Test species and origin: Daphnia magna

• Species life stage

• Test conditions: static test system

• Test duration/total exposure duration: 48 hours

• Test design: test concentrations at the start of exposure ranged from 1.30 to 15.5 mg/L (measured)

Results:

• EC50 at 48 hours: 8.2 mg/L (meas. geom. mean) (95% confidence interval: 7.16 – 9.46 mg/L); NOEC at 48 hours: 1.33 mg/L

1.3.3 Algal growth inhibition tests

[Study 1: (ECHA Registration dossier: DuPont Haskell Global Centers for Health & Environmental Sciences, 2007)]

Study reference:

H-28078: Static, 72-Hour Growth Inhibition Toxicity Test with the Green Alga, Pseudokirchneriella su / DuPont Haskell Global Centers for Health & Environmental Sciences / study report

Detailed study summary and results:

The toxicity study with Pseudokirchneriella subcapitata was conducted according to OECD TG 201 under GLP with analytical monitoring (LC/MS) in a static test-type. The test temperature ranged from 23.8 to 24.0 °C and the pH value from 7.97 to 9.97. Nominal test concentrations were 0.200, 0.640, 2.00, 6.60, and 21.0 mg/L and the mean measured concentrations were 0.154, 0.623, 2.22, 7.10, and 23.5 mg/L. Four replicates were used per concentration (3 for the test and 1 for analytical sampling). The photoperiod was 24 hours light per day (6670 to 6980 lux). The resulting 72h-E_rC_{50} was 14.8 mg/L (measured). The NOE_rC was 2.22 mg/L (measured). All validity criteria were fulfilled.

Test type:

OECD Guideline 201 (Alga, Growth Inhibition Test) before 23 March 2006 (no deviations; GLP; analytical monitoring: LC/MS; no vehicle)

Test substance:

• Test material used in the study is equivalent to the substance identified in the C&L dossier.

• Degree of purity

Materials and methods:

• Test species: Pseudokirchneriella subcapitata (previous names: Raphidocelis subcapitata, Selenastrum capricornutum)

• Initial cell concentration: 10000 cells/mL

• Test conditions:

- *Static test type*
- *Test temperature 23.8 to 24.0 °C*
- *pH 7.97 to 9.97 (e.g. temperature, lighting, test medium, pH, test system, solubilising agent, etc.)*
- *Adjustment of pH: nutrient medium pH was adjusted to 7.49*
- *Photoperiod: 24 hrs light*
- *Light intensity and quality: 6670 to 6980 lux*

• Test duration/total exposure duration: 72 hours

• Test design:

- *test concentrations: 0.200, 0.640, 2.00, 6.60, and 21.0 mg/L, number/type of controls, number of replicates, etc)*
- *No. of vessels per concentration (replicates): 4 (3 used for test, 1 for analytical sample)*
- *No. of vessels per control (replicates): 4 (3 used for test, 1 for analytical sample)*

Results:

• Observations in the controls: control end cells density mean 430000 cells/mL

• Details on the determination of algal biomass: Growth was determined by counting the number of cells in an approximate 10-µL sample from each vial at approximately 24, 48, and 72 hours after the definitive test initiation. The counts were conducted using a haemocytometer and a compound microscope.

• Growth curves: exponential growth in the control (for algal test): Healthy cell counts increased in the blank control by at least a factor of 16 in 72 hours and the coefficient of variation of average specific growth rates during the whole test period (0-72 hour) in blank control replicates did not exceed 7%, thereby satisfying the appropriate test acceptance criteria

• Monitoring of test concentrations: 0, 0.154, 0.623, 2.22, 7.10, and 23.5 mg/L (mean measured)

• ErC50 at 72 hours: 14.8 mg/L (meas.; 95 confidence interval 9.85 to 19.8 mg/L), EbC50 at 72 hours: 7.3 mg/L (cell count; meas.; 95 confidence interval 5.16 – 10.3 mg/L), NOErC at 72 hours: 2.22 mg/L (meas.); NOEbC at 72 hours: 0.623 mg/L (meas.)

[Study 2: (ECHA Registration dossier: Safepharm Laboratories Ltd., UK, 2005)]

Study reference:

C6-2AL: ALGAL INHIBITION TEST / Safepharm Laboratories Limited / study report

Detailed study summary and results:

The toxicity study with Desmodesmus subspicatus was conducted according to OECD TG 201 under GLP with analytical monitoring in a static test-type. Mean measured concentrations ranged from 1.13 to 13 mg/L (1.3, 2.3, 3.1, 6.7 and 13). The resulting 72h-E_rC_{50} was 7.8 mg/L (measured). The NOE_rC was 1.3 mg/L (measured). All validity criteria were fulfilled according to the registrant.

Test type:

OECD Guideline 201 (Alga, Growth Inhibition Test) before 23 March 2006 (no deviations; GLP; analytical monitoring; no vehicle)

Test substance:

• Test material used in the study is equivalent to the substance identified in the C&L dossier.

• Degree of purity: 99.8%

Materials and methods:

• Test species: Desmodesmus subspicatus (previous name: Scenedesmus subspicatus)

• Test conditions: the culture solution contained additional sodium bicarbonate to provide a source of CO2 required for algal growth in sealed test vessels; static test type

• Test duration/total exposure duration: 72 hours

• Test design: mean measured concentrations ranged from 1.13 to 13 mg/L (1.3, 2.3, 3.1, 6.7 and 13)

Results:

• ErC50 at 72 hours: 7.8 mg/L (meas.; 95% confidence interval 6.8 – 8.9 mg/L), EbC50 at 72 hours: 3.8 mg/L (meas.; 95% confidence interval 3.4 – 4.3 mg/L), NOErC at 72 hours: 1.3 mg/L

[Study 3: (ECHA Registration dossier: Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan, 2007)]

Study reference:

Algae Growth Inhibition Study of 13F-EtOH with Pseudokirchneriella subcapitata / Kurume Laboratory / study report

Detailed study summary and results:

The toxicity study with Pseudokirchneriella subcapitata was conducted according to OECD TG 201 with analytical monitoring in a static test-type. Mean measured concentrations ranged from 0.0966 to 9.45 mg/L. The resulting 72h-E_rC_{50} was greater than 5.19 mg/L (mean measured). The NOE_rC was 1.47 mg/L (measured). All validity criteria were fulfilled according to the registrant.

Test type:

OECD Guideline 201 (Alga, Growth Inhibition Test) before 23 March 2006 (no deviations; GLP: not specified; analytical monitoring)

Test substance:

• Test material used in the study is equivalent to the substance identified in the C&L dossier.

• Degree of purity: not reported

Materials and methods:

• Test species: Pseudokirchneriella subcapitata (previous names: Raphidocelis subcapitata, Selenastrum capricornutum)

• Test duration/total exposure duration: 72 hours

• Test design: test concentrations: five exposure levels, ranging from 0.0966 to 9.45 mg/L measured at study start; static test type (e.g. test concentrations, number/type of controls, number of replicates, etc)

Results:

• ErC50 at 72 hours: > 5.19 mg/L (meas), NOErC at 72 hours: 1.47 mg/L (meas.)

1.4 Chronic toxicity

1.4.1 Chronic toxicity to aquatic invertebrates

[Study 1: (ECHA Registration dossier: DuPont Haskell Global Centers for Health & Environmental Sciences, 2007)]

Study reference:

H-29849: 21-Day Chronic Static-Renewal, Zero Headspace Toxicity Test with the Cladoceran, Daphnia m / DuPont Haskell Global Centers for Health & Environmental Sciences / study report

Detailed study summary and results:

One long-term toxicity test to the aquatic invertebrate Daphnia magna is available conducted according to OECD TG 211 under GLP with analytical monitoring (LC/MS/MS) in a semi-static test-type. The test temperature ranged from 20.6 to 21.6 °C, the pH value from 7.6 to 8.1 and the dissolved oxygen concentration from 5.4 to 9.0 µg/L. The nominal test concentrations amounted to 0, 0.65, 1.3, 2.5, 5, and 10 mg/L and the mean measured concentrations amounted to 0, 0.557, 1.11, 2.16, 4.46, and 8.57 mg/L. Two organisms per test vessel werever used and the control was composed of 5 replicates. The number of replicates for the test concentrations was not described in the RSS but it would be likely that this replicate number was also used for the test concentrations. The photoperiod was 16 hours light per day (17-40 lux). The test resulted in a NOEC of 2.16 mg/L (based on mean measured concentrations) (basis for the effect: adult survival, total live young per female, total immobile young per surviving female and length and weight of surviving females at day 21). According to the registrant all validity criteria were fulfilled.

Test type:

EPA OTS 797.1330 (Daphnid Chronic Toxicity Test) as well as OECD Guideline 211 (Daphnia magna Reproduction Test) (no deviations; GLP; analytical monitoring LC/MS/MS; no vehicle)

Test substance

• Test material used in the study is equivalent to the substance identified in the C&L dossier

• Degree of purity: 99.94%

• Impurities: 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10, 10-heptadecafluoro- (CAS 678-39-7) - 0.0143 wt%; Decane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-heptadecafluoro-10-iodo- (CAS 2043-53-0) - 0.0015 wt%; Octane, 1,1,1,2,2,3,3,4,4,5,5,6,6-tridecafluoro-8-iodo- (CAS 2043-57-4) - 0.0127 wt%; Unknowns - 0.0315 wt%

Materials and methods:

• Test species and origin: Daphnia magna (Parent Daphnia magna were reared at Haskell Laboratory in 1000-mL Pyrex beakers which contained 1000 mL of filtered fish tank water at approximately 20°C. Each beaker contained 8-10 adults.)

• Species life stage: age of parental stock = 24 days

• Test conditions: semi-static test system;

- *Food type: during test: green algae (Pseudokirchneriella subcapitata) and yeast, cereal leaves and trout chow (YCT) mixture; Amount: during test: 62,500 cells/mL and 3 mL/L YCT on renewal days, 62,500 cells/mL on nonrenewal days; Frequency: daily;*
- *hardness: Total alkalinity range: 80 - 88 mg/L as CaCO3; EDTA hardness range: 117 - 131 mg/L as CaCO3*
- *Test temperature: range of 20.6 - 21.6ºC*
- *pH: range of 7.6 - 8.1*
- *Dissolved oxygen: range of 5.4 - 9.0 µg/L*

• Preliminary test

• *Test duration*

• *Test design:*

- *test concentrations 0, 0.65, 1.3, 2.5, 5 and 10 mg/L (nominal),*
- *Test vessel: Glass bottles (250 mL) containing approximately 250 mL of test solution capped with a Mininert™ valve closure*
- *Renewal rate of test solution: renewed every Monday, Wednesday, and Friday*
- *No. of organisms per vessel: 2*
- *No. of vessels per concentration (replicates):*
- *No. of vessels per control (replicates): 5 number of controls, number of replicates, number of animals, etc.)*
- *Photoperiod: 16 hours light, 8 hours dark with approximately 25-30 minutes of transitional light preceding and following the 16 hour light interval*
- *Light intensity: approximately 17 - 40 Lux*

Results:

• *Monitoring of test concentrations: 0, 0.557, 1.11, 2.16, 4.46, and 8.57 mg/L*

• *NOEC of 21 days: 2.16 mg/L (arithm. mean meas.; endpoints: adult survival, total live young per female, total immobile young per surviving female and length and weight of surviving females at day 21), EC50 of 21 days: 3.87 mg/L (arithm. Mean meas.; mortality); LOEC of 21 days: 3.1 mg/L (arithm. Mean meas.)*

1.4.2 Chronic toxicity to algae or aquatic plants

[See short-term toxicity]