CLH Report

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Ammonium pentadecafluorooctanoate, (APFO)

EC Number: 223-320-4

CAS Number: 3825-26-1 (APFO)

The classification of Ammonium pentadecafluorooctanoate, (APFO), a salt of Perfluorooctanic acid (PFOA), was agreed in the former TC C&L group. New data on developmental toxicity were available after the final conclusion on classification was reached in the former TC C&L group. There data are included in the CLH-report section 5.9.2. The discussion and conclusions from the TC C&L group on the classification of APFO is included in Annex I of this CLH dossier.

Annex I: Summary Record of PFOA and it salts from the TC C&L group meeting 21-24 March 2006 and 4-5 October 2006.

Submitted by : Climate and Pollution Agency (Norway)

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PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Ammonium pentadecafluorooctanoate, (APFO)

EC Number: 223-320-4

CAS number: 3825-26-1

Registration number (s):

Purity: 98%

Impurities: -

Proposed classification based on Directive 67/548/EEC criteria:

R-phrase(s):

Carc. Cat 3; R40

Repr. Cat. 2: R61

T; R48/23

Xn; R48/22, R20/22,

Xi; R36

Proposed classification based on GHS criteria:

Carc. 2, H351

Repr. 1B, H360D

STOT RE 1, H372

STOT RE 2, H373

Acute Tox. 3, H331

Acute Tox. 3, H301

Eye Irrit. 2, H319

Proposed labelling based on Directive 67/548/EEC:

Class of danger: Toxic; irritant

R phrases: 40-61-48/23-48/22-20/22-36
S phrases: 53-45

Proposed labelling based on CLP Regulation:
Pictogram: GHS07, GHS08
Signal word: Danger
Hazard statement codes: H351, H360D, H372, H373, H331, H301, H319
Precautionary statements: Not required as PS are not included in Annex VI

Proposed specific concentration limits (if any): -

Proposed notes (if any):

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical Name: Ammonium pentadecafluorooctanoate, (APFO)

EC Name: Ammonium pentadecafluorooctanoate, (APFO)

CAS Number: 3825-26-1

IUPAC Name: Ammonium pentadecafluorooctanoate

1.2 Composition of the substance

Chemical Name: Ammonium pentadecafluorooctanoate, (APFO),

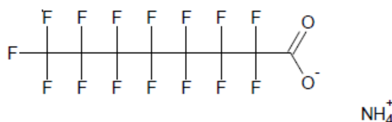
EC Number: 223-320-4

CAS Number: 3825-26-1

IUPAC Name: Ammonium pentadecafluorootanoate

Molecular Formula: $C_8H_4NF_{15}NO_2$

Structural Formula: APFO



Molecular Weight: APFO: 431.10

Typical concentration (% w/w): 98 % impurities not known.

Concentration range (% w/w): No information found.

1.3 Physico-chemical properties

Table 1: Summary of physico-chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	Reference
VII, 7.1	Physical state at 20℃ and 101.3 KPa	3.1	APFO is a solid.	Kirk-Othmer, 1994
VII, 7.2	Melting/freezing point	3.2	APFO: 157-165 °C (decomposition starts above 105 °C) APFO: 130 (decomposition)	Lines and Sutcliff, 1984 3M Company, 1987
VII, 7.3	Boiling point	3.3	Decomposition	Lines and Sutcliff, 1984 (IUCLID 2.2)
VII, 7.4	Relative density	3.4 density	APFO: 0,6-0,7 g/ml, 20 °C	Griffith and Long, 1980
VII, 7.5	Vapour pressure	3.6	APFO: 0.0081 Pa at 20 °C, calculated from measured data	Washburn et al., 2005
VII, 7.6	Surface tension	3.10		
VII, 7.7	Water solubility	3.8	**conc. at sat. (g/l)** APFO: > 500	**Temperature (°C)** 20 °C (3M Company, 1987)
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	**Experimental** No data **Calculated** No data.	
VII, 7.9	Flash point	3.11	No data found.	
VII, 7.10	Flammability	3.13	No data found.	
VII, 7.11	Explosive properties	3.14	No data found.	
VII, 7.12	Self-ignition temperature			
VII, 7.13	Oxidising properties	3.15	No data found.	
VII, 7.14	Granulometry	3.5		
IX, 7.15	Stability in organic solvents and identity of relevant degradation products	3.17		
IX, 7.16	Dissociation constant	3.21	Dissociation Constants: pKa = 2.80 in 50% aqueous ethanol pKa = 2.5	Brace, 1962 Ylinen et al., 1990
IX, 7.17,	Viscosity	3.22		
	pH value in water at 23 °C		Approx. 5	3M, 1987, (reliability not assignable)
	Auto flammability	3.12		

	Reactivity towards container material	3.18		
	Thermal stability	3.19		

2 MANUFACTURE AND USES

2.1 Manufacture

2.2 Identified uses

PFOA is used as a group name for PFOA and its salts , and PFOA is mainly produced and used as its ammonium salt, ammoniumpentadecafluorootanoate (APFO, CAS Number: 3825-26-1). However, the perfluorooctanoate anion is the molecule of primary interest. APFO and PFOA are sometimes used interchangeably as both PFO-anion and PFOA (neutral species) exist in solution.

2.3 Uses advised against

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex I of Directive 67/548/EEC

APFO is not included in Annex I of Directive 67/548/EEC

3.2 Self classification(s)

4 ENVIRONMENTAL FATE PROPERTIES

Not relevant for this dossier

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

A summary of the toxicokinetics of APFO/PFOA is described in the OECD Draft SIDS (2006) Initial Assessment Report of APFO and PFOA and is included below:

Limited information is available concerning the pharmacokinetics of PFOA and its salts in humans. Preliminary results of a 5-year half-life study in 9 retired workers indicate that the mean serum elimination half-life of PFOA in these workers was 3.8 years (1378 days, 95% CI, 1131-1624 days) and the range was 1.5 - 9.1 years.

Metabolism and pharmacokinetic studies in non-human primates has been examined in a study of 3 male and 3 female cynomolgus monkeys administered a single i.v. dose of 10 mg/kg potassium PFOA. In male monkeys, the average serum half-life was 20.9 days. In female monkeys, the average serum half-life was 32.6 days. In addition, 4-6 male cynomolgus monkeys were administered APFO daily via oral capsule at 10 or 20 mg/kg-day for six months, and the elimination of PFOA was monitored after cessation of dosing. For the two 10 mg/kg-day recovery monkeys, serum PFOA elimination half-life was 19.5 days, and the serum PFOA elimination half-life was 20.8 days for the three 20 mg/kg-day monkeys.

Studies in adult rats have shown that the ammonium salt of PFOA (APFO) is absorbed following oral, inhalation and dermal exposure. Serum pharmacokinetic parameters and the distribution of PFOA have been examined in the tissues of adult rats following administration by gavage and by i.v. and i.p. injection. PFOA distributes primarily to the liver, serum, and kidney, and to a lesser extent, other tissues of the body. It does not partition to the lipid fraction or adipose tissue. PFOA is not metabolized and there is evidence of enterohepatic circulation of the compound. The urine is the major route of excretion of PFOA in the female rat, while the urine and feces are both main routes of excretion in male rats.

There are gender differences in the elimination of PFOA in adult rats following administration by gavage and by i.v. and i.p.injection. In female rats, following oral administration, estimates of the serum half-life were dependent on dose and ranged from approximately 2.8 to 16 hours, while in male rats estimates of the serum half-life following oral administration were independent of dose and ranged from approximately 138 to 202 hours. In female rats, elimination of PFOA appears to be biphasic with a fast phase and a slow phase. The rapid excretion of PFOA by female rats is believed to be due to active renal tubular secretion (organic acid transport system); this renal tubular secretion is believed to be hormonally controlled. Hormonal changes during pregnancy do not appear to cause a change in the rate of elimination in rats.

Several recent studies have been conducted to examine the kinetics of PFOA in the developing Sprague-Dawley rat. These studies have shown that PFOA readily crosses the placenta and is present in the breast milk of rats. The gender difference in elimination is developmentally regulated; between 4-5 weeks of age, elimination assumes the adult pattern and the gender difference becomes readily apparent. Distribution studies in the postweaning rat have shown that PFOA is distributed primarily to the serum, liver, and kidney.

5.2 Acute toxicity

5.2.1 Acute toxicity: oral

Table 2: Acute toxicity, oral

Species	LD50 (mg/kg)	Observations and Remarks	Ref.

CD rats (5/sex/ group)	680 (male) 430 (female)	Vehicle: Acetone (40%), corn oil (60%). The following doses of APFO were tested: 100, 215, 464, 1000 and 2150 mg/kg in a volume of 10 mL/kg. Animals were observed for mortality and pharmacotoxic signs during the first four hours after dosing, at 24 hours and daily thereafter for a total of 14 days. The study was not performed according to GLP..	Dean and Jessup, 1978; Griffith and Long, 1980
Sprague-Dawley rats (5/sex/ group)	> 500 (male) Between 250 and 500 (females)	APFO was tested at doses of 250 and 500 mg/kg in a volume of 10 mL/kg. Vehicle was water. Clinical observations were made at 1, 2.5 and 4 hours after treatment and each day for 14 days. GLP. Yes. The study was performed according to OECD test guidelines.	Glaza, 1997
Sherman-Wistar rats (5/sex/ Group)	< 1000 (male and female)	Vehicle: 50% water. The dose-level was 1000 mg/kg. 14 days observation period. GLP. No. Test substance: T-1585, identified by 3M.	Gabriel, 1976c
Rat (10/sex/ group)	470 (male) 482 (female)	Vehicle: Corn oil. No further details available. No information found on the test substance used, PFOA or APFO.	Du Pont, 1981d
Rat (5/sex/ group)	1800 (male) 600 (female)	Vehicle: Water. No further details available. No information found on the test substance used, PFOA or APFO.	Hazleton, 1997
Mouse (10 sex/group	457	Vehicle: Corn oil. No further details available. No information found on the test substance used, PFOA or APFO.	Du Pont, 1981e
Guinea Pig (10/sex/ group)	178 (male) 217 (female)	Vehicle: Corn oil. No further details available. No information found on the test substance used, PFOA or APFO.	Du Pont, 1981f
New born rats less than 2 days old	Approximately 250	No further details available. No information found on the test substance used, PFOA or APFO.	Du Pont, 1983a
Weanling and adult rats	340-580	No further details available. No information found on the test substance used, PFOA or APFO.	Du Pont, 1983a

5.2.2 Acute toxicity: inhalation

Table 3: Acute toxicity, inhalation

Species	LC50 (mg/l)	Exposure time	Observations and Remarks	Ref.

		(h/day)		
Sprague-Dawley rats 5/sex/ group	> 18.6	1 hour	No mortality was reported in male and female Sprague-Dawley rats following inhalation exposure to 18.6 mg/L APFO for one hour. (18.6 divided with 4 hours = 4.6 mg/l 4 hours). The animals were observed for abnormal signs at 15-minutes intervals during the exposure, upon removal from the chamber, hourly for 4 hours after removal from test chamber, and daily thereafter for 14 days.	Rusch, 1979; Griffith and Long, 1980
Rat (6/sex/group)	0.98	4 hours	4 hour exposure. APFO was administered to rats by inhalation (head only) as dust. The concentrations of APFO ranged from 0.38 to 5.7 mg/l. All deaths occurred within 48 hours.	Kennedy et al., 1986

5.2.3 Acute toxicity: dermal

Table 4: acute toxicity, dermal

Species	LD50 (mg/kg)	Observations and Remarks	Ref.
New Zealand White rabbits (5/sex/group)	Greater than 2000	Aqueous paste. Only one dose tested, 2000 mg/kg. No vehicle. The rabbits had their hair clipped from their backs before the appropriate amount of the test substance was applied to intact skin. The area of application was covered with a gauze patch and an occlusive dressing. After 24 hour exposure, the collars and dressings were removed. The test site was washed with tap water. Clinical observations and mortality checks were made at approximately 1, 2.5, and 4 hours after test material application and twice daily thereafter for 14 days. All animals appeared normal and exhibited body weight gains throughout the study. GLP. Yes. The test substance used was identified as T-6342.	Glaza, 1995
New Zealand White male Rabbits (5)	4300	Four groups of rabbits were treated with 1500, 3000, 5000 and 7500 mg APFO/kg bw. Dosing sites were wrapped. The contact time was 24 hours at which time the application sites were washed with water and rabbits were observed for clinical signs of response for a 14-day recovery/observation period. LD50 values were calculated from the mortality data.	Kennedy, 1985
Crl:CD Rat (5/sex/group)	7000 (male) Greater than 7500 (female)	Three groups of male and two groups of female rats were treated with 1500, 3000, 5000 and 7500 mg APFO/kg bw. Dosing sites were wrapped. The contact time was 24 hours at which time the application sites were washed with water and rats were observed for clinical signs of response for a 14-day recovery/observation period. LD50 values were calculated from the mortality data.	Kennedy, 1985

5.2.4 Acute toxicity: other routes

5.2.5 Summary and discussion of acute toxicity

Oral:

Following oral exposure APFO (in some of the studies no information regarding the test substance used was given) is considered to be of moderate acute toxic. Guinea Pigs seem to be more susceptible to the test substance than other rodents with LD50 values around 200 mg/kg in males and females. The LD50 values in male rats were reported between approximately 500 and 1000 mg/kg, and in female rats between 250 and 1000 mg/kg. New born rats appeared to be more sensitive to the test substance used than adult rats. Based on the data and Directive 67/548/EEC classification criteria a classification as harmful with Xn R22 (Harmful if swallowed) is proposed. According to CLP criteria APFO is proposed classified as Acute tox. 3 H301 since LD50 values are reported between 50 mg/kg bw < ATE ≤ 300 mg/kg which are the limit ATE values for Acute toxicity Category 3.

Inhalation:

Following inhalation exposure of APFO a LC50 of 0.98 mg/L (4 hour exposure), and a LC50 > 18.6 mg/L (1 hour exposure) was reported. Based on the data and according to Directive 67/548/EEC classification criteria APFO is considered classified as harmful with Xn R20 (Harmful by inhalation). According to CLP criteria APFO is considered classified as Acute tox. 3, H331 since LC50 values are reported between 0.5 mg/l < ATE ≤ 1.0 mg/l which are the limit ATE values for Acute toxicity Category 3.

Dermal:

Following dermal exposure APFO/PFOA (test substance not identified) LD50 values greater than 2000 mg/kg were reported in New Zealand rabbits. Following dermal exposure to APFO a LD50 value at 4300 mg/kg was reported in male New Zealand rabbits, and a LD50 value at 7000 mg/kg in male rats and a LD50 value greater than 7500 mg/kg in female rats. Based on the data and Directive 67/548/EEC classification criteria no classification for acute toxicity following dermal exposure is proposed. According to CLP criteria APFO is not proposed classified for acute toxicity following dermal exposure since the LD50 values were higher than 2000 mg/kg.

5.3 Irritation

5.3.1 Skin

Table 5: Irritation, skin

Species	No. of animals	Exposure time (h/day)	Conc.	Dressing: occlusive semi-occlusive open	Observations and remarks	Ref.
Rabbit, female	3/ exposure period	3 minutes, 1 and 4 hours	0.5 gram	occluded	APFO produced irreversible tissue damage following a 3-minute, 1- and 4-hour contact period. Moderate erythema and edema, as well as chemical burn, eschar, and necrosis were produced following all three contact periods. Inadequate information was presented in the report to evaluate the quality of the study and validity of the conclusions.	Markoe, 1983
Rabbit	6	24 hours	0.5 gram	occluded	APFO as powder was applied to dry and moistened abraded skin. No information regarding washing of the test site was given. The skin test sites were scored according to the Draize method after 24 hours and 48 hours. No irritation was observed. The primary skin irritation score was 0.	Griffith and Long, 1980
Rabbit (male)	6	24 hours	0.5 gram	occluded	APFO was applied to shaved intact skin as an aqueous paste for 24 hours. Observation for dermal irritation was performed after removal of patches and after 24 hours (48 hours after dose application). APFO caused mild erythema (color deep pink) in 3 rabbits and moderate erythema	Kennedy, 1985; Hazleton, 1990

					(redness deepened, dose-site outline sharp) in 3 rabbits. Of 6 rabbits 4 had evidence of oedema (1 mild and 3 slight) at 24 hours. At 48 hours the reactions were still present although the degree and number of affected animals were reduced (erythema - 2 moderate, 3 mild and 1 slight; oedema – 1 mild, 2 slight and 3 not present).	

5.3.2 Eye

Table 6: Irritation, eye

Species	No. of animals	Exposure time (h/day)	Conc.	Observations and remarks	Ref.
Rabbit	6, single dose		0.1 gram	The eyes were examined 1,24, 48 and 72 hours and 5 and 7 days after installation. Installation of APFO caused moderate corneal opacity, iritis, and conjunctivitis. The effect was most pronounced at the one hour reading (mean score 14, highest possible score 110). Scoring was made by the method: Illustrated Guide for Grading Eye Irritation by Hazardous Substances. Corneal opacity and area = 4 Iris = 2 Conjunctival redness = 2 Conjunctival chemosis = 4 Conjunctival discharge = 3 The irritation was persistent but by day 7 the mean score was 2. A subsequent wash out study with 6 albino rabbits was performed. After installation of 0.1 g APFO the eyes of 3 rabbits were washed with 200 ml water after 5 seconds and the 3 other rabbits were washed similarly after 30 seconds. The eyes were examined and scored the same way as the eyes that were not washed. In the wash-out study the ocular effects were limited to conjunctival irritation. Those eyes washed after 5 seconds had a maximum score of 5.3 noted at 72 hours and after 5 and 7 days. The mild conjunctival effects were immediate and persistent.	Griffith and Long, 1980
Rat	6/sex/group	4 hours	0.81 mg/L	In rats exposed to APFO particulate (0.81 mg/L) during a 4 hours inhalation period (head only) exhibited corneal opacity and ulceration, which was microscopically evident 42 days post-exposure.	Kennedy et al., 1986

5.3.3 Respiratory tract

No data available.

5.3.4 Summary and discussion of irritation

Skin irritation:

APFO caused moderate skin irritation in two studies, however, inadequate information was given regarding the quality of the studies. In one study where the skin irritation was scored according to the Draize method, the primary irritation scores were zero. Due to the equivocal results from the studies and limited information available from some of these studies it is difficult to drawn conclusion regarding the classification of PFOA for skin irritation.

Eye irritation:

APFO caused eye irritation in two studies. The effects on eye irritation was on the borderline between Xi; R41 and Xi; R36. However, this effect was discussed in the former TC C&L group and concluded on a classification according to Directive 67/548/EEC with Xi; R36 . We therefore propose the classification already agreed by the former TC C&L group. According to CLP criteria APFO is proposed classified as Eye irrit. 2, H319.

5.4 Corrosivity

No data available.

5.5 Sensitisation

5.5.1 Skin

Table 7: Sensitisation, skin

Species	Type of test	No. of animals	Incidence of reactions observed	Ref.
Guinea pigs	Buhler test	No data.	In a dermal sensitization test (Buhler test) PFOA/APFO was shown to be negative (no clear information was given regarding the identity of the test substance).	Moore, 2001

5.5.2 Respiratory system

No data available.

5.5.3 Summary and discussion of sensitisation

Based on the insufficient data and according to Directive 67/548/EEC classification criteria and CLP criteria no classification for skin sensitization is proposed.

5.6 Repeated dose toxicity

5.6.1 Repeated dose toxicity: oral

Table 8: Repeated dose toxicity, oral

Species	Dose mg/kg/day bw, mg/kg diet, ppm	Duration of treatment	Observations and Remarks	Ref.
ChR-CD mice (5/sex/group)	0, 30, 100, 300, 1000, 3000, 10 000 and 30 0000 ppm APFO, corresponding to approximately 1.5 to 1500 mg/kg bw/day	28 days	All animals in the 1000 ppm group and higher died before the end of day 9. All animals in the 300 ppm group died within 26 days except one male. One animal in each of the 30 and 100 ppm groups died prematurely. Clinical signs were reported in mice exposed to 100 ppm and higher. There was a statistically significant dose-related reduction in mean body weight in all treated groups from 30 ppm. Relative and absolute liver weights were statistically significantly increased in mice fed 30 ppm and more.	Christopher and Marisa, 1977; Griffith and Long, 1980

			Treatment related changes were reported in the livers among all treated animals including enlargement and/or discoloration of 1 or more liver lobes. Histopathologic examination of all surviving treated mice revealed diffuse cytoplasmic enlargement of hepatocytes throughout the liver accompanied by focal to multifocal cytoplasmic lipid vacuoles of variable size which were random in distribution from 30 ppm. The LOAEl was 30 ppm based on hepatocellular hypertrophy, hepatocellular degeneration and/or necrosis; cytoplasmic vacuoles; increased absolute and relative liver weight; body weight loss.	
ChR-CD rats (5/sex/group)	0, 30, 100, 300, 1000, 3000, 10 000 and 30 000 ppm APFO corresponding to approximately 1.5 to 1500 mg/kg bw/day	28 days	All animals in the 10 000 and 30 000 ppm groups died before the end of the fist week. There were no premature deaths or unusual behaviour reactions in the other groups. Body weight gain was reduced as the dose increased. The reduction in body weight gain was statistically significant for males from 1000 ppm and females from 3000 ppm. Absolute liver weights were increased in males from 30 ppm and in females from 300 ppm. Treatment-related morphological changes were reported in the livers of all test animals. These lesions consisted of focal to multifocal cytoplasmic enlargement (hypertrophy) of hepatocytes in animals in the control, 30 and 100 mg/kg bw/day dose groups, and multifocal to diffuse enlargement of hepatocytes among animals exposed to 300, 1000 and 3000 ppm APFO The severity and degree of tissue involvement were more pronounced in males than in females. LOAEL 30 ppm based on increased liver weight and hepatocyte hypertrophy.	Metrick and Marisa, 1977; Griffith and Long, 1980
ChR-CD rats (5/sex/group)	0, 10, 30, 100, 300 and 1000 ppm APFO corresponding to 0, 0.056, 1.72, 5.64, 17.9 and 63.5 mg/kg bw/day in males and 0, 0.74, 2.3, 7.7, 22.36, 76.47 mg/kg bw/day in females	90 days	One female in the 100 and 300 ppm group died, however, this was not considered to be treatment related. No treatment-related changes in behaviour or appearance were reported. In males a statistically significant decrease in body weight was reported at 1000 ppm. The relative kidney weights were significantly increased in males from 100 ppm. However, absolute kidney weights were comparable among groups, and there were no histopathological lesions. Absolute liver weights were significantly increased in males from 30 ppm and in females at 1000 ppm. Relative liver weights were significantly increased in males from 300 ppm and in females at 1000 ppm. Hepatocellular hypertrophy (focal to multifocal in the centrilobular to midzonal regions) was reported in 4/5, 5/5 and 5/5 males in the 100, 300 and 1000 ppm groups, respectively. Hepatocyte necrosis was reported in 2/5, 2/5, 1/5 and 2/5 males in the 30, 100, 300 and 1000 ppm groups, respectively.	Goldenthal, 1978a; Griffith and Long, 1980
ChR-CD male rats (45-55 per group)	0, 1, 10, 30 and 100 ppm APFO corresponding to 0, 0.06, 0.64, 1.94 and 6.50 mg/kg bw/day. Two control groups (a non-pair fed	13 weeks. 15 animals per group were sacrificed following 4, 7 and 13 weeks of	When analysing the data, animals exposed to 1, 10, 30 and 100 ppm were compared to the control animals in the non-pair fed group, while data from the pair-fed control group were compared to animals exposed to 100 ppm. No treatment clinical signs were reported. At 100 ppm a significant reduction in bw was reported compared to the pair-fed control group during week 1 and the non pair-fed control group during weeks 1-13. Bw data in the other dosed-groups were comparable to controls. At 100 ppm mean body weight gains were	Palazzolo, 1993

	group and a pair-fed group to the 100 ppm dose group). Following 13 weeks exposure, 10 rats/group were fed control diet for a 8-week recovery period	treatment. 10 animals per group were sacrificed after 13 weeks of treatment and after a 8 weeks recovery period.	significantly higher than the pair-fed control group during week 1 and significantly lower than the non pair-fed control group during weeks 1-13. At 10 and 30 ppm, mean body weight gains were significantly lower than the non-pair-fed control group at week 2. These differences in body weight and body weight gains were not reported during the recovery period. A significant increase in absolute and relative liver weights and hepatocellular hypertrophy were reported at weeks 4, 7 and 13 in the 10, 30 and 100 ppm groups. There was no evidence of any degenerative changes or abnormalities associated with the hypertrophy. Hepatic palmitoyl CoA oxidase activity (indicating peroxisome proliferation) was significantly increased at weeks 4, 7, and 13 in the 30 and 100 ppm groups. At 10 ppm, hepatic palmitoyl CoA oxidase activity was significantly increased at week 4 only. During the recovery period none of the liver effects were reported, indicating that these treatment-related liver effects were reversible.	
Rhesus monkeys (2/sex/group)	0, 3, 10, 30 and 100 mg APFO/kg ba/day by gavage.	90 days	All monkeys in the 100 mg/kg bw/day, and 3 monkeys in the 30 mg/kg bw/day group died during the study. Clinical signs (anorexia, pale and swollen face, black stools, marked diarrhoea) were reported in the 3 and 10 mg/kg bw/day. No changes in bw at 3 and 10 mg/kg bw/day, however, significant reduction in bw in the one male left in the 30 mg/kg bw/day group. Absolute and relative organ weight changes were reported in the heart (from 10 mg/kg bw/day in females, brain (from 10 mg/kg bw/day in females) and pituitary (from 3 mg/kg bw/day in males), however, no morphological changes were reported in the organs. The male from the 30 mg/kg bw/day group that survived had slight to moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen. No treatment related lesions were reported in the organs of animals in the 3 and 10 mg/kg bw/day dose groups.	Goldenthal, 1978b; Griffith and Long, 1980
Cynomolgus male monkeys (4-6 animals/group)	0 (6), 3 (4), 10 (6) and 30 (6) mg/kg bw/day APFO by oral capsule.	26 weeks	Dosing of animals in the 30 mg/kg bw/day group was stopped on day 11-21 due to severe toxicity. From day 22 these animals received 20 mg/kg bw/day, and this group was called the 30/20 mg/kg bw/day dose group. At the end of the 26 weeks treatment period, 2 animals in the control group and 10 mg/kg bw/day groups were observed for a 13-week recovery period. One male from the 30/20 and 3 mg/kg bw/day dose groups were sacrificed in moribund conditions during the study. The cause of the deaths was not determined, but APFO treatment could not be excluded. Of the 5 remaining animals in the highest dose group only 2 animals tolerated this dose level for the rest of the study. In 3 animals from the highest dose group the treatment was halted on day 43, 66 and 81, respectively. Clinical signs in these animals included low or no food consumption and weight loss. The animals appeared to recover from compound-related effects within 3 weeks after cessation of treatment. At terminal sacrifice at 26 weeks a significant increase in mean absolute liver weights and liver-to-body weight percentages in all dose groups, considered to be	Thomford, 2001b; Butenhoff et al., 2002

			treatment-related, and due, in part to hepatocellular hypertrophy. However, there was no evidence of peroxisome proliferators-activated receptor alpha activity (PPARα). At recovery sacrifice, no treatment-related effects on terminal body weights or on absolute or relative organ weight were reported, indicating that these effects were reversible over time.	

5.6.2 Repeated dose toxicity: inhalation

Table 9: Repeated dose toxicity, inhalation

Species	**Conc.** **mg/l or mg/m^3**	**Exposure** **Time (h/day)**	**Duration of treatment**	**Observations and remarks**	**Ref.**
Crl:CD rats 24 males	0, 1, 8, 84 mg/m^3 APFO (head only exposure)	6 h/day	5 days per week, for 2 weeks followed by 28 – 84-day recovery	Mortality (2) was reported in the highest dose group. One rat was killed after the third day of exposure due to severe weight loss, respiratory distress and lethargy. The other rat died during the fourth exposure. A statistically significant reduction in body weight was reported on test day 5 that recovered by day 16. A statistically significant increase in absolute and relative liver weight and serum alkaline phosphatase was reported from 8 mg/m^3 that persisted through 28 days of recovery. Hepatocellular atrophy, and necrosis was reported from 8 mg/m^3. These included panlobular and centrilobular hepatocellular hypertrophy and necrosis. Panlobular hepatocellular hypertrophy was reported only in rats killed immediately after the last exposure; the affected livers contained entire lobules with uniformly enlarged hepatocytes. This change was limited to the centrilobular hepatocytes following a 14- or 28-day recovery period and was absent after either 42 or 84 days. Focal or multi-focal hepatocellular necrosis was seen in 2/5 rats from the high-dose group (one killed on day 0 and one of day 14 of recovery), in 3/5 rats from the mid-dose group (one each on day 0, 42 and 84 of recovery), and in 1/5 control rats (on recovery day 28). (Five rats from each group were given a complete histopathologic examination). The authors of the study considered the hepatocellular necrosis to be treatment related since hepatocellular necrosis rarely is encountered as a spontaneous lesion	Kennedy et al., 1986

				in young male rats.	

5.6.3 Repeated dose toxicity: dermal

Table 10: Repeated dose toxicity, dermal

Species	Dose mg/kg/day	Exposure time (h/day)	Duration of treatment	Observations and remarks	Ref.
Crl:CD Rat (15 males)	20-2000 mg/kg APFO, 10 applications dermal and 84 days recovery.	6 hours/day	2 weeks, 5 days/week	Skin irritation and reversible reduction in bw at doses from 200 mg/kg. Increased liver weight, increased AST and ALT, as well as hepatocellular hypertrophy and necrosis from 20 mg/kg. Affected livers contained one or more foci of coagulative necrosis. The Kupffer cells within the foci of hepatocellular necrosis contained large vesicular nuclei and were markedly increased in number. Inflammatory cells were occasionally present within and at the periphery of the necrotizing lesions. All of the treatment-related toxicity findings resolved during a 42-day recovery period.	Kennedy, 1985
Rabbit (10 males/ females)	100 mg/kg, 10 applications dermal and 14 days recovery.	6 hours/day	2 weeks, 5 days/week	Reversible reduction in body weight. The only information regarding the identity of the test substance was T-2618.	Riker, 1981

5.6.4 Other relevant information

5.6.5 Summary and discussion of repeated dose toxicity:

Oral:

Increased mortality and liver toxicity in mice, rats and monkeys following exposure to APFO was reported. Hepatocellular hypertrophy, degeneration and/or focal to multifocal necrosis were reported with increases in severity between doses of 1.5 to 15 mg/kg bw/day in rats and mice. The effects on repeated dose toxicity following oral exposure was on the borderline between Xn; R48/22 and T; R48/25. However, this effect was discussed in the former TC C&L group and concluded on a classification according to Directive 67/548/EEC with Xn; R48/22 . We therefore propose the classification already agreed by the former TC C&L group. According to CLP criteria APFO is proposed classified as STOT RE 2, H373 since the guidance value for STOT RE 2 oral exposure is $10 < C \leq 100$ mg/kg bw/day.

Inhalation:

Based on the increased mortality and severe liver toxicity in rats following exposure to APFO at doses from 0.008 mg/litre a classification according to Directive 67/548/EEC criteria with T; R 48/23 is proposed. According to CLP criteria APFO is considered classified as STOT RE 1, H372 since the guidance value for STOT RE 1 inhalation exposure is C ≤ 0.02 mg/litre.

Dermal:

Based on the limited data available on repeated dose toxicity following dermal exposure to APFO, 2 week study with 84 days recovery period in rats, no clear conclusion can be drawn regarding a classification for repeated dermal exposure to APFO. This effect was discussed in the former TC C&L group and concluded no classification of APFO for repeated dose toxicity following dermal exposure.

5.7 Mutagenicity

5.7.1 In vitro data

Table 11: Mutagenicity, in vitro data

Test	Species	Conc. (mg/l)	Metabolic activ.	Observations and Remarks	Ref.
Bacterial reverse mutation assay	*Salmonella Typhimurium* (TA 1535, TA 1537, TA 1538 and TA 100) and *S. cerevicia* D4 yeast	No data.	+/-	APFO did not induce mutations +/- metabolic activation in *Salmonella Typhimurium* and in *S. Cervicia*.	Litton, 1978
Bacterial reverse mutation assay	*Salmonella Typhimurium* (TA 1535, TA 1537, TA 98 and TA 100) and *E. coli* (WP2uvrA)	No data.	+/-	The ammonium salt of PFOA (APFO) was tested twice in *Salmonella Typhimurium* and *E. Coli*. One positive response was seen at one dose level with *Salmonella* Typhimurium TA 1537 when tested without metabolic activation, however, the response was not reproducible. It was concluded that *Salmonella Typhimurium* and *E. coli* did not induce mutations +/- metabolic activation.	Lawlor, 1995; 1996
Chromosomal aberrations (CA)	Human lymphocytes	Range finding assay: 0.167 to 5000 µg/mL. Confirmatory trial: 62.5 to 3000 µg/mL.	+/-	APFO did not induce CA in human lymphocytes up to cytotoxic concentrations when tested with and without metabolic activation. The test was performed according to GLP.	Murli, 1996c; NOTOX, 2000
Chromosomal	Chinese Hamster	Range	+/-	APFO was tested twice for CA in CHO	Murli,

aberrations (CA)	Ovary (CHO) cells	finding assay: 0.169 to 5080 µg/mL. Initial study: 62.5 to 4000 µg/mL. Confirmatory study: 50 to 3000 µg/mL.		cells. In the first assay APFO induced both CA and polyploidy when tested +/- metabolic activation at toxic concentrations. In the second assay no significant increase in CA were reported without metabolic activation, however with metabolic activation a significant increase in CA and polyploidy was reported at highly toxic concentrations. The test was performed according to GLP.	1996b; 1996d
Gene mutations	K-1 line of Chinese hamster ovary (CHO) cells	No data	+/-	APFO did not induce gene mutation when tested with and without metabolic activation.	Sadhu, 2002
Cell transformation and cytotoxicity assay	C_3H $10R_{1/2}$ mouse embryo fibroblasts	0.1, 1.0, 10, 50, 100 and 200 µg/mL.	None.	The cell transformation was determined as both colony transformation and foci transformation potential. In this assay no evidence of transformation was reported following exposure to APFO with both the colony or foci method. Cytotoxic concentration (LD50) was 50 µg/mL. GLP. No.	Garry and Nelson, 1981

5.7.2 In vivo data

Table 12: Mutagenicity, in vivo data

Test	Species	Conc. (mg/l)	Metabolic activ.	Observations and Remarks	Ref.
Micronucleus assay	Mouse 5/sex	1250, 2500 and 5000 mg/kg	-	The bone marrow was evaluated after 24, 48 and 72 h, The test with APFO was negative. The test was performed according to GLP.	Hazleton, 1995b
Micronucleus assay	Mouse 5/sex	500, 1000 and 2000 mg/kg	-	APFO was tested twice in the mouse micronucleus assay, and APFO did not induce and significant increase in micronuclei when evaluated after 24, 48 and 72 h, and the test was considered negative. The test was performed according to GLP.	Murli, 1996a; Hazleton, 1996e

5.7.3 Human data

5.7.4 Other relevant information

5.7.5 Summary and discussion of mutagenicity

Based on the available *in vitro* and *in vivo* studies APFO is considered not mutagenic, and no classification according to Directive 67/548/EEC criteria or CLP criteria for mutagenicity is proposed.

5.8 Carcinogenicity

5.8.1 Carcinogenicity: oral

Table 13: Carcinogenicity, oral

Species	Dose (mg/kg bw/day)	Duration of treatment	Observations and remarks	Ref.
Sprague-Dawley rats 50/sex/group. Groups of 15 additionally rats/sex were fed 0 or 300 ppm and evaluated after 1 year	0, 30 or 300 ppm APFO in the diet corresponding to 1.3 and 14.2 mg/kg/day in males and 1.6 and 16.1 mg/kg/day in females	2 years	A dose-related decrease in bw in males, and to a lesser extent in females was reported, and the decrease was considered treatment-related. There were no differences in mortality between treated and untreated groups. Histologic evaluation showed lesions in the liver, testis and ovary. **Liver;** At the 1-year sacrifice a diffuse hepatomegalocytosis (12/15) portal mononuclear cell infiltration (13/15) and hepatocellular necrosis (6/15) were reported in the high-dosed males, whereas the incidences in the control group were 0/15, 7/15 and 0/15, respectively. At 2-year sacrifice megalocytosis was found at an incidence of 0%, 12% and 80% in the males, and at 0%, 2% and 16% in the females, in the controls, low- and high dose groups, respectively. Hepatic cystoid degeneration was reported in 14% and 56% of the low and high dose males, as compared to 8% in controls. The incidence of hyperplastic nodules was slightly increased in the high-dosed males, 6%, as compared to 0% in controls. **Testis**; At 1-year sacrifice, testicular masses were found in 6/15 high dosed and 1/15 low-dosed rats compared to 0/15 in controls. Furthermore, marked aspermatogenesis was found in 2/15 in high-dosed males but not in the controls. At the 2-year sacrifice, vascular mineralization was reported in 18% of high-dosed males and 6% in low-dosed males, however, not in control males. The testicular effects reached statistically significance in the high-dose group. Furthermore, at 2-year sacrifice a significant increase in the incidence of	Sibinski, 1987;

			testicular Leydig cell (LCT) adenomas in the high-dosed group was reported [0/50 (0%), 2/50 (4%) and 7/50 (14%)] in control, low- and high dose group, respectively). The historical control incidence was 0.82% (from 1 340 Sprague-Dawley rats used in 17 carcinogenicity studies (Chandra et al., 1992). The spontaneous incidence of LCT in 2-year old Sprague-Dawley rats is reported to be approximately 5% (Clegg et al., 1997). **Ovary;** In females at 2-year sacrifice a dose-related increase in the incidence of ovarian tubular hyperplasia was reported, 0%, 14% and 32% in control, low-, and high dose groups, respectively. However, recently the slides of the ovaries were re-evaluated, and more recently nomenclature was used (Mann and Frame, 2004). The ovarian lesions were diagnosed and graded as gonadal stromal hyperplasia and/or adenomas, which corresponded to the diagnoses of tubular hyperplasia or tubular adenoma by the original study pathologist. Whit this evaluation no statistically significant increase in hyperplasia (8, 16 and 15 in the control, 30 ppm and 300 ppm group, respectively), adenomas (4, 0 and 2 in the control, 30 ppm and 300 ppm group, respectively or hyperplasia/adenoma combined (12, 16 and 17 in the control, 30 ppm and 300 ppm groups, respectively) were seen in treated groups compared to controls. There was also a significant increase (P<0.05) in the incidence of mammary fibroadenomas [10/47 (21%), 19/47 (40%) and 21/49 (43%) in controls, 30 and 300 ppm groups, respectively]. The historical control incidence was 19% observed in 1329 Sprague-Dawley rats used in 17 carcinogenicity studies (Chandra et al., 1992). However, the compared to other historical control data at 24% from a study of 181 female rats terminally sacrificed at 18 month (which was considered an inappropriate historical reference), and the historical control incidence of 37% in 947 female rats in the Haskell laboratory (Sykes, 1987), the evidence of mammary fibroadenomas were considered equivocal.	
Sprague-Dawley male rats, 156 rats in the treatment group and 80 rats in the control group	300 ppm APFO	2 years	This study was performed to confirm the induction of LCT, reported in the study by Sibinski, 1987. A significant increase in the incidence of LCT in treated rats (8/76, 11%) compared to controls 0/80 (0%) was reported. The tumours may be a result of endocrine changes, because a reduced aromatase activity and a sustained increase in serum estradiol were reported. In addition, the treated group had a significant increase	Cook et al., 1994; Biegel et al., 2001

			in the incidence of liver adenomas (2/80 and 10/76 in the control and 300 ppm group, respectively) and pancreatic acinar cell tumours (PACT) (0/80 and 7/76 in the control and 300 ppm group, respectively). There was one pancreatic acinar cell carcinoma in the treated group and none in the control group. Biegel et al., 2001 also studied the temporal relationship between relative liver weights, hepatic β-oxidation, and hepatic cell proliferation and hepatic adenomas following exposure for 1, 3, 6, 9, 12, 15, 18, 21 and 14 months. Relative liver weights and hepatic β-oxidation were increased at all time-points. The liver end-points (weight, β-oxidation, and cell proliferation) were all elevated well before the first occurrence of liver adenomas, which occurred after 12 month of treatment. In the study by Sibinski, 1987, no increase in the incidence of PACT was reported (0/33, 2/34 and 1/34 in the control, 30 and 300 ppm groups, respectively). Therefore, the histological slides from both studies were reviewed by an independent pathologist. This review indicated that PFOA produced increased incidences of proliferative acinar cell lesions in the pancreas in both studies at 300 ppm. The differences reported were quantitative rather than qualitative; more and larger focal proliferative acinar cell lesions and greater tendency for progression of lesions to adenoma of the pancreas were reported in the second study. It was concluded that the difference between pancreatic acinar hyperplasia (reported in Sibinski, 1987) and adenoma (reported in Cook et al., 1994; Biegel et al., 2001) in the rat was a reflection of arbitrary diagnostic criteria and nomenclature by the different pathologists.	

5.8.2 Carcinogenicity: inhalation

5.8.3 Carcinogenicity: dermal

5.8.4 Carcinogenicity: human data

5.8.5 Other relevant information

5.8.6 Summary and discussion of carcinogenicity

In the two carcinogenicity studies APFO induced liver adenomas, Leydig cell adenomas, and pancreatic acinar cell tumours in male Sprague-Dawley rats, and mammary fibroadenomas in the female rats.

The mammary fibroadenomas were originally considered equivocal since the incidences were comparable to some historical control data from another laboratory. However, as the Sprague-Dawley rats, represent an outbreed rat strain, the frequencies of spontaneous tumours will vary considerably from laboratory to laboratory. Thus, it is inappropriate to use historical control data from other laboratories. The most appropriate control group is the concurrent control group. The mammary gland findings in the Sibinski (1987) study were re-examined by a Pathology Working Group (Hardisty, 2005) The Pathology Working Group concluded that there were no statistically significant differences in the incidence of fibroadenoma, adenocarcinoma, total benign neoplasms or total malignant neoplasms of the mammary glands between control and treated animals. There was also no significant difference in combined benign and malignant neoplasms between control and treated groups. The primary difference between the original reported findings and the Pathology Working Group evaluation involved findings initially reported as lobular hyperplasia which the working group classified as fibroadenoma resulting in incidences of mammary fibroadenoma in the control, low- and high-dose groups of 32%, 32%, and 40%, respectively.

Regarding liver carcinogenicity, there is evidence to indicate that APFO is a PPARα agonist and that the liver carcinogenicity (and toxicity) of APFO is mediated by binding to the PPARα in the liver in rodents. It has been well documented that APFO is a potent peroxisome proliferator, inducing peroxisome proliferation in the liver of mice and rats (Ikeda et al., 1985; Pastoor et al., 1987; Sohlenius et al., 1992). Due to uncertainties and limitation of the data it can, however, not be concluded that PPARα agonism is the sole mode of action for the rat liver tumour induction. Thus, in contrast to what would be predicted, administration of APFO, but not the prototype PPARα agonist WY-14,643, increased liver weights in PPARα receptor knockout mice, i.e. in mice where PPARα activation was precluded, raising the possibility that the APFO-induced liver tumours could occur by PPARα independent effects (Yang et al., 2002). Moreover, there is as yet no published evidence that the induction of PPARα by APFO results in clonal expansion of pre-neoplastic foci which is considered a critical step in the proposed mode of action, also there are no data demonstrating increased cell proliferation and/or decreased apoptosis in the liver of APFO-treated rats. In addition, the available data for children have not been adequately characterized to be able to conclude that the PPARα mode of action is not operative in this young age group.

The modes of carcinogenic action of APFO induced Leydig cell adenomas and pancreatic acinar cell tumours have not been fully elucidated. There is insufficient evidence to link these tumours to

PPARα. The induction of Leydig cell adenomas may involve a hormonal mechanism whereby APFO either inhibits testosterone biosynthesis and/or increases serum estradiol via induction of hepatic aromatase activity. The induction of pancreatic acinar cell tumours are probably related to an increase in serum level of the growth factor, CCK (cholecystokinin-33 [human], cholecystokinin [rat]), that appears to be secondary to changes in the liver. At the Specialised Experts meeting January 22-23, 2004 it was concluded that non-genotoxic chemicals causing Leydig cell tumours in rats by perturbating the HPT axis should be classified in Carc.Cat 3 according to Directive 67/548/EEC, (this should be the classification in the absence of additional carcinogenicity data) unless the mechanism of perturbation of the axis can be proven not to relevant for human Leydig cell carcinogenesis.

To conclude, the rat liver tumours cannot be disregarded as not relevant for humans although PPARα agonism is involved in the induction of liver toxicity. Because available data are insufficient to characterize the mode of action for APFO-induced Leydig cell adenomas and pancreatic acinar cell tumours, the responses at these sites are presumed to be relevant to humans. Consequently, it is proposed that APFO should be classified according to Directive 67/548/EEC criteria with Carc. Cat. 3; R40 and according to CLP criteria APFO is proposed classified as Carc 2 H351.

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

Table 14: Reproduction, effects on fertility

Species	Route	Dose	Number of generations exposed	Observations and Remarks	Ref.
Sprague-Dawley rats (30 rats/group)	Oral by gavage	0, 1, 3, 10 and 30 mg/kg/ Day APFO	2 generations	***F0 males***: In the highest dose group one male was sacrificed on study day 45 due to adverse clinical signs. No treatment-related effects were reported at any dose level for any of the mating and fertility parameters assessed. At necropsy a statistically significant reduction in terminal body weight was reported from 3 mg/kg/day (6%, 11%, and 25% decrease from controls in the 3, 10 and 30 mg/kg/day, respectively. Absolute weights of the left and right epididymis, left cauda epididymis, seminal vesicles, prostate, pituitary, left and right adrenals and thymus were statistically significantly reduced at 30 mg/kg /day, however, the organ-to- body weight ratios were either normal or increased. The absolute weight of the liver was significantly increased in all dose groups, and the absolute weights of the kidneys were significantly increased at 1, 3 and 10 mg/kg/day, and significantly deceased at 30 mg/kg/day. Organ weight-to-body weight ratios for the liver and kidneys were significantly increased in all treated groups. No histopathology was performed on the liver and kidney. Dose-related histopathologic changes were reported in the adrenals. No treatment-related effects were reported at necropsy	York, 2002; Butenh off et al., 2004)

				on the reproductive organs, with the exception of increased thickness and prominence of the zona glomerulosa and vacuolisation of the cells of the adrenal cortex in 2/10 males and 7/10 males in the 10 and 30 mg/kg/day dose group. The LOAEL was 1 mg/kg/day based on increased absolute and relative liver weight. ***F0 females****:* No treatment-related effects were reported on oestrus cyclisity, mating or fertility parameters. No treatment-related effects on body weights or organ weights. The NOAEL was 30 mg/kg/day. ***F1 generation****:* At 30 mg/kg/day one pup died on Lactation Day (LD) 1. Additionally, on LD 6 and 8 a significant increase in the numbers of pups found dead were reported at 3 and 30 mg/kg/day. Pup body weight on a per litter basis was significantly reduced up to lactation day 15 in the high dose group (LD 1; 5.5 vs 6.3 in controls, LD 8; 11.9 vs. 13.3 in controls, and LD 15; 22.9 vs. 25.0 in controls). Of the pups necropsied at weaning no absolute or relative organ weight changes were reported. ***F1 males:*** A significant increase in treatment-related deaths (5/60 rats) was reported in the high dose group between day 2-4 post-weaning. Significant increases in clinical signs of toxicity were also reported during most of the post-weaning period at all dose levels. A significant dose-related reduction in mean body weight gain for the entire dosing period (days 1-113). Absolute food consumption was significantly reduced from 10 mg/kg/day during the entire pre-cohabitation period (days 1-70 post-weanling), while relative food consumption values were significantly increased. Significant delays in sexual maturation (the average of preputial separation) were reported at 30 mg/kg/day (52.2 days of age vs. 48.5 days of age in controls). When the body weight was co-varied with the time to sexual maturation, the time to sexual maturation showed a dose-related delay that was statistically significant at $p \leq 0.05$. No treatment-related effects were reported at any dose level for any of the mating and fertility parameters assessed. Necroscopic examination revealed significant effects on the liver and kidney from 3 mg/kg/day. Terminal body weight was significantly dose-related decreased from 1 mg/kg/day (6%, 6%, 11%, and 22% decreased from controls at 1, 3, 10 and 30 mg/kg/day, respectively. The absolute and relative liver weights were significantly increased in all treated groups and were accompanied by histopathological changes. All other organ weight changes reported (thymus, spleen, left adrenal, brain, prostate, seminal vesicles, testes and epididymis) were probably due to body weight reductions, since the relative weights of these organs were either normal or increased. However, the biological significance of the weight changes observed in the adrenal is unclear since	

				histopathological changes were also reported. The NOAEL developmental effects were 3 mg/kg/day and the LOAEL for F1 adult effects was 1 mg/kg/day. ***F1 females**:*: A significant increase in treatment-related deaths (6/60 rats) was reported in the high dose group between day 2-8 post-weaning. Significantly decrease in body weights were reported in the high dose group during post-weaning, pre-cohabitation, gestation and lactation Body weight gain was significantly reduced during day 1-15 pos-weanling. Decreased absolute food consumption was reported during days 1-22 post-weaning, pre-cohabitation, gestation and lactation in the highest dose group. Relative food consumption values were comparable across all treated groups. Significant delays in sexual maturation (the average of vaginal patency) were reported at 30 mg/kg/day (36.6 days of age vs. 34.9 days of age in controls). When the body weight was co-varied with the time to sexual maturation, the time to sexual maturation showed a dose-related delay that was statistically significant at $p \leq 0.05$. No treatment-related effects were reported at any dose level for any of the mating and fertility parameters assessed. All natural delivery observations were unaffected by treatment at any dose level. No effect on terminal body weights was reported. The absolute weight of the pituitary, the pituitary weight-to-terminal body weight ratio and the pituitary weight-to-brain ration was significantly decreased from 3 mg/kg/day. No histopathologic changes were reported in the pituitary. The NOAEL developmental effects were 10 mg/kg/day and the NOAEL for F1 adult effects was 10 mg/kg/day. ***F2 generation:*** No treatment related adverse clinical signs were reported. Dead or stillborn pups were noted in both the control and treated groups. The deaths occurred on lactation day 1-8 with the majority occurring on days 1-6, however, there was no dose-relationship. No effect on body weights or organ weights, as well as AGD was reported. The NOAEL was set at 30 mg/kg/day.	

5.9.2 Developmental toxicity

Table 15: Reproduction, developmental toxicity

Species	Route	*Dose mg/kg/day ppm **Conc. (mg/l)	Exposure period: - number of gene- rations or - number of days	Observations and Remarks	Ref.

			during pregnancy		
Sprague-Dawley rats (22/group)	Oral by gavage	0, 0.05, 1.5, 5 and 150 mg/kg/day APFO	Gestation day 6-15	*Maternal toxicity:* In the high dose group 3 dams died, and a significant reduction in maternal body weights on gd 9, 12 and 15 was reported. The NOAEL for maternal toxicity was 5 mg/kg/day. *Developmental toxicity:* No significant differences were found between treated and control groups. The NOAEL for developmental toxicity was 150 mg/kg/day.	Gortner, 1981
Rabbits (18 /group)	Oral by gavage	0, 1.5, 5 and 50 mg/kg/day APFO	Gestation day 6-18	*Maternal toxicity*: Six dams died during the study, however, 5 of the 6 deaths were attributed to gavage errors. Transient reduction in body weight gain on gd 6-9, however, they returned to control levels on gd 12-29. No other effects were reported.No clinical or other treatment related signs were reportedThe NOAEL for maternal toxicity was 50 mg/kg/day. *Developmental toxicity:* A dose-related increase in a skeletal variation, extra ribs or 13th rib, which reached statistically significance at 50 mg/kg/day (38%, 30%, 20% and 16% in the 50, 5, 1.5 mg/kg/day and control group, respectively). The NOAEL for developmental toxicity was 5 mg/kg/day.	Gortner, 1982
Sprague-Dawley rats (25/ group in the first trial, 12/group in the second trial)	Oral by gavage	0 and 100 mg/kg/day APFO	Gestation day 6-15. In trial 1 the dams were sacrificed on gd 21, in trial 2 the dams were allowed to litter and the pups were sacrificed on postpartum day 35.	*Trial 1 maternal toxicity*: Three dams died at 100 mg/kg/day during gestation (one on GD 11 and two on GD 12). Food consumption and body weight was reduced in treated dams compared to controls. No other effects were reported on reproductive parameters such as maintenance of pregnancy or incidence of resorptions. *Trial 1 developmental toxicity*: No effects reported. *Trial 2 maternal toxicity*: The same as in trial 1. *Trial 2 developmental toxicity*: No effects reported.	Staples et al., 1984
Sprague-Dawley rats (12/ group in trial 1 and 2)	inhalation	0, 0.1, 1, 10 and 25 mg/m^3 APFO (whole body dust inhalation), 6 hours/day	Gestation day 6-15. In trial 1 the dams were sacrificed on gd 21, in trial 2 the dams were allowed to litter and the pups were sacrificed on postpartum day 35	*Trial 1 maternal toxicity*: Treatment-related clinical signs were reported in the two highest dose groups (chromodacryorrhea, chromorhinorrhea, a general unkempt appearance, and lethargy in four dams in the high dose group only). 3 dams died in the high dose group on gd 12, 13 and 17. In the two highest dose groups a statistically significant reduction in food consumption was reported, however, no significant differences were seen between treated and pair-fed groups.Iin the highest dose group a statistically significant reduction in body weight and increase in mean liver weight was reported. The NOAEL for maternal toxicity was 1 mg/m^3. *Trial 1 developmental toxicity*: A statistically significant reduction in mean foetal body weight was reported at 25 mg/m^3 and in the control group pair-fed 25 mg/m^3. However, interpretation of the decreased foetal body weight is difficult due to mortality in dams. The NOAEL for developmental	Staples et al., 1984

				toxicity was 10 mg/m^3. *Trial 2 maternal toxicity*: Similar as to trial 1. Two dams died during treatment in the highest dose group. *Trial 2 developmental toxicity*: A statistically significant reduction in pup body weight on day 1 post partum (PP) (6.1 g at 25 mg/m^3 vs 6.8 g in controls). Days 4 and 22 PP pup body weights continued to remain lower than controls, although the difference was not statistically significant. No significant effects were reported following external examinations of the pups or with ophthalmoscopic examination of the eyes. Interpretations of the effects reported are difficult due to the incidence of maternal mortality.The NOAEL for developmental toxicity was 10 mg/m^3.	
CD-1 mice	Oral by gavage	0 (45), 1 (17), 3 (17), 5 (27), 10 (26), 20 (42) or 40 (9) mg/kg bw/day APFO (number in brackets is number of dams examined)	From gestation day 1 to 17, at gestation day 18, some dams were sacrificed for maternal and foetal examination, and the rest were allowed to give birth.	*Maternal toxicity:* Statistically significant (st sign) reduction in body weight gain in the 20 and 40 mg/kg bw/day dose groups. The maternal weight gain on GD 18 was approximately 22, 24, 28, 21, 17, 5 and minus 5 gram in the control animals, 1, 3, 5, 10, 20 and 40 mg/kg bw/day exposed groups, respectively. In addition APFO treatment led to a dose-depended st. sign. increase in liver weight from 1 mg/kg bw/day. The maternal serum level of APFO increased in a dose-dependent manner. No NOAEL for maternal toxicity could be derived. The LOAEL at 1 mg/kg bw/day is based on a st. sign. increased liver weight. *Developmental toxicity*: No changes in the number of implantations were reported. However, a st. sign. increase in the incidence of full litter resorption from 5 mg/kg bw/day (6.7, 11.8, 5.9, 25.9, 46.1, 88.1 and 100% in the 0, 1, 3, 5, 10, 20 and 40 mg/kg bw/day dose group, respectively) was reported. The number of live foetuses per litter was st. sign. reduced at 20 mg/kg bw/day. The foetal body weight was st. sign. decreased at 20 mg/kg bw/day. Reduced ossification of sternebrae, caudal vertebrae, metacarpals, metatarsals, phalanges, calvaria, supraoccipital and huoid as well as enlarged fontanel in the 10 and 20 mg/kg bw/day dose groups was reported as well. Most offspring were born alive, but the incidence of stillbirth and neonatal mortality was increased markedly, particularly in the 10 and 20 mg/kg bw/day dose groups. At 10 and 20 mg/kg bw/day most of the pups did not survive the first day of life. Postnatal survival was comparable to controls in the two lowest dose groups. Among survivors, a trend towards growth retardation was noted in the APFO-treated neonates, leading to 25-30 % lower body weights from 3 mg/kg bw/day at weanling. Corresponding to the early postnatal growth deficits, development of the mice exposed *in utero* was impaired, evident as st. sign. delays in eye opening	Lau et al., 2006

				from 5 mg/kg bw/day, by as much as 3 days. The onset of puberty of male pups was markedly advanced. The preputial separation in the 1mg/kg bw/day dose group was almost 4 days earlier than in control pups, and this accelerated pubertal malformation took place despite a body weight reduction of 25-30%. No acceleration in female pubertal onset was reported. No NOAEL for developmental effects could be determined. The LOAEL at 1 mg/kg bw/day is based on increases in the onset of sexual maturation in males.	
Sprague-Dawley rats (30 rats/group)	Oral by gavage	0, 1, 3, 10 and 30 mg/kg/ day APFO	2 generations	***F0 males***: In the highest dose group one male was sacrificed on study day 45 due to adverse clinical signs. No treatment-related effects were reported at any dose level for any of the mating and fertility parameters assessed. At necropsy a statistically significant reduction in terminal body weight was reported from 3 mg/kg/day (6%, 11%, and 25% decrease from controls in the 3, 10 and 30 mg/kg/day, respectively. Absolute weights of the left and right epididymis, left cauda epididymis, seminal vesicles, prostate, pituitary, left and right adrenals and thymus were statistically significantly reduced at 30 mg/kg (day, however, the organ-to- body weight ratios were either normal or increased. The absolute weight of the liver was significantly increased in all dose groups, and the absolute weights of the kidneys were significantly increased at 1, 3 and 10 mg/kg/day, and significantly deceased at 30 mg/kg/day. Organ weight-to-body weight ratios for the liver and kidneys were significantly increased in all treated groups. No histopathology was performed on the liver and kidney. Dose-related histopathologic changes were reported in the adrenals. No treatment-related effects were reported at necropsy on the reproductive organs, with the exception of increased thickness and prominence of the zona glomerulosa and vacuolisation of the cells of the adrenal cortex in 2/10 males and 7/10 males in the 10 and 30 mg/kg/day dose group. The LOAEL was 1 mg/kg/day based on increased absolute and relative liver weight. ***F0 females****:* No treatment-related effects were reported on oestrus cyclisity, mating or fertility parameters. No treatment-related effects on body weights or organ weights. The NOAEL was 30 mg/kg/day. ***F1 generation****:* At 30 mg/kg/day one pup died on Lactation Day (LD) 1. Additionally, on LD 6 and 8 a significant increase in the numbers of pups found dead were reported at 3 and 30 mg/kg/day. Pup body weight on a per litter basis was significantly reduced up to lactation day 15 in the high dose group (LD 1; 5.5 vs 6.3 in controls, LD 8; 11.9 vs. 13.3 in controls, and LD 15; 22.9 vs. 25.0 in controls). Of the pups necropsied at weaning no absolute or relative organ weight changes were reported.	York, 2002; Butenhoff et al., 2004

				F1 males: A significant increase in treatment-related deaths (5/60 rats) was reported in the high dose group between day 2-4 post-weaning. Significant increases in clinical signs of toxicity were also reported during most of the post-weaning period at all dose levels. A significant dose-related reduction in mean body weight gain for the entire dosing period (days 1-113). Absolute food consumption was significantly reduced from 10 mg/kg/day during the entire pre-cohabitation period (days 1-70 post-weanling), while relative food consumption values were significantly increased. Significant delays in sexual maturation (the average of preputial separation) were reported at 30 mg/kg/day (52.2 days of age vs. 48.5 days of age in controls). When the body weight was co-varied with the time to sexual maturation, the time to sexual maturation showed a dose-related delay that was statistically significant at $p \leq 0.05$. No treatment-related effects were reported at any dose level for any of the mating and fertility parameters assessed. Necroscopic examination revealed significant effects on the liver and kidney from 3 mg/kg/day. Terminal body weight was significantly dose-related decreased from 1 mg/kg/day (6%, 6%, 11%, and 22% decreased from controls at 1, 3, 10 and 30 mg/kg/day, respectively. The absolute and relative liver weights were significantly increased in all treated groups and were accompanied by histopathological changes. All other organ weight changes reported (thymus, spleen, left adrenal, brain, prostate, seminal vesicles, testes and epididymis) were probably due to body weight reductions, since the relative weights of these organs were either normal or increased. However, the biological significance of the weight changes observed in the adrenal is unclear since histopathological changes were also reported. The NOAEL developmental effects were 3 mg/kg/day and the LOAEL for F1 adult effects was 1 mg/kg/day. ***F1 females***:***:*** A significant increase in treatment-related deaths (6/60 rats) was reported in the high dose group between day 2-8 post-weaning. Significantly decrease in body weights were reported in the high dose group during post-weaning, pre-cohabitation, gestation and lactation Body weight gain was significantly reduced during day 1-15 pos-weanling. Decreased absolute food consumption was reported during days 1-22 post-weaning, pre-cohabitation, gestation and lactation in the highest dose group. Relative food consumption values were comparable across all treated groups. Significant delays in sexual maturation (the average of vaginal patency) were reported at 30 mg/kg/day (36.6 days of age vs. 34.9 days of age in controls). When the body weight was co varied with the time to sexual maturation, the time to sexual maturation showed a dose-related delay that was statistically significant at $p \leq 0.05$. No treatment-related effects were reported at any dose level for any of the mating and fertility	

				parameters assessed. All natural delivery observations were unaffected by treatment at any dose level. No effect on terminal body weights was reported. The absolute weight of the pituitary, the pituitary weight-to-terminal body weight ratio and the pituitary weight-to-brain ration was significantly decreased from 3 mg/kg/day. No histopathologic changes were reported in the pituitary. The NOAEL developmental effects were 10 mg/kg/day and the NOAEL for F1 adult effects was 10 mg/kg/day. ***F2 generation:*** No treatment related adverse clinical signs were reported. Dead or stillborn pups were noted in both the control and treated groups. The deaths occurred on lactation day 1-8 with the majority occurring on days 1-6, however, there was no dose-relationship. No effect on body weights or organ weights, as well as AGD was reported. The NOAEL was set at 30 mg/kg/day.	

Studies in animals and humans on the developmental toxicity of APFO in mice performed and published after the final discussion of the classification proposal in the TC C&L meeting in Arona in 4-5 October 2006.

Animal studies:

Four studies (Wolf et al., 2007; White et al., 2007 and 2009; Fenton et al. 2009) address the developmental toxicity observed in mice and elaborate on the importance of *in utero* versus lactational exposure and the potential existence of sensitive window(s) of exposure. One additional study by Yang et al. (2009), address the effects of PFOA on mammary gland development in two different species of mice. The studies in mice are shortly described below.

In a study with CD-1 mice by Wolf et al. (2007), the contributions of gestational and lactational exposures and the impact of restricting exposure to specific gestational periods to the developmental toxicity of APFO (>98% pure) was examined. This study used two exposure regiments; a) cross-foster study where pregnant mice were dosed on gestation days (GD) 1–17 with 0, 3, or 5 mg APFO/kg bw, and pups were fostered at birth to give seven treatment groups: unexposed controls, pups exposed *in utero* (3U and 5U), lactationally (3L and 5L), or *in utero* + lactationally (3U + L and 5U + L) and b) a restricted exposure study were pregnant mice received 5 mg APFO /kg bw from GD7–17, 10–17, 13–17, or 15–17 or 20 mg on GD15–17. In all APFO -treated groups, dam weight gain, number of implantations, and live litter size were not adversely affected and relative liver weight increased. Treatment with 5 mg/kg bw on GD1–17 increased the incidence of whole litter loss and pups in surviving litters had reduced birth weights, but effects on pup survival from birth to weaning were only affected in 5U + L litters. *In utero* exposure (5U), in the absence of lactational exposure, was sufficient to produce postnatal body weight deficits and developmental delay in the pups. In the restricted exposure study, birth weight and survival were reduced by 20 mg/kg bw on GD15–17. Birth weight was also reduced by 5 mg/kg bw/day on GD7–17 and 10–17. Although all APFO -exposed pups had deficits in postnatal weight gain, only those exposed on GD7–17 and 10–17 also showed developmental delay in eye opening and hair growth. The observations suggest that the postnatal developmental effects of APFO in mice are mainly due to gestational exposure and that exposure earlier in gestation produces stronger responses.

In two studies by White et al. (2007, 2009), the effects of APFO (> 98% pure) on the development of mammary gland following restricted gestational exposure was reported. In the former study, timed-pregnant CD-1 mice were orally dosed with 5 mg APFO /kg bw/day on gestation days (GD) 1–17, 8–17, 12–17, or vehicle on GD 1–17. APFO exposure had no effect on maternal weight gain or number of live pups born. Mean pup body weights on postnatal day (PND) 1 in all APFO -exposed groups were significantly reduced and decrements persisted until weaning. Mammary glands from lactating dams and female pups on PND 10 and 20 were scored based on differentiation or developmental stages. A significant reduction in mammary differentiation among dams exposed GD 1–17 or 8–17 was evident on PND 10. On PND 20, delays in normal epithelial involution and alterations in milk protein gene expression were observed. All exposed female pups displayed stunted mammary epithelial branching and growth at PND 10 and 20. While control litters at PND 10 and 20 had average scores of 3.1 and 3.3, respectively, all treated litters had scores of 1.7 or less, with no progression of duct epithelial growth evident over time. Body weight was an insignificant covariate for these effects. In the 2009 study, timed pregnant CD-1 dams received APFO by oral gavage over various gestational durations. Cross-fostering studies identified the 5 mg/kg bw/day dose, under either lactational- or intrauterine-only exposures, to delay mammary gland development as early as PND 1, persisting beyond PND 63. Intrauterine exposure during the final days of pregnancy caused adverse mammary gland developmental effects similar to that of extended gestational exposures. These two studies suggest that there is a window of mammary gland sensitivity in late fetal and early neonatal life and that the effects might be persistent.

In a study by Yang et al. (2009), the effects of peripubertal exposure (21 through 50 days of age) to APFO (> 98% pure) on mammary gland development was examined in two different strains of mice. The effects of APFO (0.1–10 mg/kg bw/day) were examined in Balb/c and C57BL/6 mice. APFO treatment caused hepatocellular hypertrophy and delayed vaginal opening in both mouse strains. While Balb/c mice exhibited inhibition of mammary gland and uterine development at the two highest doses (5, 10 mg/kg bw), C57BL/6 mice exhibited stimulatory effects in both organs at 5 mg/kg bw and inhibition at the highest dose. This study confirms the effects of APFO exposure on mammary gland development in two additional strains of mice, but underscores that there are strain differences in sensitivity.

In a study by Fenton et al (2009), the disposition of APFO (> 98% pure) in the pregnant and lactating dam and her offspring was studied following a single exposure by oral gavage. Time-pregnant CD-1 mice received a single dose of 0, 0.1, 1, or 5 mg APFO/kg bw (n = 25/dose group) on GD17. Biological samples were collected on PNDs 1, 4, 8 and 18. Unlike studies using multiple gestational exposures, there was no change in pup body weight, dam liver weight, and dam liver:bw ratios, within the APFO dose range administered in this study. Pup serum PFOA concentration was evaluated on PNDs 1, 4, 8, and 18. In comparing the average PFOA concentrations in PND1 pups vs. their respective dams, it appeared that circulating pup serum PFOA concentrations were significantly higher than those measured in dams, regardless of dose. PFOA body burden (adjusted for weight) rose through the peak of lactation and had begun to decline by PND18, demonstrating an inverse U-shaped curve. The PFOA burden of pups was proposed to increase due to milk-borne PFOA intake. The distribution of milk:serum PFOA varied by dose and time, but was typically in excess of 0.20.

Abbott et al. (2007) studied the influence of PPARα on PFOA-induced developmental toxicity using WT and PPARα (KO) mice (129S1/SvlmJ). Timed-pregnant mice were dosed by daily gavage from gestation days 1-17 with water (control) or 0.1, 0.3, 0.6, 1, 3, 5, 10 or 20 mg APFO (> 98% pure)/kg bw/day. Endpoints evaluated included maternal weight, embryonic implantation number, pup weight, neonatal survival, and eye opening. APFO did not affect maternal weight, embryonic implantation, number, or weight of pups at birth. There was a trend across dose for reduced pup weight in both WT and KO mice on several postnatal days, but only WT mice exposed to 1 mg/kg were significantly different from control (PND7–10 and 22). The incidence of full litter resorptions increased at the 5 mg/kg bw/day dose in both WT and KO mice. Neonatal survival was reduced only in the WT mouse starting at the 0.6 mg/kg dose, and eye opening was delayed in WT starting at the 1 mg/kg dose. This study indicates that several of the developmental effects in mice are influenced by PPARα (post-natal lethality, delayed eye opening and deficits in postnatal weight gain) although other mechanisms may contribute. In contrast, early pregnancy loss appeared to be independent of PPARα expression.

The incidence of complete litter loss was increased in several of the developmental studies in mice mentioned above and this effect seems to be independent of PPARα. The observed increased postnatal pup mortality, reduction in pup body weight and postnatal growth and development indicate direct embryotoxicity. PPARα appears to contribute to some of the developmental effects of PFOA.

Human studies:

In a pilot study (Midasch 2007) levels of PFOS and PFOA in 11 maternal and umbilical cord plasma sample pairs were examined. In the case of PFOA slightly higher PFOA concentrations within the analyzed sample pairs was observed in maternal versus cord plasma (median: 2.6 _g/l vs. 3.4 g/l for maternal and cord plasma samples, respectively). Thus, PFOA appears to cross the placental barrier unhindered in humans and in mice and a slight accumulation of PFOA in the embryo/neonate is indicated. Several human epidemiological studies analysing a possible association between concentrations of PFOA in maternal or fetal blood to birth outcomes are considered inconclusive and thus not relevant for classification purposes. As regards the renal clearances of PFOA in humans a study by Harada et al., 2005 showed that the renal clearances of PFOA were almost negligible in both sexes in humans, in clear contrast to the large active excretion in the female rat. Due to the similar lack of sex-difference in PFOA elimination among humans and mice, more weight should be put on the findings reported in the mice studies in the decision on classification of PFOA/APFO for developmental effects in offspring.

5.9.3 Human data

See the human studies on the developmental toxicity of APFO performed and published after the final discussion of the classification proposal in the TC C&L meeting in Arona in 4-5 October 2006 described above.

5.9.4 Other relevant information

5.9.5 Summary and discussion of reproductive toxicity

Fertility

In a 2-generation study in rats no effects on mating and fertility parameters were reported in the F0 and F1generation exposed to up to 30 mg/kg/day APFO in the diet. In the F0 generation a statistically significant decrease was reported in the absolute weights of the left and right epididymis, left cauda epididymis, seminal vesicles, prostate, pituitary, left and right adrenals and thymus at 30 mg/kg /day, however, due to an statistically significant reduction in body weight at the same dose level, the organ-to- body weight ratios were either normal or increased. There were no treatment-related effects for any of the mating and fertility parameters assessed up to and including the highest tested dose level of 30 mg/kg.

In a chronic 2-year study in rats at 1 year sacrifice testicular masses were found in 6/15 rats exposed to 14.2 mg/kg/day (high dose) and in 1/15 rats exposed to 1.3 mg/kg/day (low dose), compared to 0/15 in control rats (Sibinski et al., 1987). Furthermore, marked aspermatogenesis was found in 2/15 high dosed males compared to 0/15 in controls. At the 2-year sacrifice, vascular mineralization was reported in 18% of high-dosed males and 6% in low-dosed males, however, not in control males. The testicular effects reached statistically significance in the high-dose group. Furthermore, at 2-year sacrifice a significant increase in the incidence of testicular Leydig cell (LCT) adenomas in the high-dosed group was reported [0/50 (0%), 2/50 (4%) and 7/50 (14%) in control, low- and high dose group, respectively]. The tumours may have be a result of endocrine changes, because a reduced aromatase activity and a sustained increase in serum estradiol were reported in the study by Biegel et al., 2001.

In several repeated dose toxicity studies in mice, rats and monkeys with durations up to 90 days no effects on the male or female reproductive organs were reported (see section 5.6, Repeated dose toxicity).

Due to the lack of effects on fertility parameters in the 2-generation study and lack of effects on the reproductive organs in experimental animal studies in males and females with durations up to 90 days no classification for fertility is proposed.

Developmental toxicity:

In an oral 2-generation study (York, 2002; Butenhoff et al., 2004) in rats in the 30 mg/kg/day dose group one pup died on Lactation Day (LD) 1. Additionally, on LD 6 and 8 significant increases in the number of pups found dead were reported at 3 and 30 mg/kg/day. Pup body weight on a per litter basis was significantly reduced up to lactation day 15 in the 30 mg/kg/day dose group (LD 1; 5.5 vs 6.3 in controls, LD 8; 11.9 vs. 13.3 in controls, and LD 15; 22.9 vs. 25.0 in controls). Furthermore, significant delays in sexual maturation (the average of preputial separation in males and vaginal patency in females) were reported at 30 mg/kg/day (52.2 days of age vs.48.5 days of age in controls in males, and 36.6 days of age vs. 34.9 days of age in female). When the body weights were co varied with the time to sexual maturation, the time to sexual maturation in both males and females showed still a dose-related delay that was statistically significant at $p \leq 0.05$. These effects were reported in the absence of maternal toxicity. However, in rat developmental toxicity studies following oral or inhalation exposure to APFO minimal effects were reported in the offspring.

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

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

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

In the study by Abbott et al., 2007 it was shown that several of the developmental effects in mice may be influenced by PPARα (post-natal lethality, delayed eye opening and deficits in postnatal weight gain) although other mechanisms may contribute. In contrast, reduced pup weight and early pregnancy loss appeared to be independent of PPARα expression.

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

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

5.10 Other effects

Table 16. Exposure of workers

Exposure of workers	Ref.
3M and DuPont have measured the PFOA in serum of occupationally exposed workers from 1995 to 2002. The serum concentration in µg/mL (arithmetic mean) ranged from 0.106 to 6.8 µg/mL in the bio-monitoring data from 3M (Olsen et al., 1998c; 1999; 2000; 2001a and c; 2003 a, b, e and f). In bio-monitoring data from DuPont the serum concentrations in µg/mL (arithmetic mean) ranged from 1.53 to 3.21 µg/mL (DuPont, 2001a and b). 3M and Dupont have conducted several epidemiology and medical surveillance studies of the workers at their plants in various cities of U.S. From these studies it can be concluded that no remarkable health effects that can be directly attributed to PFOA exposure were reported in fluorochemical production workers. However, in a study by Gilliland and Mandel, 1993 a statistically significant association with length of employment in the Chemical Division and prostate cancer mortality was found. An update of this study was conducted in which more	Olsen et al., 1998c; 1999; 2000; 2001a and c; 2003 a, b, e and f. DuPont2001a and b. Gilliland and Mandel, 1993;

specific exposure measures were used, and in this study no significant association for prostate cancer was observed (Alexander, 2001).	Alexander, 2001

Table 17. Exposure of the general population

Exposure of general population	Ref
Data on PFOA levels in the general population include both pooled and individual serum samples. In pooled samples from commercial sources of blood (n=35 lots) the arithmetic mean was 0.003 µg/mL (3M Company, 1999a) and from blood banks, 1998 (n=18 lots, 340-680 donors) the arithmetic mean was 0.017 µg/mL (3M Company, 1999b). In individual samples from the American Red Cross banks, 2000 (n=645) the arithmetic mean was 0.0056 µg/mL and geometric mean 0.0046 µg/mL (Olsen et al., 2002a and 2003d). In elderly people (65-96 years), 2000 (n=238) the geometric mean was 0.0042 µg/mL (arithmetic mean was not reported) (Olsen et al., 2002b and 2004a). In children (2-12 years), 1995 (n=598) the arithmetic mean was 0.0056 µg/mL and the geometric mean was 0.0049 µg/mL (Olsen et al., 2002c and 2004b). In 23 pooled serum samples collected in USA from 1990 through 2002 the median concentration was 0.0116 µg/ml PFOA, and the 90th percentile concentration was 0.0223 µg/ml. In serum samples collected in 2003 from 44 residents in Peru the 90th percentile concentration was 0.0001 µg/ml (Calafat et al., 2006).	3M Company, 1999a and b; Olsen et al., 2002 a, b and c; Olsen et al., 2003 d; Olsen et al., 2004a and b. Calafat et al., 2006
In a recent study, fifty-seven pooled archived human serum samples were analyzed to assess the time trends as well as influence of age and gender on selected perfluorinated compounds (PFCs) in Norwegian residents. The study comprised determinations of 19 PFCs in serum samples pooled according to year of collection in the period 1976 to 2007. An approximately 9-fold increase in the serum concentrations of PFOA in males age 40-50 years was seen from 1977 to the mid 1990s where the concentration reached a plateau before it started to decrease around year 2000. The PFOA concentration observed in serum in year 2000 (4.5 ng/ml) were approximately two times higher than what was found in 2006 (2.7 ng/ml) (Haug et al. 2009). In a recent Danish study (Joensen et al., 2009), levels of 10 different PFAAs were related to reproductive hormones and semen quality. Serum samples from 105 Danish men (median age, 19 years) were analysed and the median PFOA levels were found to be 4.9 ng/ml.	Haug et al, 2009; Joensen et al, 2009

5.11 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response

Not relevant for this type of dossier.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

6.1 Explosivity

Not relevant for this dossier

6.2 Flammability

Not relevant for this dossier

6.3 Oxidising potential

Not relevant for this dossier

7 ENVIRONMENTAL HAZARD ASSESSMENT

Not relevant for this dossier

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

The classification of the salt of PFOA, APFO, was concluded in the former TC C&L group in October 2006. The agreed classification was: Carc. Cat 3; R40, Repr. Cat. 2: R61, T; R48/23, Xn; R48/22, R20/22, Xi; R36. Since this was agreed to be the hamonized classification for APFO/PFOA, we consider it important to includ the complete result on the agreed classification of APFO/PFOA from the discussion in the TC C&L group into Annex VI of the CLP regulation. See Annex I of this report (Summary Record from the TC C&L group meeting 21-24 March 2006 and 4-5 October 2006) for the dicussion and conclusion of the TC C&L group.

OTHER INFORMATION

It is suggested to include here information on any consultation which took place during the development of the dossier. This could indicate who was consulted and by what means, what comments (if any) were received and how these were dealt with. The data sources (e.g registration dossiers, other published sources) used for the dossier could also be indicated here.

REFERENCES

Abbott BD, Wolf CJ, Schmidt JE, Das KP, Zehr RD, Helfant L, Nakayama S, Lindstrom AB, Strynar MJ and Lau C. 2007. Perfluorooctanoic acid-induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator-activated receptor-alpha. Toxicological science, 98; 571-581.

Alexander BH. 2001. Mortality study of workers employed at the 3M Cottage Grove facility. Final Report, April 2, 2001. Division of Environmental and Occupational Health, School of Public Health, University of Minnesota, US EPA AR226-1030a018.

Biegel LB, Hurtt ME, Frame SR, O'Conner JC and Cook JC. 2001. Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. Toxicol. Sci., 60; 44-55.

Brace NO. 1962. Long chain alkanoic and alkenoic acids with perfluoroalkyl terminal segments. Journal of Organic Chemistry, 27; 4491-4498.

Butenhoff J, Costa G, Elcomb C, Farrar D, Hansen K, Iwai H, Jung R, Kennedy G, Lieder P, Olsen G and Thomford P. 2002. Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. Toxicol. Sci., 69; 244-257.

Butenhoff JL, Kennedy GL, Frame SR, O'Conner JC and York RG. 2004. The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology, 196; 95-116.

Calafat AM, Needham LL, Kuklenyik Z, Reidy Jam Tully JS, Aguilar-Villalobos M and Naeher LP. 2006. Perfluorinated chemicals in selected residents of the American continent. Chemosphere, 63; 490-496.

Chandra M, Riley MGI and Johnson DE. 1992. Spontaneous neoplasm in aged Sprague-dawley rats. Arch. Toxicol., 66; 496-502.

Clegg ED, Cook JC, Chapin RE, Foster PMD and Daston GP. 1997. Leydig cell hyperplasia and adenoma formation: mechanisms and relevance to humans. Reproduct. Toxicol., 11; 107-121.

Christopher B and Marisa AJ. 1977. 28-day oral toxicity study with FC-143 in albino mice, Final Report, Industrial Bio-Test Laboratories, Inc. Study No. 8532-10655, 3M Reference No. T-1742CoC, Lot 269.

3M Company. Environmental Laboratory. 1987. Unpublished data, U.S. Environmental Protection Agency Administrative Record 226-0511, Microbics Microtox Test (April 29).

3M Company. 1987. Fluorad® Fluorochemical Surfactant FC-143. 3M Company Technical Bulletin.

3M Company (Minnesota Mining and Manufactoring Company). 1999a. Letter (dated May 26, 1999) re: TSCA 8(e) Supplemental Notice: Sulfonate-based and carboxylic-based fluorochemicals- Docket Nos. 8EHQ-1180-373; 8EHQ-1180-374; 8EHQ-0381-0394; 8EHQ-0699-373.

3M Company (Minnesota Mining and Manufactoring Company). 1999b. “The science of Organic Fluorochemistry” and “Perfluorooctanesulfonate: current summary of human sera, health, and toxicology data” February 5, 1999. 8EHQ-0299-373.

3M Company. 2000. Voluntary use and exposure information profile for perfluorooctanoic acid and salts. Submission to USEPA, June 8.

Cook JC, Hurtt ME, Frame SR and Biegel LB. 1994. Mechanisms of extrahepatic tumor induction by peroxisome proliferators in Crl:CD BR (CD) rats. Toxicologist, 14; 301 (abstract No. 1169).

Dean WP and Jessup DC. 1978. Acute oral toxicity (LD50) study in rats. International Research and Development Corporation study No. 137-091, May 5, 1978. US EPA AR226-0419.

Du Pont Company. 1979a. Acute dermal toxicity. Haskell Laboratory unpublished report. Newark, DE.

Du Pont Company. 1979b. Eye irritation test in rabbits. Haskell Laboratory unpublished report. Newark, DE.

Du Pont Company. 1981d. Oral LD_{50} test in rats. Haskell Laboratory unpublished report. Newark, DE.

Du Pont Company. 1981e. Oral LD_{50} test in mice. Haskell Laboratory unpublished report. Newark, DE.

Du Pont Company. 1981f. Oral LD_{50} test in Guinea Pigs. Haskell Laboratory unpublished report. Newark, DE.

Du Pont Company. 1983a. Acute oral toxicity of FC-143 as a function of age. Haskell Laboratory unpublished report. Newark, DE.

Dupont (Haskell Laboratory). 2001a. Letter to Charles Auer from Gerald Kennedy, dated January 25, 2001. FYI-00-001378. US EPA AR226-1000.

Dupont (Haskell Laboratory). 2001b. Letter from Gerald Kennedy re: FYI-010-01378 anf FYI-0301-01378, dated May 29, 2001. . US EPA AR226-1023.

DuPont, Teflon. 2006. Online: http://www.teflon.com/NASApp/teflon/

Gabriel K. 1976a. Primary eye irritation study in rabbits. Biosearch, Inc., March 4, 1976. US EPA AR226-0422.

Gabriel K. 1976b. Primary skin irritation study in rabbits. Biosearch, Inc., March 4, 1976. US EPA AR226-0423.

Gabriel K. 1976c. Acute oral toxicity – rats. Biosearch, Inc., September 16, 1976. US EPA AR226-0425.

Gabriel K. 1976d. Primary eye irritation study in rabbits. Biosearch, Inc., September 16, 1976. US EPA AR226-0426.

Garry VF and Nelson RL. 1981. An assay of cell transformation and cytotoxicity in C3H10T1/2 clonal cell line for the test chemical T-2942 CoC. Stone Research Laboratories, Minneapolis, MN, March 4, 1981. US EPA AR226-0428.

Glaza S. 1995. Acute dermal toxicity study of T-6342 in rabbits. Corning Hazelton Inc., Madison, WI. Project ID: HWI 50800374. 3M Company. St. Paul, MN.US EPA AR226-0427.

Glaza SM. 1997. Acute oral toxicity study of T-6669 in rabbits. Corning Hazelton Inc., Study No. CHW 61001760, January 10, 1997. US EPA AR 226-0420.

Gilliland FD and Mandel JS. 1993. Mortality among employees of a perfluorooctanoic acid production plant. J. Occup. Med., 35; 950-954.

Goldenthal EI. 1978a. Final report, Ninety day sub-acute rat toxicity study on Flurado® Fluorochemical FC-143, International Research and development corporation, Study No. 137-089, 3M Reference No. T-3141, November 6, 1978. US EPA AR226-0441.

Goldenthal EI. 1978b. Final report, Ninety day sub-acute Rhesus Monkey toxicity study, International Research and development corporation, Study No. 137-090, November 10, 1978. US EPA AR226-0447.

Gortner EG. 1981. Oral teratology study of T-2998CoC in rats. Safety Evaluation Laboratory and Riker Laboratories, Inc. Experiment Number: 0681TR0110, December, 1981.

Gortner EG. 1982. Oral teratology study of T-3141CoC in rabbits. Safety Evaluation Laboratory and Riker Laboratories, Inc. Experiment Number: 0681TB0398, February, 1982.

Griffith FD and Long JE. 1980. Animal toxicity studies with ammonium perfluorooctanoate. Am. Ind. Hyg. Assoc. J., 41: 576-583.

Harada K, Inoue K, Morikawa A, Yoshinaga T, Saito N and Koizumi A. 2005. Renal clearance of perfluorooctane sulfonate and perfluorooctanoate in humans and their species-specific excretion. Environmental Research, 99; 253-261.

Hardisty JF;. 2005. PWG report: Pathology peer review and pathology working group review of mammary glands from a chronic feeding study in rats with PFOA. Experimental Pathology Laboratories, Inc. Research Triangle Park, NC. June 17, 2005.

Haug LS, Thomsen C and Becher G. 2009. Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. Environ. Sci. Technol. 43; 2131-2136.

Hazleton, Inc., 1990. Primary irritation dermal irritation/corrosion study in rabbits. HLA number 91201740. US EPA Public Docket AR-226-0666, Washington DC.

Hazleton, Inc., 1995b. Mutagenicity test with T-6342 in an in vivo mouse micronucleus assay. Corning Hazleton, Inc. Final Report CHV Study No. 17073-0-455. December 14, 1995. US EPA Public Docket AR-226-0435, Washington DC.

Hazleton, Inc., 1996e. Mutagenicity test with T-6342 in an in vivo mouse micronucleus assay. Corning Hazleton, Inc. Final Report CHV Study No. 17750-0-455. December 14, 1996. US EPA Public Docket AR-226-0430, Washington DC.

Hazleton, Inc., 1997.Acute oral toxicity study of T-6669 in rats. Corning Hazleton, Inc. CHW 61001760. US EPA Public Docket AR-226-0420, Washington DC.

Hinderliter PM, Mylchreest E, Gannon SA, Butehoff JL and Kennedy GL. 2005. Perfluorooctanoate: Placental and lactational transport pharmacokinetics in rats. Toxicology, 211; 139-148.

Ikeda T, Aiba K, Fukuda K and Tanaka M. 1985. The induction of peroxisome proliferation in rat liver by perfluorinated fatty acids, metabolically inert derivatives of fatty acids. J. Biochem., 98; 475-482.

Joensen UN, Bossi R, Leffers H, Astrup AA, Skakkebæk NE and Jørgensen N. 2009. Do perfluoroalkyl compounds impair human semen quality. Environmental Health Perspectives, 117 (number 6); 923-927.

Kemper RA and Jepson GW. 2003. Pharmacokinetics of perfluorooctanoic acid in male and female rats. Toxicol. Sci., 72; 148.

Kennedy GL Jr. 1985. Dermal toxicity of ammonium perfluorooctanoate. Toxicol. Appl. Pharmacol. 81: 348-355.

Kennedy GL Jr., Hall CT, Brittelli MR, Barnes JR and Chen HC. 1986. Inhalation toxicity of ammonium perfluorooctanoate. Food Chem. Toxicol. 24: 1325-1329.

Kirk-Othmer. 1994. Encyclopaedia of Chemical Technology. 4th ed. Volumes 1: New York, NY. John Wiley and Sons, 1991-Present., p. V11 (1994) 551.

Lau C, Thibodeaux JR, hanson RG, Narotsky MG, Rogers JM, Lindstrom AB and Strynart MJ. 2006. Effects of perfluorooctanoic acid exposure during pregnancy in the mous. Toxicological Science, 90 (2); 510-518.

Lawlor TE. 1995. Mutagenicity test with T-6342 in the *Salmonella- Escherichia coli*/mammalian-microsome reverse mutation assay. Laboratory number: 17073-0-409. Corning Hazleton Inc., Vienna, VA. 3M Company. St. Paul, MN. USA EPA AR226-0436.

Lawlor TE. 1996. Mutagenicity test with T-6564 in the *Salmonella- Escherichia coli*/mammalian-microsome reverse mutation assay with a confirmatory assay. Corning Hazleton Inc., Final report. CHV Study No. 17750-0-409R. September 13, 1996. USA EPA AR226-0432.

Lines D and Sutcliffe H. 1984. Preparation and properties of some salts of perfluorooctanoic acid. J. Fluorine Chem. 25; 505-512.

Litton Bionetics. 1978. Mutagenic evaluation of T-2015 CoC in the Ames Salmonella microsome plate test. Final Report LBI Project No. 20838, February 1978. US EPA Public Docket AR-226-0429, Washington DC.

Mann PC and Frame SR. 2004. FC-143: Two year oral toxicity-oncogenicity study in rats: Peer review of ovaries. Du Pont Project ID 15261, June 25, 2004. US EPA AR226.

Markoe DM. 1983. Primary skin irritation test with T-3371 in albino rabbits. Riker Laboratories, Study No. 0883EB0079, July 13, 1983.US EPA AR226-0424.

Merck MSDS online version for PFOA.

Online:
http://www.chemdat.merck.de/pls/pi03/web2.zoom_in?text=807114&screen=110&cid=xcsfs&pg=0&s=&lang=1

Metrick M and Marias AJ. 1977. 28-day oral toxicity study with FC-143 in albino Rats, Final Report, Industrial Bio-Test Laboratories, Inc. Study No. 8532-10654, 3M Reference No. T-1742CoC, Lot 269, September 29, 1977.

Midasch O, Drexler H, Hart N, Beckmann MW and Angerer J. 2007. Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study. Int Arch Occup Environ Health, 80; 643-648.

Moore GE. 2001. H-24291: Dermal sensitization test-Buhler method. Product Safety Labs, Study No. 11321. December 17. Dayton, NJ. Available from GLK on request.

Murli H. 1996a. Mutagenicity test on T-6564 in an in vivo mouse micronucleus assay. Corning Hazleton Inc., Vienna, VA. Study number 17750-0-455, November 1, 1996. US EPA AR 226-0430.

Murli H. 1996b. Mutagenicity test on T-6564 measuring chromosomal aberrations in Chinese Hamster ovary (CHO) cells with a confirmatory assay with multiple harvests. Corning Hazleton Inc., Vienna, VA. Study number 17750-0-437CO, September 16, 1996. US EPA AR 226-0431.

Murli H. 1996c. Mutagenicity test on T-6342 measuring chromosomal aberrations in human whole blood lymphocytes with a confirmatory assay with multiple harvests. Corning Hazleton Inc., Vienna, VA. Study number 17073-0-449CO, November 1, 1996. US EPA AR 226-0433.

Murli H. 1996d. Mutagenicity test on T-6342 measuring chromosomal aberrations in Chinese Hamster ovary (CHO) cells with a confirmatory assay with multiple harvests. Corning Hazleton Inc., Vienna, VA. Study number 17073-0-437CO, September 16, 1996. US EPA AR 226-0434.

NOTOX. 2000. Evaluation of the ability of T-7524 to induce chromosomal aberrations in cultured peripheral lymphocytes. NOTOX Project Number 292062. Hertogenbosch, the Netherlands.

OECD Draft SIDS. Initial assessment report of ammonium perfluorooctanoate after SIAM 22 Paris April 18-21. 2006.

Olsen GW; Burris JM, Mandel JH and Zobel LR. 1998c. An epidemiologic investigation of clinical chemistries, hematology and hormones in relation to serum levels of perfluorooctane sulfonate in male fluorochemical production employees. 3M Company. Final Report. August 22, 1998. US EPA AR226-0030.

Olsen GW. Logan PW, Simpson CA, Hansen KJ, Burris JM, Burlew MM Schumpert JC and Mandel JH. 1999. Fluorochemical exposure assessment of Decatur chemical and film plant employees. 3M Company. Final Report. August 11, 1999. US EPA AR226-0950.

Olsen GW, Burris JM, Burlew MM and Mandel JH. 2000. Plasma cholecystokinin and hepatic enzymes, cholesterol and lipoproteins in ammonium perfluorooctanoate production workers. Drug Chem. Tox., 23 (4); 603-620.

Olsen GW, Logan PW, Simpson CA, Burris JM, Burlew MM, Lundberg JK and Mandel JH. 2001a. Descriptive summary of serum fluorochemical levels among employee participants of the year 2000 Decatur fluorochemical surveillance program. . 3M Company. Final Report. March 19, 2001. US EPA AR226-1030a020a.

Olsen GW, Madsen DC, Burris LM and Mandel JH. 2001c. Descriptive summary of serum fluorochemical levels among 236 building employees. 3M Company. Final Report. March 19, 2001. US EPA AR226-1030a020c.

Olsen GW, Burris JM, Lundberg JK, Hansen KJ, Mandel JH and Zobel LR. 2002a. Identification of fluorochemicals in human sera. I. American Red Cross adult blood donors. 3M Company. Final Report. February 25, 2002. US EPA 226-1083.

Olsen GW, Burris JM, Lundberg JK, Hansen KJ, Mandel JH and Zobel LR. 2002b. Identification of fluorochemicals in human sera. II. Elderly participants of the Adult Changes in Thought Study, Seattle, Washington. 3M Company. Final Report. February 25, 2002. US EPA 226-1084.

Olsen GW, Burris JM, Lundberg JK, Hansen KJ, Mandel JH and Zobel LR. 2002c. Identification of fluorochemicals in human sera. III. Pediatric participants in a Group A Streptococci clinical trial investigation. 3M Company. Final Report. February 25, 2002. US EPA 226-1085.

Olsen GW and Mandel JH. 2003a. Descriptive analysis of serum fluorochemical concentrations from Cottage Grove employee participants of the 2002 Medical Surveillance Program. 3M Company. Final Report. US EPA AR226-1353.

Olsen GW and Mandel JH. 2003b. Descriptive analysis of serum fluorochemical concentrations from Decatur employee participants of the 2002 Medical Surveillance Program. 3M Company. Final Report. US EPA AR226-1352.

Olsen GW, Church TR, Miller JP, Burris JM, Hansen KJ, Lundberg JK, Armitage JB, Herron RM, Medhdizadehkashi Z, Nobiletti JB, O'Neill EM, Mandel JH and Zobel LR. 2003d. Perfluorooctanesulfonate and other fluorochemicals in the serum of American Red Cross adult blood donors. Environ. Health Perspect., 111 (16); 1892-1901.

Olsen GW, Burris JM, Burlew MM and Mandel JH. 2003e. Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS9 and perfluorooctanoate (PFOA) concentrations and meical surveillance examinations. JOEM 45(3); 260-270.

Olsen GW, Butenhoff JL and Mandel JH. 2003f. Assessment of lipid, hepatic and thyroid function in relation to an occupational biologic limit value for perfluorooctanoate. 3M Company. Final Report. June 9, 2003. US EPA 226-1351.

Olsen GW, Church TR, Larson EB, van Belle G, Lundberg JK, Hansen KJ, Burris JM, Mandel JH and Zobel LR. 2004a. Serum concentrations of perfluorooctanesulfonate and other fluorochemicals in an elderly population from Seattle, Washington, Chemosphere. 54; 1599-1611.

Olsen GW, Church TR, Hansen KJ, Butenhoff JL, Mandel JH and Zobel LR. 2004b. Quantitative evaluation of perfluorooctanesulfonate (PFOS) and other FC's in the serum of children. J. Children's health. 2; 1-24.

Palazzolo MJ. 1993. Thirteen week dietary study with T-5180, ammonium perfluorooctanoate (CAS No. 3825-26-1) in male rats. Final report. Laboratory project Identification HWI 6329-100. Hazelton Wisconsin, Inc. US EPA AR226-0449.

Pastoor TP, Lee KP, Perri MA and Gillies PJ. 1987. Biochemical and morphological studies of ammonium perfluorooctanate-induced hepatomegaly and peroxisome proliferation. Exp. Mol. Pathol., 47; 98-109.

Riker. 1981. Final Report: Repeat application 28-day percutaneous absorption study with T-2618 CoC in albino rabbits. Riker Laboratories Report 09790AB0485, March 15, 1981. US EPA Public Docket AR 226-0446, Washington, DC.

Rusch G. 1979. An acute inhalation study of T-2305 CoC in the rat. Bio/dynamics, Inc. Study No. 78-7184, May 3, 1979. US EPA AR226-0417.

Sadhu D. 2002. CHO/HGPRT forward mutation assay – ISO (T6.889.7). Toxicon Corporation, Bedford, MA. Reprot No. 01-7019-G1, March 3, 1979. US EPA AR226-0417.

Sibinski LJ. 1987. Final report of a two year oral (diet) toxicity and carcinogenicity study of fluorochemical FC-143 (perfluorooctane ammonium carboxylate) in rats. Vol. 1-4, 3M Company/Riker exp. No. 0281CR0012; 8EHQ-1087-0394, October 16, 1987.

Sohlenius AK, Andersson K and DePierre JW. 1992. The effects of perfluoro-octanoic acid on hepatic peroxisome proliferation and related parameters show no sex-related differences in mice. Biochem., 34; 146-152.

Staples RE, Burgess BA and Kerns WD. 1984. The embryo-fetal toxicity and teratogenic potential of ammonium perfluorooctanoate (APFO) in the rat. Fundam. Appl. Toxicol., 4; 429-440.

Sykes G. 1987. Two-year toxicology/carcinogenicity study of fluorochemical FC-143 in rats. Memo. From G Sykes to C Reinhardt, Haskell Lab. Toxicol. Ind. Med., October 29, 1987.

Thomford PJ. 2001b. 26-week capsule toxicity study with ammonium perfluorooctanoate (APFO) in cynomolgus monkeys. Study performed by Convance Laboratories Inc., Madison Wisconsin 53704-2592 for APME ad-hoc APFO Toxicology Working Group. Study No. Covance 6329-231, Completion date December 18, 2001, 463 pp. US EPA AR226-1052a.

Washburn ST, Bingman TS, Braithwaite SK, Buck RC, Buxton LW, Clewell HJ, Haroun LA, Kester JE, Rickard RW and Shipp AM. 2005. Exposure assessment and risk characterization of perfluorooctanoate in selected consumer articles. Environ. Sci. Technol. 39; 3904-3910 (supplement).

Yang Q, Xie Y, Alexson SE, Nelson BD, and DePierre JW. 2002. Involvement of theperoxisome proliferator-activated receptor alpha in the immunomodulation caused by peroxisome proliferators in mice. Biochemical Pharmacology, 63:1893-1900.

Yang C, Tan YS, Harkema JR and Haslam SZ. 2009. Differential effects of peripubertal exposure to perfluorooctanoic acid on mammary gland development in C75BI/6 and Balb/c mouse strains. Reproductive toxicology, 27 (3-4); 299-306.

Ylinen M, Kojo A, Hanhijãrvi H and Peura P. 1990, Deposition of perfluorooctanoic acid in the rat after single and subchronic administration. Bulletin of Environmental Contamination and Toxicology, 44; 46-53.

York RG. 2002. Oral (gavage) two-generation (one litter per generation) reproduction study of ammonium perfluorooctanoic (APFO) in rats. Argus Research Laboratories, Inc. Protocol Number: 418-020, Sponsor Study Number: T-6889.6, March 26, 2002. US EPA AR226-1092.

White SS, Calafat AM, Kuklenyi, Z, Villanueva L, Zehr RD, Helfant L, Strynar MJ, Lindstrom AB, Thibodeaux JR, Wood C and Fenton SE. 2007. Gestational PFOA exposure of mice is associated with altered mammary gland development in dams and female offspring. Toxicological Science , 96; 133-144.

White SS, Kalto K, Jia LT, Basden BJ, Calafat AM, Hines EP, Stanko JP, Wolf CJ, Abbott BD and Fento SE. 2009. Effects of perfluorooctanoic acid om mouse mammary gland development and differentiation resulting from cross-foster and restricted gestational exposures. Reproductive toxicology, in press.

Wolf CJ, Fenton SE, Schmid JE, Calafat AM, Kuklenyik Z, Bryant XA, Thibodeaux J, Das KP, White SS, Lau CS and Abbott BD. 2007. Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures. Toxicological Science, 95; 462-473.

ANNEX 1

Summary record from the TC C&L meeting in Arona, 21-24 March 2006 (ECBI/90/06 Rev.8)

Perfluorooctanic acid (PFOA) [1] and its salts (N003)

Ammonium salt of PFOA, APFO [2]

Sodium salt of PFOA [3]

Potassium salt of PFOA [4]

Silver salt of PFOA [5]

Fluoride acid of PFOA [6]

Methyl ester of PFOA [7]

Ethyl ester of PFOA [8]

(EC number : 206-397-9 [1],

CAS number : 335-67-1 [1]

CAS number : 3825-26-1 [2]

CAS number : 335-95-5 [3]

CAS number : 2395-00-8 [4]

CAS number : 335-93-3 [5]

CAS number : 335-66-0 [6]

CAS number : 376-27-2 [7]

CAS number : 3108-24-5 [8])

Not in Annex 1.

Classification proposal: Carc Cat 3; R 40, Repr Cat 2; R 61, Repr Cat 3; R 62, T; R 48/23, X n; R 20/22, R 48/22, Xi; R 36.

ECBI/18/06 ADD 1

Norway introduced its proposal for the classification of PF0A and its salts by reviewing the various end points and the suggestions for classification.

In Norway's view the classification for acute toxicity and irritancy were straightforward. Classification as Xn; R 48/22 was based on liver toxicity in both mice and rats as demonstrated in several studies. Classification with T; R 48/23 was proposed on the basis of a single study showing liver toxicity at a low doses in rats. The proposal to classify as a carcinogen category 2; R 45 was based on two studies which Norway acknowledged were borderline cases between category 2/3. In the context of fertility Repr Cat 3; R 62 was proposed on the basis of the evidence during two-year carcinogenicity studies where testicular damage had been observed. For developmental toxicity Repr Cat 2; R 61 was proposed based on a two-generation study in which there had been deaths of pups during feeding together with signs of delayed development in the absence of maternal toxicity. Norway made the general point that this substance was related to PFOS for which decisions had already been made in terms of developmental toxicity.

Discussion by the member-states commenced with Germany raising the issue of the substances for which evidence was available. Whilst it was clear that there is a close relationship between the behaviour of the acid and the salts classification should take into account the compound tested. Industry reported that most of the tests had been carried out on the ammonium salt of of PFOA which is the main commercialised product. Both Norway and Industry agreed to provide further information on the identification of the substances used in the different tests.

Notwithstanding the need for further clarification on the above issue the Chair suggested that it would be appropriate to review the various end points and try to reach provisional conclusions on classification.

Irritancy

On this basis TC C&L agreed that Xi; R 36 should be assigned to the ammonium salt on which most of the evidence was based.

Repeat dose toxicity

It was also agreed that Xn; R 48/22 was appropriate for the ammonium salt. In discussion of T; R48/23 industry argued that T was not appropriate. After discussion there was Member States agreement that T; R48/23 would be provisionally assigned. Further comments from industry on this end point will be provided. Meanwhile TC C&L provisionally agreed on Xn; R48/22 and T; R48/23 for the ammonium salt.

Carcinogenicity

In discussion of the carcinogenicity proposal Norway acknowledged that peroxisome proliferation was a possible relevant issue and this would slightly diminish the weight of evidence. However based on work by US EPA Norway had concluded that classification should also take into account the mammary and pancreatic tumours. On the basis of the range of tumours and the number of studies Norway had concluded that Carc Cat 2; R 45 was appropriate. The Chair drew attention to the fact that the original Norwegian proposal was for Carc Cat 3; R 40. Norway was asked to formally present a new proposal. In commenting on the carcinogenicity industry noted that PFOA could be regarded as a mixed inducer and that the observed liver tumours derived from peroxisome proliferation. Industry noted that the Norwegian proposal had stated that the mammary tumours were based on equivocal evidence and argued that there was no increase in the incidence. However Industry acknowledged that the pancreatic tumours could not easily be explained and for this reason agreed to Carc Cat 3; R 40 classification.

Reproductive toxicity

In discussion of reproductive toxicity and the proposal for Repr Cat 3; R 62 Germany commented that the findings were minimal and confined to a few animals with the possibility of age related effects. As a result classification was not appropriate. This position was supported by the United Kingdom and the Netherlands. Denmark indicated a preference for Repr Cat 3 but a majority of The Group agreed no classification for fertility.

On developmental toxicity the Norwegian proposal for Repr Cat 2; R 61 was adjourned.

Conclusion:

It was agreed that further discussion on this substance, and the various end points, will take place at the next meeting.

The meeting was then concluded. ECB thanked the participants for their valuable contributions and reminded of the deadlines for the next meeting.

Summary record from the TC C&L meeting in Arona, 4-5 October 2006 (ECBI/13/07 Rev.2)

Perfluorooctanic acid (PFOA) [1] (N002a)

(EC number : 206-397-9 [1], CAS number : 335-67-1 [1])

Salts of PFOA (N002b):

Ammonium salt of PFOA, APFO [2]

Sodium salt of PFOA [3]

Potassium salt of PFOA [4]

Silver salt of PFOA [5]

Fluoride acid of PFOA [6]

Methyl ester of PFOA [7]

Ethyl ester of PFOA [8]

(CAS number : 3825-26-1 [2]

CAS number : 335-95-5 [3]

CAS number : 2395-00-8 [4]

CAS number : 335-93-3 [5]

CAS number : 335-66-0 [6]

CAS number : 376-27-2 [7]

CAS number : 3108-24-5 [8])

Not in Annex 1.

Classification proposal: Carc Cat 3; R 40, Repr Cat 2; R 61, Repr Cat 3; R 62, T; R 48/23, X n; R 20/22, R 48/22, Xi; R 36.

ECBI/18/06 REV. 1 N, REVISED C&L PROPOSAL FOR PFOA
ECBI/18/06, ADD 1
ECBI/18/06, ADD 2
ECBI/18/06, ADD 3

In **March 2006** it was agreed that further discussion on this substance, and the various end points, will take place at the next meeting.

ECB reported that there was already a discussion going on and that **N** had prepared a new proposal. There was also a document on data that was requested by the MS.

Carcinogenicity:

N started with carcinogenicity and explained the data base. When one compared the historical controls, the substance was a peroxisome proliferater. However compared with a classical peroxisome proliferater the substance in addition increased the liver weight. They stated that with regard to findings of Leydig cell tumours and pancreatic tumors they could not be disregarded to be important for humans.

UK preferred classification with Carc. Cat. 3. Leydig cell tumours in rats did not raise concern. The pancreatic tumors were not really relevant according to them. The whole data base was not robust enough for Carc. Cat 2.

NL and **IT** agreed to the position of the UK.

S and **DK** agreed with N and preferred classification with Carc. Cat. 2 based on the present data.

DE said that there were only tumours found in one species, and the criteria then said that Carc. Cat. 3 should be applied. **FR** agreed to that.

N replied that there were two species. Looking at the tumours for one strain there was a high background but for the other strain not. Also the adenomas cannot be dismissed.

NL asked about the mechanism and said that it it looked like a non-genotoxic mechanism only at high doses.

N replied that little was known about the mechanism and it was of course a borderline case between Carc. Cat. 2 and Carc. Cat. 3.

IND had submitted an abstract about the outcome of a pathology group. There is on-going work on the mechanism. PFOA is a phenobarbital inducer. That is why we have liver growth. The peroxisome proliferation is still under investigation. And also the pancreatic tumours are under discussion. **IND** agreed to Carc. Cat 3.

IND continued and wanted to comment on the nature of the substances. The test material tested 3 M FC143 that contained some branched chain isomers.

ECB replied that the intention would be to treat all substances similar.

NL said that there were some difference and the TC C&L should reflect on whether it would be possible to use the data for the ammonium salt for the other substances.

IND said that the only significant salt is the ammonium salt. We should not get into testing the other salts because it is not worth it.

Reprotoxicity:

N said that there was a new mouse study included in the revised proposal. The effects in the mouse were more severe than those in the rat. There was statistical significant litter absorption. Most of the offspring was alive but at 5 mg did not survive the first day. Delay in eye opening. She quoted the outcome of ECBI/18/06 Add. 3. The renal clearance in mice is lower in mice than in rats and in humans its even lower. That is why the mouse study should be considered.

UK said that the findings were confounded by marked maternal toxicity. They would therefore support Cat 3 for developmental effects.

S supported N as the maternal toxicity was not the reason for the findings. **DK** agreed to this.

DE said that the mouse reacts with absorptions to maternal toxicity and there is also effects at low doses were there is no maternal toxicity and the pup mortality is increased. The pup mortality is very rare in mouse. They therefore ended up with classification in Category 2

IND said the effects in mice were compromised by maternal toxicity.

NL agreed with DE and supported N because of the effects at the low doses.

UK pointed out that maternal toxicity was seen at all doses.

The **TC C&L** on the reasoning referred to above and supported by a majority of the experts agreed to Category 2 for development R61

At the last meeting co classification for fertility had already been agreed.

Acute Toxicity:

ECB said that Xn; R20/22 was agreed already for the ammonium salt.

NL said that for inhalation for ammonium and sodium salt would probably be possible to read across but for silver and fluoride acid and for the esters listed the inhalation route could be different.

FIN said that probably some of the substances were not on the market and it would be necessary only to classify those that were.

DE thought it was better to cover the toxicology for similar compounds as the market was changing and new similar products very well could be introduced.

ECB asked whether there should be split the entries for different compounds.

IND reported about the use pattern. The again stressed that the main use was ammonium salt. They thought it might be convenient to read-across to inhalation toxicity in this case as there was no intention from IND to conduct any further studies on the different compounds listed in the currently drafted entry.

ECB summarised that the TC C&L then would agree to read across inhalation toxicity. **NL** stressed that it should be minuted that the read-across was made out of practical reasons as referred to above and this should not be used as an example for read-across.

The acute toxicity by oral route was agreed without further discussion for all salts.

Repeated dose Toxicity:

IND said that there was an inhalation study where mortality occurred. They said that this would trigger R48/20.

N reported the data again and said that R48/23 was warranted.

DE agreed to the N proposal based on the presented data.

IND said that this was a question of interpretation. There was some uncertainty. The study had to be transformed as there was an outlier.

The **TC C&L** agreed to T; R48/23 as suggested by N. They also agreed to Xn; R48/22 agreed based on the N proposal.

S also wanted to discuss R48/24.

N did not suggest classification for dermal route since they thought there was not enough data. But they volunteered to have an additional look at the data available. Perhaps the data would rather justify R48/21.

IND said that the substance was absorbed through rat skin but this was not demonstrated in humans. There were significant differences. **IND** would send in data on this during the Follow-up period.

Irritancy:

The TC C&L agreed to Xi; R36 without further comments.

Conclusion :

The TC C&L agreed to the following classification proposal: Carc. Cat. 3; R40 - Repr. Cat 2; R61 - T; R48/23 - Xn; R20/22 -Xn; R48/22 - Xi; R36, further the following labeling was agreed: Symbol: T; R-phrases: 61-20/22-36-40-48/22-48/23 and S-phrases: 53-45.

All substances as listed in the draft entry were thereby classified but the read across was done based on pragmatism as no further data would be assumed to be available for these substances. The read across had not been discussed on the basis of different physical chemical properties and structure relationships between the different substances considered.