Committee for Risk Assessment
RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at Community level of
Perfluorooctanoic acid (PFOA)

ECHA/RAC/ CLH-O-0000002227-78-01/A1

EC number: 206-397-9
CAS number: 335-67-1

Adopted

2 December 2011

CLH Report

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Perfluorooctanoic acid (PFOA)

EC Number: 206-397-9

CAS Number: 335-67-1

The classification of Perfluorooctanic acid (PFOA) was agreed in the former TC C&L group. The discussion and conclusions from the TC C&L group on the classification of PFOA is included in Appendix I of this CLH dossier.

Appendix 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)

CONTENTS

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING....4

JUSTIFICATION....6

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES....6

1.1 Name and other identifiers of the substance....6

1.2 Composition of the substance....6

1.3 Physico-chemical properties....8

2 MANUFACTURE AND USES....10

2.1 Manufacture....10

2.2 Identified uses....10

2.3 Uses advised against....10

3 CLASSIFICATION AND LABELLING....10

3.1 Classification in Annex I of Directive 67/548/EEC....10

3.2 Self classification(s)....10

INFORMATION EXTRACTED FROM THE BACKGROUND DOCUMENT OF APFO....12

4 ENVIRONMENTAL FATE PROPERTIES....12

5 HUMAN HEALTH HAZARD ASSESSMENT....12

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)....12

5.2 Acute toxicity....13
5.2.1 Acute toxicity: oral....13
5.2.2 Acute toxicity: inhalation....15
5.2.3 Acute toxicity: dermal....15
5.2.4 Acute toxicity: other routes....16
5.2.5 Summary and discussion of acute toxicity....16

5.3 Irritation....18
5.3.1 Skin....18
5.3.2 Eye....19
5.3.3 Respiratory tract....19
5.3.4 Summary and discussion of irritation....20

5.4 Corrosivity....21

5.5 Sensitisation....22
5.5.1 Skin....22
5.5.2 Respiratory system....22
5.5.3 Summary and discussion of sensitisation....22

5.6 Repeated dose toxicity....22
5.6.1 Repeated dose toxicity: oral....22

5.6.2 Repeated dose toxicity: inhalation ... 25
5.6.3 Repeated dose toxicity: dermal ... 26
5.6.4 Other relevant information ... 27
5.6.5 Summary and discussion of repeated dose toxicity: ... 27

5.7 Mutagenicity ... 29
5.7.1 In vitro data ... 30
5.7.2 In vivo data ... 31
5.7.3 Human data ... 31
5.7.4 Other relevant information ... 31
5.7.5 Summary and discussion of mutagenicity ... 31

5.8 Carcinogenicity ... 32
5.8.1 Carcinogenicity: oral ... 32
5.8.2 Carcinogenicity: inhalation ... 35
5.8.3 Carcinogenicity: dermal ... 35
5.8.4 Carcinogenicity: human data ... 35
5.8.5 Other relevant information ... 35
5.8.6 Summary and discussion of carcinogenicity ... 35

5.9 Toxicity for reproduction ... 42
5.9.1 Effects on fertility ... 42
5.9.2 Developmental toxicity ... 44
5.9.3 Human data ... 53
5.9.4 Other relevant information ... 53
5.9.5 Summary and discussion of reproductive toxicity ... 53

5.10 Other effects ... 60

5.11 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response ... 61

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES ... 62

6.1 Explosivity ... 62

6.2 Flammability ... 62

6.3 Oxidising potential ... 62

7 ENVIRONMENTAL HAZARD ASSESSMENT ... 63

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS ... 64

REFERENCES ... 65

APPENDIX 1 ... 79

APPENDIX I Summary records for APFO/PFOA from the former TC C&L group ... 79

TABLES

Table 1: Summary of physico-chemical properties ... 8
Table 2: Acute toxicity, oral ... 13
Table 3: Acute toxicity, inhalation ... 15
Table 4: acute toxicity, dermal ... 15
Table 5: Irritation, skin ... 18
Table 6: Irritation, eye ... 19
Table 7: Sensitisation, skin ... 22
Table 8: Repeated dose toxicity, oral ... 22
Table 9: Repeated dose toxicity, inhalation ... 25
Table 10: Repeated dose toxicity, dermal ... 26
Table 11: Mutagenicity, in vitro data ... 30
Table 12: Mutagenicity, in vivo data ... 31
Table 13: Carcinogenicity, oral ... 32
Table 14: Reproduction, effects on fertility ... 42
Table 15: Reproduction, developmental toxicity ... 44
Table 16. Exposure of workers ... 60
Table 17. Exposure of the general population ... 60

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Perfluorooctanoic acid (PFOA)

EC Number: 206-397-9

CAS number: 335-67-1

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. 4, H332

Acute Tox. 3, H301

Eye Irrit. 2, H319

Proposed labelling:

Category 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

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 of primary interest. APFO and PFOA are sometimes used interchangeably as both PFO-anion and PFOA (neutral species) exist in solution.

For systemic effects it might be assumed that both substances (APFO and PFOA) are mainly available to cells with its physiological pH in form of the corresponding anion (PFO). That might be the central justification for read across for systemic effects.

For local effects available literature indicates that PFOA and APFO in water yield acidic pH values. The differences in the pH values are considered small and therefore read across for local effects is considered relevant. In addition no studies on the human health hazard of PFOA are performed. Therefore, we suggest basing the CLH-proposal for PFOA on a read-across from APFO. See the CLH dossier for APFO for the assessment of human health hazard for PFOA.

We have only included the CLH-proposal for the ammonium salt (APFO) at this stage because most of the studies are performed with APFO. Furthermore, we found it important to reach agreement on a harmonised classification of APFO/PFOA first, and then as a possible further step it could be considered to make CLH-proposals for the other salts as well. The other salts are as following: Sodium salt of PFOA CAS No: 335-95-5; Potassium salt of PFOA CAS No: 2395-00-8; Silver salt of PFOA, CAS No: 335-93-3; Fluoride acid of PFOA CAS No: 335-66-0; Methyl ester of PFOA CAS No: 376-27-2 and ethylester of PFOA CAS No: 3108-24-5.

1.1 Name and other identifiers of the substance

Chemical Name: Perfluorooctanic acid (PFOA)

EC Name: Pentadecafluorooctanoic acid (PFOA)

CAS Number: 335-67-1

IUPAC Name: Pentadecafluorooctanoic acid

1.2 Composition of the substance

Chemical Name: Perfluorooctanic acid (PFOA)

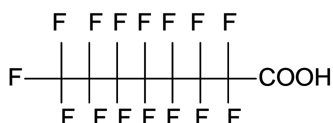
EC Number: 206-397-9 (PFOA)

CAS Number: 335-67-1 (PFOA)

IUPAC Name: Pentadecafluorooctanoic acid

Molecular Formula: C8HF15O2 (PFOA)

Structural Formula: PFOA



Molecular Weight:	PFOA: 414.09
Typical concentration (% w/w):	98% , impurities: not known.
Concentration range (% w/w):	

1.3 Physico-chemical properties

Table 1: Summary of physico-chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	[enter comment/reference or delete column]
VII, 7.1	Physical state at 20°C and 101.3 KPa	3.1	PFOA is a solid.	Kirk-Othmer, 1994
VII, 7.2	Melting/freezing point	3.2	PFOA: 52 – 54 °C PFOA: 54.3 °C	Kirk-Othmer, 1994 Lide, 2003
VII, 7.3	Boiling point	3.3	PFOA: 189 °C PFOA: 189-192 °C/736 mm Hg	Kirk-Othmer, 1994. Boit, 1975
VII, 7.4	Relative density	3.4 density	PFOA: Density/specific gravity. 1.792 g/ml	Kirk-Othmer, 1994
VII, 7.5	Vapour pressure (Pa)	3.6	PFOA: 4.2 (25 °C) extrapolation from measured data PFOA: 2.3 (20 °C) extrapolation from measured data PFOA: 128 (59.3 °C) measured	Kaiser et al., 2005; Washburn et al., 2005 Washburn et al., 2005 Washburn et al., 2005
VII, 7.6	Surface tension	3.10		
VII, 7.7	Water solubility (g/L)	3.8	 PFOA: 3.4 PFOA: 9.5 PFOA: 4.14	**Temperature (°C)** 20 °C (Merck, undated) 25 °C (Kauck and Diesslin, 1951) 22 °C (Prokop et al., 1989)
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	3.17		

	degradation products			
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		2.6, 1 g/l (20 °C)	Merck, 2005, (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

Industrial:

PFOA is used primarily to produce its salts, which are used as essential processing aids in the production of fluoropolymers and fluoroelastomers (68 FR 18626 (4/16/2003, available from http://www.epa.gov.). PFOA is used in fire-fighting applications, cosmetics, grease and lubricants, paints, polishes and adhesives, and in herbicide and insecticide formulations (Moody and Field, 2000). PFOA is also used to make Teflon (DuPont, Teflon, 2006).

General public:

PFOA is used in a variety of commercial applications as refrigerants, surfactants and polymers, and as components of pharmaceuticals, fire retardants, lubricants, adhesives, paints, cosmetics, paper coatings, and insecticides (3M company, 2000).

2.3 Uses advised against

3 CLASSIFICATION AND LABELLING

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

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

3.2 Self classification(s)

General aspects

The classification proposal for PFOA is restricted to the assessment of human health hazards. For PFOA studies on human health hazards are not available. The PFOA proposal completely refers to the classification proposal for its salt APFO which has been extensively tested in a broad spectrum of toxicological studies.

Reference to APFO data

Dossier submitter

The dossier submitter states that both substances (PFOA and APFO) are mainly available to cells and tissues (with its physiological pH) in form of the corresponding carboxylate anion (PFO). This matter of fact is considered to be the key justification for directly using the toxicological data from APFO for the PFOA assessment.

The dossier submitter indicated that the proposed DSD classification is identical to the classification proposal that was concluded by the former TC C&L group in October 2006.

The PFOA CLH report is no stand-alone document. There is full reference to the toxicological information in the APFO document.

Public consultation

There was no comment in the public consultation that addressed or questioned the validity of directly using the toxicological data from APFO for the assessment of PFOA.

Some of the comments referred to endpoint-specific classification proposals. However, these comments are not specific for PFOA; they relate to the toxicological APFO data and were submitted identically in the context of the APFO public consultation.

RAC conclusion

RAC takes note of the dossier submitter's proposal to establish a human health hazard classification for PFOA that is identical to the corresponding classification for its salt APFO. Testing substances in toxicological studies have generally been identified as APFO, but not as PFOA. The dossier submitter considers the APFO data directly relevant for the assessment of the systemic and local toxicity of PFOA.

This rationale is supported by RAC. RAC emphasises that both substances share a common active structure. Both substances will be available to cells and tissues in the form of the corresponding carboxylate anion.

The main difference between APFO and PFOA is the initial pH value when coming into contact with body surfaces. However, it is reported that both PFOA and APFO yield acidic pH values in water; possible differences in these local pH values at first sight do not question the validity of the approach for local toxicity as well.

Thus, although the dossier submitter and the TC C&L group did not discuss the possible impact of different physico-chemical properties of PFOA and APFO (e.g. solubility characteristics) on

relative systemic and local toxicity in detail, RAC accepts the basic justification that APFO and PFOA share a common active chemical structure (the carboxylate anion) and supports the dossier submitter's proposal to identically classify PFOA and APFO for human health hazards.

RAC concludes to use the final APFO classification proposal in order to finalise the classification proposal for PFOA. RAC recognises that the PFOA dossier is not a stand-alone document because it does not contain any toxicological data but completely refers to the corresponding chapters of the APFO document.

Given that RAC concluded that PFOA warrants the same classification as APFO, the rationale for classifying APFO is included in this opinion. RAC concludes, as mentioned above, that the argumentation is valid also for PFOA.

INFORMATION EXTRACTED FROM THE BACKGROUND DOCUMENT OF APFO

The following text is inserted from, and identical to, the same sections in the APFO background document.

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.

Additional information on toxicokinetics will be available in the Annex XV Report (in preparation):

PFOA has been found in human blood from all around the world and elevated concentrations are observed following specific exposure either via the environment (contaminated drinking water) or occupationally. The time trend studies show that PFOA levels are significantly associated with the time working as a ski waxer (Freberg et al., 2010, Nilsson et al., 2010b; Nilsson et al., 2010a). and some recent studies strongly indicate that PFOA levels increase with age (Haug et al., 2010, Haug et al., 2011).

PFOA has been shown to be readily transferred to the fetus through the placenta both in laboratory animals and humans. Further, breast milk is an important source of exposure to breast-fed infants and the PFOA exposure for these infants is considerably higher than for adults. Gestational and lactational exposure is of special concern as the foetus and newborn babies are highly vulnerable to toxicant exposure. [*]

5.2 Acute toxicity

5.2.1 Acute toxicity: oral

Table 2: Acute toxicity, oral

Species	LD_{50} (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

[*] Text added to the original report by the rapporteurs

			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 *(no info on TG used).* *All animals exhibited body weight gain throughout the study. All animals treated at 250 mg/kg appeared normal during the study except for two females that exhibited red-stained faces and/or wet urogenital area within 24 hours of test material administration. Clinical signs of toxicity observed in the animals treated with 500 mg/kg were: red-stained face, yellow stained or wet urogenital area, hypoactivity, hunched posture, staggered gait, and excessive salivation (clinical findings also cited from Kudo and Kawashima, 2003). There were no test-material related lesions observed at necropsy, although at 250 mg/kg, one male had a cannibalized right flank, one female had multiple dark brown areas in the glandular mucosa of the stomach, and a second female had a clear fluid in the lumen of the bilateral horns of the uterus. No more details regarding mortality was reported.**	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	LC_{50} (mg/l)	Exposure time (h/day)	Observations and Remarks	Ref.
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	LD_{50} (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 LD_{50} values around 200 mg/kg in males and females. The LD_{50} 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 the CLP criteria APFO is proposed to be classified as Acute Tox. 3 (H301) since LD_{50} values are reported between 50 mg/kg bw < ATE ≤ 300 mg/kg which are the limit ATE values for Acute Tox. 3.

Inhalation:

Following inhalation exposure of APFO an LC_{50} of 0.98 mg/l (4 hour exposure), and an LC_{50} > 18.6 mg/l (1 hour exposure) was reported. Based on the data and according to the Directive 67/548/EEC classification criteria APFO is considered to be classified as harmful with Xn; R20 (Harmful by inhalation). According to the CLP criteria the APFO dossier submitter originally proposed to classify as Acute Tox. 3 (H331). Later on the dossier submitter revised his proposal and suggested to classify as Acute Tox. 4 (H332) since LC_{50} values are reported between 0.5 mg/l < ATE ≤ 1.0 mg/l which are the limit ATE values for Acute Tox. 3.

Dermal:

Following dermal exposure, APFO/PFOA (test substance not identified) LD_{50} values greater than 2000 mg/kg were reported in New Zealand rabbits. Following dermal exposure to APFO an LD_{50} value at 4300 mg/kg was reported in male New Zealand rabbits, and an LD_{50} value at 7000 mg/kg in male rats and an LD_{50} value greater than 7500 mg/kg in female rats. Based on the data and the Directive 67/548/EEC classification criteria no classification for acute toxicity following dermal exposure is proposed. According to the CLP criteria APFO is not proposed to be classified for acute toxicity following dermal exposure since the LD_{50} values were higher than 2000 mg/kg.

RAC evaluation of Acute toxicity

Summary of the dossier submitter's proposal

Oral

According to the CLP criteria APFO is proposed to be classified as Acute Tox. 3, H301 since LD_{50} values are reported between 50 mg/kg bw < ATE ≤ 300 mg/kg, which are the limit ATE values for Acute Tox. 3.

Based on the data and the Directive 67/548/EEC criteria a classification as harmful with Xn; R22 (Harmful if swallowed) is proposed.

Inhalation

Following inhalation exposure of APFO an LC_{50} of 0.98 mg/L (4 hour exposure), and an LC_{50} > 18.6 mg/L (1 hour exposure) was reported. According to the Directive 67/548/EEC classification criteria the APFO dossier submitter proposed classification as harmful with Xn; R20 (Harmful by inhalation) as agreed at TC C&L. According to the CLP criteria the APFO dossier submitter originally proposed Acute Tox. 3 (H331) but in the revised version considered to classify as Acute Tox. 4 (H332) since relevant LC_{50} values were considered to be between 1.0 mg/l < ATE ≤ 5.0 mg/l by the TC C&L group.

Dermal
Based on the data and the Directive 67/548/EEC classification criteria no classification for acute toxicity following dermal exposure is proposed by the dossier submitter. According to the CLP criteria APFO is not proposed to be classified for acute toxicity following dermal exposure since the LD_{50} values were higher than 2000 mg/kg.

Comments received during public consultation

Several member states agreed in general to the proposed classification. In occasions where specific comments were given these were addressed further on.

Oral
One Member State expressed its agreement on R20/22, but raised concern on the CLP classification as Acute Tox. 3. As also requested in the accordance check the dossier submitter highlights the borderline situation between classes.
Others did not specifically refer to the classification proposal, most likely as it was already agreed by TC C&L in 2006.

Inhalation
One member state expressed its preference for Acute Tox. 4 (H332) based on discrepancies in LC_{50} (>4.5 (calculated from 18.6 mg/l at 1 hour exposure) and 0.98 mg/l/4 hr), which were also relevant for DSD classification as Xn; R20 (1< $LC_{50} \leq 5$ mg/l/4 hr).

Dermal
No specific comments.

Outcome of the RAC assessment - comparison with the criteria and justification

Oral
In the study of Glaza (1977) the lowest LD_{50} was reported to be between 250 and 500 mg/kg for female rats. Minor clinical signs such as coloured faeces and wet urogenital area were reported in females at 250 mg/kg, but no other signs of toxicity or mortalities were reported. Moribundity was reported for animals at 500 mg/kg. Details on the used test guideline and on whether mortalities occurred at all are unknown.

Other limited studies give indications on LD_{50} in the range of 200-250 mg/kg, also these studies are of limited validity due to lack of information. An LD_{50} at approximately 250 mg/kg was derived in newborn rats (Du Pont, 1983a). In Guinea pigs the LD_{50} was below 200 mg/kg(Du Pont, 1981f)..

In the most reliable study of Glaza no definitive mortalities below 300 mg/kg, the borderline dosage between category 3 and 4 (CLP) has been identified and other studies have neither characterised substance identity nor were conducted according to guideline protocols, RAC decided to propose Acute Tox. 4. Thus the original proposal of the dossier submitter on Acute Tox. 3 was not supported.

Based on the guidance value of 200 mg/kg a classification as harmful with Xn; R22 (Harmful if swallowed) is proposed along the Directive 67/548/EEC criteria.

Inhalation
Following inhalation exposure to APFO an LC_{50} of 0.98 mg/L (4 hour exposure) was identified at the borderline from Cat. 3 to Cat. 4. Another LC_{50} was > 18.6 mg/l after 1 hour inhalation, which corresponds to 4.6 mg/l for 4 hours and supports Cat. 4 as more appropriate.

Beyond the evidence from acute testing, data from repeated dose study could be taken into consideration. Mortalities observed on day 3 and during the fourth exposure in the repeated inhalation study on rats (Kennedy et al., 1986) are more relevant for acute toxicity than for chronic toxicity and support argumentation that Acute Tox. 3 (H331) could remain as proposed by dossier submitter. 84 mg/m³ caused mortality after third day (6 h/day) (84 mg/m³ x 18 h/4 h = 378 mg/m³ (0.378 mg/l). A value in this range can also be derived for the second death during the fourth exposure.

However RAC gave more weight to the supporting evidence from 1 hour testing than from mortalities after 18 hours of (interrupted) treatment. Although the exact value of 1 mg/l is the upper limit for Cat 3, RAC came to the overall conclusion was that LC_{50} is considered to be 1 mg/l and above.

With respect to the CLP criteria RAC decided to propose classification as Acute Tox. 4 (H332), since relevant LC_{50} values were considered to be in the range of 1.0 mg/l <ATE ≤ 5.0 mg/l. According to Directive 67/548/EEC RAC

agreed with the dossier submitter who proposed classification as harmful with Xn R20 (Harmful by inhalation) as agreed at TC C&L.

Dermal
RAC agrees that no classification should be proposed.

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 (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	Kennedy, 1985; Hazleton, 1990

					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 draw a conclusion regarding the classification of APFO (PFOA) for skin irritation.

Eye irritation:

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

RAC evaluation of skin and eye irritation
Summary of the dossier submitter's proposal **Skin** The dossier submitter concluded that data do not allow to draw a conclusion on the need for classification with regard to skin irritation. **Eye** The dossier submitter considered effects on eye irritation as borderline between Xi; R41 and Xi; R36 and referred to the decision of the TC C&L group who concluded on a classification as Xi; R36 (DSD). Accordingly APFO is proposed to be classified as Eye Irrit. 2 (H319) (CLP). ***Comments received during public consultation*** **Skin** No specific comments received. **Eye** One Member State expressed agreement with the CLP classification as Eye Irrit. 2 (H319) and DSD classification Xi, R36 as agreed by TC C& L. ***Outcome of the RAC assessment - comparison with the criteria and justification*** **Skin** Differences in the applied form of the test sample do not enable to explain the different outcome of the studies. Griffith and Long applied the test substance as dry and as moistened samples, while Kennedy (1995) applied an aqueous paste that resulted in mild to moderate erythema. The negative study of Griffith and Long as well as the mean values from Kennedy do not justify classification. In contrast, the study of Markoe (1983) revealed skin irritant effects including necrosis from 3 minutes of exposure that would require classification as corrosive. No more details are available (no access to the study report). RAC followed the argumentation that data are inconclusive. At present no proposal for classification was given. **Eye** RAC discussed the adequacy of a category 2 classification (CLP) and decided to deviate from the proposal of the dossier submitter due to consistent evidence from two studies. Although these studies were not compliant to the test guideline, corneal opacity (grade 4) and iris effects (grade 2) (observed in rabbits of the Griffith study) are lead effects that in combination with observed corneal ulceration (acute inhalation study, Kennedy et al., 1986) justify Eye Dam. 1 (CLP) and for the DSD Xi; R41 accordingly.

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 the Directive 67/548/EEC classification criteria and CLP criteria no classification for skin sensitisation is proposed.

RAC evaluation of skin sensitisation
Summary of the dossier submitter's proposal No classification for skin sensitisation is proposed due to insufficient data (skin) or lack of data (respiratory tract). ***Comments received during public consultation*** No relevant comments received. ***Outcome of the RAC assessment - comparison with the criteria and justification*** RAC agrees to not propose classification of the endpoint.

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	Duration of	Observations and Remarks	Ref.

	diet, ppm	treatment		
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. *After four days, rough hair coat and muscular weakness were evident in animals fed 3000 ppm or more APFO. Similar reactions and cyanosis were present in the 1000 ppm group after six days and in the 300 ppm group after nine days. Some 100 ppm animals had slight cyanosis on days 10 and 11 but appeared normal thereafter.*[*] 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. 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.	Christopher and Marisa, 1977; Griffith and Long, 1980
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 ppm 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,	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	Goldenthal, 1978a; Griffith and Long, 1980

[*] Text added to the original report by the rapporteurs

	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		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.	
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 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	13 weeks. 15 animals per group were sacrificed following 4, 7 and 13 weeks of treatment. 10 animals per group were sacrificed after 13 weeks of treatment and after a 8 weeks recovery period.	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 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.	Palazzolo, 1993
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	Goldenthal, 1978b; Griffith and Long, 1980

			mg/kg bw/day dose groups.	
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 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.	Thomford, 2001b; Butenhoff et al., 2002

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	Kennedy et al., 1986

				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 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, 200, 2000 mg/kg APFO, 10 applications dermal (6 hours/day, 5 days/week)* *5 rats/group killed at the end of treatment, on day 14 and on day 42 of 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 was seen in all groups at the end of treatment, in the two higher groups after 14 day recovery period and at the top dose at 42 days of recovery. 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-*	Kennedy, 1985

[*] Text from the original report modified by the rapporteurs

				related toxicity findings of clinical pathology resolved during a 42-day recovery period. After 10th treatment of 20, 200 and 2000 mg/kg incidences of rats with liver lesions were 2, 3 and 3 out of 5 rats per group. No data on severity, multifocal appearance or extension of lesions in the liver were reported. The number of animals with liver lesions as reported above decreased during recovery, but was still present in 1 of 5 rats at 20 and 2000 mg/kg. *Blood organofluoride concentrations were increased in all test groups with the concentrations decreasing during revovery. 52 ppm was obtained after 10th treatment in rats at 20 mg/kd bw/d APFO. This value is higher than values observed for comparable oral doses (300 ppm in feed (corresponding to 17.9 mg/kg in this dossier) for 90 days resulted in 38 ppm blood concentration in the oral study of Griffith and Long (1980).* *	
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 were 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 the 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/l a classification according to the Directive 67/548/EEC criteria with T; R

48/23 is proposed. According to the CLP criteria APFO is considered to be classified as STOT RE 1 (H372) since the guidance value for STOT RE 1 (inhalation exposure) is C ≤ 0.02 mg/l.

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.

RAC evaluation of repeated dose toxicity

Summary of the dossier submitter's proposal

Oral:

The dossier submitter considered that the effects reflecting repeated dose toxicity were on the borderline between Xn; R48/22 and T; R48/25, but referred to the decision of the former TC C&L group which concluded on a classification according to Directive 67/548/EEC with Xn; R48/22 . The proposal according to the CLP criteria is to classify as STOT RE 2, H373 since the guidance value for STOT RE 2 (oral exposure) is 10 < C ≤ 100 mg/kg bw/day.

Inhalation:

As agreed by TC C&L, the dossier submitter's proposal is based on the increased mortality and severe liver toxicity in rats at doses from 0.008 mg/l and proposes a classification according to the Directive 67/548/EEC criteria as T; R 48/23. The proposal according to the CLP criteria is STOT RE 1 (H372) since the guidance value for STOT RE 1 inhalation exposure is C ≤ 0.02 mg/l.

Dermal:

The dossier submitter suggested no classification for the route since no clear conclusion can be drawn from a 2 week study with 84 days recovery period in rats.

Comments received during public consultation

One Member State suggested to delete STOT RE 2 since it is covered by STOT RE 1 (H372) and informed that the route only needs to be specified if proven that no other routes causes hazardous effects.

Reflecting the liver as the target organ one Member State suggested modifying the hazard statement H372 for STOT RE 1: "Causes damage to organs (liver) through prolonged or repeated exposure."

Outcome of the RAC assessment - comparison with the criteria and justification

With respect to the CLP Regulation, the RAC agreed to propose classification as STOT RE 1 and hazard statement H372 to be phrased: "Causes damage to organs (liver) through prolonged or repeated exposure".

RAC also agreed with the proposal on a classification according to the Directive 67/548/EEC criteria as T; R 48/23 for the inhalation route and as Xn; R48/22 for the oral route.

Adverse effects that are of relevance for the oral route are mortalities, reduced body weight gain, cyanosis and liver cell degeneration and necrosis. Effects that are expected to be related to peroxisome proliferation such as liver weight increase, liver cell hypertrophy were not regarded and

would not if occurring alone will justify classification (see CLP guidance, 3.9.2.5.3). Remaining effects that justify classification are: Delayed mortalities at ≥300 ppm (15 mg/kg/d), reduced body weigh gain liver cell degeneration and necrosis at ≥30 ppm (1.5 mg/kg/d) and dose-related onset of cyanosis (≥100 ppm (5 mg/kg/d) in mice (28-day study (Christopher and Marisa, 1977); reduced body weight gain in rats at 1000 ppm (50 mg/kg/d) (28-day study, Metrick and Marisa, 1977); reduced body weight gain in rats at 100 ppm (6.5 mg/kg/d) (13 week study, Palazzolo, 1993); mortalities, bad general health state and immunosuppression in Rhesus monkeys at ≥30 mg/kg/d (90-day study, Goldenthal 1978b), general toxicity and increased liver weight at 30 mg/kg/d in Cynomolgus monkeys (where PPARα should not be active). Liver cell necrosis was also observed in rats exposed to APFO for 90 days (Goldenthal, 1978a). However, no clear dose response (only 5 animals/sex/group!) was seen for this effect. Comparison with the guidance values of the classification criteria reveal that some of the observed effects may be considered to justify T, R48/25, however, lacking of data on severity and incidences from the documentation of this report do allow only rough evaluation.

According to the CLP criteria the final classification shall be the most severe classification of the three routes. This also covers that oral toxicity from repeated dose studies was also a borderline case for STOT RE 1.

The criteria say that if it is shown that classification for this endpoint is not required for a specific route, then this can be included in the hazard statement. With respect to the dermal route data are insufficient to prove that the dermal route could be excluded. The available dermal study (Kennedy, 1985) indicated that liver cell necrosis was observed from 20 mg/kg bw/d onwards after 2 weeks of treatment and remained up to 42 days of recovery. This is far below the guidance values for the dermal route which are 100 mg/kg/d (DSD) (corresponding values for 28 days: 321 mg/kg and for 14 days 643 mg/kg bw/d) respectively 200 mg/kg/d (CLP) for a 90 day-study.

Target organ and toxic effects in the dermal rat study are consistent to those seen in repeated dose tests using oral and inhalation routes. Although the study is limited (mainly due to its shortness of 14 day treatment period and lack of details on grading histopathological findings), liver findings are supporting the conclusion that all routes are effective. External doses of about 20 mg/kg bw/d resulted in comparable organofluoride concentrations after 90 days of oral exposure to that after 10 dermal applications. This fact and the observation of liver toxicity after repeated dermal exposure give evidence on the dermal route as of relevance.

Thus there is no reason to include information on the dermal route to be excluded in the hazard statement according to CLP. On the other hand toxicity by the dermal route is already covered by STOT RE 1.

Moreover RAC decided to propose R48/21 based on the observation of liver toxicity from 20 mg/kg bw/d in a dermal 14 day study in rats. The LOAEL for liver toxicity of 20 mg/kg (which is much lower than the corresponding dermal guidance values (for Cat. 1) of 60 mg/kg for a 28 day study) might also argue for a higher classification. However taking the limits of the dermal repeated dose study into account (mainly due to limited information on severity of liver lesions) the proposal of R48/21 is thought to be adequate.

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 aberrations (CA)	Chinese Hamster Ovary (CHO) cells	Range finding assay: 0.169 to 5080 µg/mL. Initial study: 62.5 to 4000 µg/mL. Confirmatory study: 50 to 3000 µg/mL.	+/-	APFO was tested twice for CA in CHO 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.	Murli, 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	$C_3H\ 10R_{1/2}$ mouse	0.1, 1.0,	None.	The cell transformation was	Garry

transformation and cytotoxicity assay	embryo fibroblasts	10, 50, 100 and 200 µg/mL.		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 (LD_{50}) was 50 µg/mL. GLP. No.	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 the Directive 67/548/EEC criteria or CLP criteria for mutagenicity is proposed.

RAC evaluation of germ cell mutagenicity (Mutagenicity)

Summary of the dossier submitter's proposal

The dossier submitter concluded that based on the available negative *in vitro* and *in vivo* studies APFO is considered not mutagenic, and no classification according to the Directive 67/548/EEC criteria or CLP criteria for mutagenicity is proposed.

Comments received during public consultation

Within a general agreement several Member State agreed on proposed non-classification as agreed by TC C&L.

Outcome of the RAC assessment - comparison with the criteria and justification

Based on negative results from in vivo Micronucleus assays and negative in vitro tests RAC agrees to not propose classification of the endpoint.

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 gain in males (high dose -21% by week 6, over 10% through 66 weeks of the study, significant until week 98. Low dose: 5% decrease in bw gain at week 6, little thereafter), and to a lesser extent in females (slightly decreased, maximum 11%, at 92 weeks) was reported, and the decrease was considered treatment-related. There were no differences in mortality between treated and untreated groups. Significant decreases in red blood cell counts, haemoglobin concentrations and hematocrit values were observed in the high dose male and female rats. Clinical chemistry changes included slight (<2fold) but significant increases in ALT, AST and AP in both treated male groups from 3-18 months, but only in high dose males at 24 months. Slight (<10%) increases in abs/rel liver and kidney weights were noted in high dose male and female rats at 1 year interim sacrifice and at terminal necropsy. Only the rel liver weights in high dose males were significant (p<0.05). 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*	Sibinski, 1987;

			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, marked aspermatogenesis was found in 2/15 in high-dosed males but not in the controls. At the 2-year sacrifice, testicular masses were found in 6/50high dosed and 1/50low-dosed rats compared to 0/50 in controls. 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 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. With 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, 76 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 induced hepatic aromatase activity (P450-19A1[1], demonstrated in a 14 day study, Liu et al, 1996) and a sustained increase in serum estradiol were reported. In addition, the treated group had a significant increase 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, and β-oxidation (but not cell proliferation)) were elevated well before the first occurrence of liver adenomas, which occurred after 12 month of treatment. No effect on peroxisomal β-oxidation in Leydig cells was observed during the study and at the end of study. There were no biologically meaningful differences in serum hormones (testosterone, FSH, prolactin, or LH concentrations) except for serum estradiol concentrations in treated rats. Pancreatic*	Cook et al., 1994; Biegel et al., 2001 Liu et al, 1996

* Text from the original report modified by the rapporteurs

[1] P450-19A1 the cytochrome P450 monooxygenase which converts androgens to estrogens

			cell proliferation was significantly increased at 15, 18, and 21 months, but no increased proliferation was observed at 9 or 12 months. *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.**	

* Text from the original report modified by the rapporteurs

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. 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. *However, a recent study show that the administration of APFO to rats leads to hepatomegaly observed as hypertrophy and hyperplasia as a result of early increases in cell proliferation (but no inhibition of apoptosis) , which ultimately leads to liver tumour formation. These data clearly demonstrate an early hepatocellular proliferative response to APFO treatment and suggest that the hepatomegaly and tumours observed after chronic dietary exposure of S-D rats to APFO likely are due to a proliferative response to combined activation of PPAR and CAR/PXR. (Elcombe et al 2010). This mode of action is unlikely to pose a human hepatocarcinogenic hazard as demonstrated in studies utilizing mice humanized with respect to the xenosensor nuclear receptors, the activation of the human PPARα, CAR, and PXR does not appear to lead to cell proliferation (Cheung et al. 2004; Gonzalez and Shah 2008; Shah et al. 2007; Ross et al. 2010).*[*]

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.

[*] Text added to the original report by the rapporteurs

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 the Directive 67/548/EEC criteria as Carc. Cat. 3; R40 and according to the CLP criteria APFO is proposed to be classified as Carc. 2 (H351).

RAC evaluation of carcinogenicity

Summary of the dossier submitter's proposal

The dossier submitter concluded that based on the liver adenomas, Leydig cell adenomas and pancreatic acinar cell tumours in rats to propose classification as Carc. 2 (H351) according to the CLP criteria, and as already proposed by TC C&L as Carc. Cat. 3; R40 according to the Dir 67/548/EEC criteria. For these tumors there are insufficient data on the mode of action to conclude that tumours are not relevant for humans.

Comments received during public consultation

Several Member States have given their consent on the dossier submitter's proposal. There are a number of concerns against classification which were raised by Industry (see the comments on additionally proposed references in Annex 2*)*.

Outcome of the RAC assessment - comparison with the criteria and justification

There are two carcinogenicity studies on APFO in Sprague-Dawley rats that showed increased liver adenomas, Leydig cell adenomas and pancreatic cell tumours in male rats. Increased rates of mammary fibroadenomas were seen in female rats. However due to high incidence in the control female group evidence for carcinogenic potential of APFO in female rats is equivocal.

Table 13A: Summary on neoplastic and non-neoplastic lesions from carcinogenicity studies in rats

Sprague-Dawley rats	Sibinsky, 1987 50 rats/sex/group 2 year 15 rats/sex/group 1 year			Cook et al., 1994, Biegel et al., 2001 76 males at 300 ppm, 80 control males		Historical control values for S-D rats#
Ppm Mg/kg bw/d	0	30 1.3	300 14.2	0	300	
Liver						
2 year study						
Liver cell adenomas				2.5% (2/80)	13% (10/76)	
Hyperplastic nodules	0%/ 0%		6%/0%			
Liver cell megalocytosis	0% / 0%[$]	12% / 2%	80% / 16%			
Cystoid degeneration	8%/0%	14%/0%	56%/0%			

1 year						
Liver cell megalocytosis	0 % / 0%*		80% / %			
Portal mononuclear cell infiltration	47% /0%		80% / 0%			
Hepatocellular necrosis	0% /0%		40% / 0%			
Hepatocellular vacuolation	- / 33%		- / 73%			
Testis						
2-year						
Testicular masses[&]	0%/-	2%/-	12%/-			
Leydig cell adenomas	0%/-	4%/-	14%[#/-]	0% (0/80)	11%* (8/76)	5% Clegg et al., 1997 0.82% Chandra et al., 1992
Leydig cell hyperplasia				14% (11/80)	46%[#] (35/76)	
Vascular mineralisation	0%/-	6%/-	18%#/-			
1 year						
Aspermatogenesis	0%/-		13%/-			
Ovary						
2-year						
(Original) Tubular hyperplasia	- / 0%	- / 14%	- / 32%#			
§Stromal hyperplasia	- / 8%	- / 16%	- / 15%			
§Stromal adenoma	- / 4%	- / 0%	- / 2%			
§Combined stromal hyperplasia and adenoma	- / 12%	- / 16%	- / 17%			
Mamma						
2-year						
Fibroadenoma	- /21%	-/ 40%#	-/ 43%#			18% or 37%

	(10/47)	(19/47)	(21/49)			Sykes, 1987 19% Chandra et al., 1992
Pancreas						
Acinar cell adenoma	0% / -	6% (2/34 males	3% (1/34 males)	0% (0/80)	9%* (7/76)	0.22% Chandra et al., 1992
Acinar cell carcinoma				0% (0/80)	1% (1/76)	
Acinar cell hyperplasia				18% (14/80)	39%* (30/76)	

$Percentages in males/females

#No data from laboratory control values

§ ovarian lesions rediagnosed in Mann and Frame, 2004

* significantly different from pair-fed control group, p<0.05

significantly different from ad-libitum control group, p<0.05

[&] There is an inconsistency in the OECD SIDS report which says that at the one year sacrifice, testicular masses were found 6/50 high-dose and 1/50 low-dose rats, but not in any of the controls. As no low dose animals were tested at the one year schedule, it is assumed to be a mistake and the effect is related to the 2-year data. No lesions corresponding to the masses were reported in groups of the 1-year sacrifice.

ά no data on incidences on females given in the CLH report

Liver tumours

Liver tumours in rodents that are conclusively linked to peroxisome proliferation are proposed not to be of relevance for humans (CLP guidance, 3.6.2.3.2 (k)).

No evidence on increased hepatic cell proliferation was estimated at interim time points (1 month – 21 months) during the carcinogenicity study (Biegel et al., 2001). While in the original CLH dossier the dossier submitter concluded that there is no (or not yet) evidence on PPARα-related clonal expansion of preneoplastic foci, a recently published study was able to show that administration of APFO to rats leads to hypertrophy and hyperplasia (without any microscopical/biochemical evidence of liver cell toxicity) as a result of early increases in cell proliferation (but no inhibition of apoptosis), which ultimately leads to liver tumour formation (Elcombe et al., 2010). These data clearly demonstrate an early hepatocellular proliferative response to APFO treatment and suggest that the hepatomegaly and tumours observed after chronic dietary exposure of S-D rats to APFO likely are due to a proliferative response to combined activation of PPAR and CAR/PXR. This mode of action is unlikely to pose a human hepatocarcinogenic hazard as demonstrated in studies

utilizing mice humanized with respect to the xenosensor nuclear receptors, the activation of the human PPARα, CAR, and PXR does not appear to lead to cell proliferation (Cheung et al. 2004; Gonzalez and Shah 2008; Shah et al. 2007; Ross et al. 2010).

Supporting evidence

In addition, there was increase in liver weights (partly due to liver cell hypertrophy), but no indication of hepatic cell proliferation and PPARα-activity in a 6-month cynomolgus monkey study (Butenhoff et al., 2002).

Evidence from PPARα-receptor knockout mice to increase liver weight gives some evidence on other modes contributing to the liver tumours. This observation is in line with findings on developmental toxicity from the study of Abbott et al. (2007), where testing in knock-out mice did not abolish the increase in liver weight.

Elcombe hypothesised that APFO increases mitochondrial mass in rats and monkeys (not shown in mice?) that may in part account for liver weight increase. In monkeys, APFO administration resulted in a marked increase in mitochondrial succinate dehydrogenase (SDH) activity that was thought to explain the dose-related liver weight increases (Butenhoff et al., 2002). However this interpretation is subject to uncertainties since increases in SDH activity did not show dose-dependency in this study. Nevertheless studies show that APFO interferes with mitochondrial activity. Livers from adult male Sprague–Dawley rats that received a 30 mg/kg daily oral dose of APFO for 28 days showed increased PPARγ coactivator-1α (Pgc-1α) protein, a regulator of mitochondrial biogenesis and transcription of mitochondrial genes, leading to a doubling of mtDNA copy number. Further, transcription of genes encoded by mtDNA was 3–4 times greater than that of nuclear encoded genes, suggestive of a preferential induction of mtDNA transcription. Implication of the Pgc-1α pathway is consistent with PPARγ transactivation by PFOA (Walters et al. 2009). Increased mtDNA copy number were already observed 3 days after a single ip injection of 100 mg/kg bw (Berthiaume and Wallace 2002).

PPARγ transactivation by APFO were also concluded from dose-related increase in PPARγ mRNA in PPARα-null mice, while only slightly in *h*PPARα-mice was observed (Nakagawa et al. 2011)

In conclusion, much of the response to APFO can be attributed to PPARα and induction of PPARα regulated genes. The impact of activation of PPARγ-regulated genes that are proposed to interfere with mitochondrial DNA transcription biogenesis and with lipid and glucose metabolism on tumour growth is not known to the rapporteurs.

Beyond the question on whether biological responses related to activation of PPARα are of relevance for humans, there is still some degree of uncertainties with the significance of other nuclear receptor activation on tumour growth and RAC follows argumentation of the dossier submitter that other mode of actions can not fully be excluded.

Leydig cell tumours

The RAC agreed with the conclusion of the dossier submitter that there is insufficient evidence to link these tumours to PPARα. Biegel et al. (2001) demonstrated that APFO did not induce peroxisomes in Leydig cells. Another not yet identified mode of action than peroxisome proliferation must be active. Increases in serum estradiol throughout the study (Biegel et al., 2001) may indicate that hormonal mechanism might be involved, while no effect on testosterone biosynthesis has been shown.

14 day gavage administration of APFO up to 40 mg/kg bw/d to rats showed that increases in serum

estradiol concentration corresponded to increased hepatic aromatase activity (Liu et al., 1996). However, studies on estrogens demonstrated proliferative effects and tumours of the Leydig cell almost exclusively in the mouse rather than in the rat (Review in Cook et al, 1999).

Pancreatic acinar cell tumours

Increased tumour rates were observed in two carcinogenicity studies. However, the original study of Sibinski reported no significant increase in tumours rather than higher incidences of acinar cell hyperplasia (no details available), while the confirmatory mechanistic carcinogenicity study of Biegel et al. revealed significantly increased rates of acinar cell tumours and of the correspondent hyperplasia.

The dossier submitter proposed that 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]). Growth factor were also discussed by Biegel et al. (2001) as stimulative for pancreatic acinar cells without giving any proof whether CCK has been changed by treatment. No evidence is given by any of the repeated dose studies to support hypothesis that APFO enhances cholesterol/triglyceride excretion, thereby increases fat content in the gut and causes tumour growth in pancreatic acinar cells.

It is not clear to which effect pancreatic acinar cells are linked in the liver. Biegel et al. mentioned cholestasis related increases in CCK plasma concentrations for other peroxisome proliferators, but no such effect was reported for APFO. For APFO it can be concluded that at present the mode of action of pancreatic cell adenomas is unknown.

> Reference is also given to the EPA Guidance document on PPAR"-Mediated Hepatocarcinogenesis in Rodents and Relevance to Human Health Risk Assessments (EPA, 2003) that stated "In addition to inducing hepatocarcinogenesis in rodents, PPAR" agonists have also been observed to induce pancreatic acinar cell and Leydig cell tumors in rats. Of 15 PPAR" agonists tested to date, nine have been shown to induce all three tumors in non-F344 rat strains but not in mice. In the case of Leydig cell tumor formation, two potential modes of action based on activation of PPAR" have been proposed. One mode of action invokes the induction of hepatic aromatase activity leading to an increase in serum estradiol level. The second mode of action purports that PPAR" agonists inhibit testosterone biosynthesis. Although agonism of PPAR" may lead to the induction of aromatase or inhibition of testosterone biosynthesis, the data available to date are insufficient to support which, if either of these two proposed modes of action is operative. For pancreatic acinar cell tumor (PACT) formation, a mode of action has been proposed in which PPAR"-agonists cause a decrease in bile acid synthesis and/or change the composition of the bile acid resulting in cholestasis. These steps increase the level of the growth factor cholecystokinin (CCK) which then binds to its receptor, CCKA, leading to acinar cell proliferation. Some evidence exists to support this proposed mode of action and there does not appear to be evidence of any other mode of action operating in the formation of PACTs after exposure to PPAR" agonists. However, the data are not considered sufficient to establish a mode of action with confidence, because it has only been described for two chemicals, PFOA and WY14643, in one laboratory. As a result, the evidence is considered insufficient to infer that this mode of action may be generalized to all PACT-inducing PPAR" agonists."

In conclusion RAC followed the proposal of the dossier submitter to propose that APFO should be classified according to the Directive 67/548/EEC criteria as Carc. Cat. 3; R40, and according to the CLP criteria APFO is proposed to be 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 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	York, 2002; Butenhoff et al., 2004)

				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≤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≤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 during pregnancy	Observations and Remarks	Ref.
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	Gortner, 1982

				(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.	
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 *Respirable particles <10 µm 77% - 90% (MMAD 1.4-3.4 µm ±4.3-6.0)*[*]	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. In 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 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.	Staples et al., 1984

[*] Text added to the original report by the rapporteurs

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. Maternal body weight including an adjustment for gravid uterine weight and liver weight produced statistically significant differences from controls only at the highest dose (20 mg/kg).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 was reported as well. The delay in ossification was especially prominent in the 10 and 20 mg/kg bw/day dose groups, but reduced limb ossification sites and reduced ossification of calvaria was observed from 1 mg/kg bw/day. 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 and significantly lower at ≥5 mg/kg bw/d. 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 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*	Lau et al., 2006

* Text from the original report modified by the rapporteurs

				*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. ***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	York, 2002; Butenhoff et al., 2004

				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≤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≤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 on 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 during early pregnancy 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. *A recent study from the same group (Zhao et al., 2010) elaborates on the mechanisms underlying the effect of PFOA/APFO on mammary gland development in C57Bl/6 mice and the possible dependence of this effect of PPARα-activity. The authors report that mammary gland stimulation in C57BL/6 mice by PFOA was observed in both PPARα KO and WT mice. PFOA treatment significantly increased serum progesterone levels in ovary-intact mice and lead to elevated mammary gland levels of several growth factor receptors, growth hormones and proliferation markers in both wild-type and PPARα knockout mice. The results indicate that PFOA stimulates mammary gland development in C57Bl/6 mice by promoting steroid hormone production in ovaries and increasing the levels of a number of growth factors in mammary glands.*[*]

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.

[*] Text added to the original report by the rapporteurs

In the Yahia et al. study (2010) exposure to pregnant ICR mice given 0, 1, 5 and 10 mg/kg PFOA (90% purity) daily by gavage from GD 0 to 17 and 18. Five to nine dam were sacrificed on GD 18 for prenatal evaluation; other 10 dams were left to give birth. No maternal deaths were observed. In dams liver weigths increased dose-dependently, hepatocellular hypertrophy and increased mitosis was observed at all concentrations, while reduced body weight gain, single cell necrosis and mild calcification was reported to occur only at 10 mg/kg. PFOA at 10 mg/kg increased serum enzyme activities (GGT, ALT, AST and ALP) with hypoproteinemia and hypolipidemia. PFOA treatment reduced the fetal body weight at 5 (-8%) and 10 mg/kg (-29%). Teratological evaluation showed delayed ossification of the sternum and phalanges and delayed eruption of incisors at 10 mg/kg. Postnatal evaluation revealed reduced neonatal survival at 5 and 10 mg/kg. At 5 mg/kg pups were born alive and active and 16% died within 4 days observation, while all died within 6 hr after birth at 10 mg/kg. [*]

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 doses of 5 mg/kg bw/day and above 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. *PFOA significantly increased relative liver weight in both WT and KO adult females and weaned pups. The lowest dose at which relative liver weight was significantly increased was 0.1 mg/kg bw/day in WT pups or 1 mg/kg bw/day in WT adult females and 3 mg/kg bw/day in the KO adults and pups. There was a trend of increased relative liver weights also in KO pups from 0.1 mg/kg bw/day, but the variation seemed to be greater in this group than in WT and adult animals. An additional group of heterozygous litters were produced in WT and KO dams and exposed to PFOA during gestation to study the effects of maternal toxicity on pup survival. Survival was significantly reduced for the heterozygote pups born to both WT and KO dams indication that pup mortality is caused by a PPARα dependent effect in the exposed pups.** 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.

In a study by Palkar et al. (2010), exposure to the two PPARα agonists clofibrate or Wy-14,643 did not cause the developmental anomalies observed in comparable developmental studies with APFO. The authors suggests that the apparent disparity between the PPARα-dependent effects observed in the PFOA-studies and the lack of effects in response to clofibrate or Wy-14,643 could be due to a possible difference in the PPARα induced gene expression and/or to differences in bioaccumulation. Clofibrate and Wy-14,643 have significantly shorter half-lives than PFOA. Thus, prenatal exposure could cause an accumulation of PFOA in fetal liver that subsequently influences postnatal development due to a sustained PPARα activity. This study underlines that the mechanisms of PPARα-associated developmental toxicity of PFOA is far from clear and that the human relevance can not be disregarded. Furthermore, a recent study (Abbott et al., 2010) examined the expression of PPARα mRNA and protein during human fetal development. PPARα

[*] Text added to the original report by the rapporteurs

was shown to be highly expressed in the human fetal liver making and interaction between PFOA and PPARα in the fetal and newborn liver highly likely.

The study by Palkar et al. (2010) provides additional information on the possible importance of PPARα-mediated, moderate hepatomegaly in dams for developmental effects in offspring. Mice, KO and WT for PPARα were exposed to the high affinity PPARα-agonists clofibrate and WY-14,643 during gestation days 1-18 to examine whether a modest activation of PPARα in dams leads to developmental toxicity. In this study, both agonists increased the relative liver weight of the dams, but they did not induce effects on pup survival and development as seen in the studies with APFO. This study strongly indicates that the APFO/PFOA induced effects on offspring are not secondary to the maternal liver effects seen at the doses leading to developmental toxicity.[*]

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 et al., 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 cord versus maternal plasma (median: 2.6 µg/l vs. 3.4 µg/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 was indicated. Several other human studies have reported detectable concentrations of PFOA *and other PFCs in umbilical cord blood (Apelberg et al., 2007 and Fei et al., 2007), and concentrations of PFOA in cord blood were highly correlated with the corresponding concentrations in maternal serum at the time of delivery (Monroy et al., 2008). In addition, transfer efficiency of PFCs from maternal to cord serum increase with shorter carbon-chain length (Kim et al., 2011), and branched isomers pass more easily than their linear counterparts. Hence, PFOA pass the placenta more readily compared to other long chained PFCs (Kim et al., 2011). **

The half-live in humans for PFOA has been estimated to be 3.8 years (Olsen et al., 2007). The compound is thus persistent and bio-accumulative in humans and the foetus which is in contrast to mice and rats with a half life of PFOA of around 30 to 60 days in mouse and from 1 to 30 days in rat (Tatum-Gibbs et al., 2011). A study by Harada et al. (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.

Serum levels of PFOA in mice or rat showing developmental toxicity are more than 1000-10000x higher than the serum concentration measured in the human general population (Olsen et al., 2009). In humans, an inverse correlation between PFOA and birth weight, ponderal index and

[*] Text added to the original report by the rapporteurs

[*] Text added to the original report by the rapporteurs

*head circumference has been reported in two larger cohort studies (Apelberg et al., 2007; Fei et al., 2007) in 293 cord samples or 214 sample pairs respectively, however, other cohorts did not find any correlation with birth outcomes, as reviewed in Olsen et al. (Olsen et al., 2009). A probable explanation may be that the human serum levels of PFOA are too low to show any correlation with birth outcomes in many of these cohort cases, and thus we cannot conclude that there is no developmental toxicity hazard connected to elevated PFOA levels in humans.**

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.

RAC evaluation of reproductive toxicity/Fertility
Summary of the dossier submitter's proposal

No classification on fertility was proposed based on the outcome of a 2-generation study (York 2002, Butenhoff et al., 2004) and the lack of supporting evidence from repeated dose toxicity studies which gave no indication on disturbances of fertility. The increased incidence of Leydig cell tumours and vascular mineralisation in testes of rats receiving APFO for 2 years were not considered to be indicative for effects on fertility.

Comments received during public consultation

Several Member States agreed on that no classification is proposed for this endpoint as previously agreed at the TC C&L.

Outcome of the RAC assessment - comparison with the criteria and justification

Based on the previously available data RAC found it conclusive that no proposal to classify for fertility effects was proposed by the dossier submitter. The only effects in the 2-generation study were increased absolute weights of epididymis and seminal vesicles that probably is linked to body weight loss. No relevant effects in male and female animals were reported from the repeated dose toxicity studies and the 2-year carcinogenicity study in rats. The latter study revealed treatment-related testes tumours, which were not related to fertility effects.

An additional study on testosterone levels and male reproductive organ effects of APFO were published after submission of the CLH dossier: In male mice, oral APFO-treatment (0, 1 and 5 mg/kg bw/day) for 6 weeks of both wt, null- or humanized PPARα mice showed a statistically significant increase ($p<0.05$) in sperm morphology abnormalities at both concentrations, an increased incidence of abnormal seminiferous tubules and a statistically significant reduction ($p<0.05$) in plasma testosterone concentration in the wt mice (at 5 mg/kg bw/day) and the hPPARα mice at both concentrations, but none of these effects were observed in the null-mice. In addition, a statistically significant reduction ($p<0.05$) of the reproductive organ (epididymis and seminal vesicle + prostate gland) weight of the wt PPARα mice treated with the highest concentration was seen (Li et al., 2011). The authors reported inconsistencies of PPARα- expressed in interstitial Leydig cells or seminiforous tubule cells of testis in m PPARα-mice, but not in testis of *h*PPARα-mice (Cheung et al., 2004).

The RAC discussed the new study published in 2011 (Li et al., 2011) indicating a potential of adverse effect on the male mice reproductive system.

RAC concluded that evidence on impaired fertility through sperm abnormalities and reduced testosterone levels are not (yet) sufficient to overwrite the negative evidence from the 2-generation study and repeated dose toxicity. Reconsideration of the endpoint is recommended.

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≤0.05. *These effects were reported in the absence of maternal toxicity in F0 females. Significant decrease in absolute food consumption (but no treatment-related effect on relative food consumption) and body weight gain were observed in F1 females at 30 mg/kg APFO during postweaning, precohabitatin, gestation and lactation. No clear treatment-related effect was observed in the F2 generation. However, in rat developmental toxicity studies following oral or inhalation exposure to APFO minimal effects (e.g. rib variation (Gortner, 1982) were reported in the offspring.*

In a mouse developmental toxicity study (Lau et al., 2006) early pregnancy loss (full litter resorption from 5 mg/kg bw/d onwards), reduced postnatal survival (≥5 mg/kg), severely compromised postnatal survival (≥20 mg/kg), delays in general growth (≥3 mg/kg), and development (delay of eye opening ≥5 mg/kg), as well as sex-specific alterations in pubertal maturation (separable prepuce indicating earlier onset of male puberty ≥1 mg/kg), were reported. Significant lower body weight gain was observed in dams at 20 mg/kg and 40 mg/kg. Significant dose-related increases in liver weight was reported for all dose groups (≥1 mg/kg APFO). [*]

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

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

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

The developmental toxicity reported in mice had a different profile compared to the developmental toxicity reported in rats. The different findings in rats and mice are likely due to the different pharmacokinetics of APFO in rats and mice. *Renal elimination is high in rat females leading to a significantly lower serum concentration of PFOA in pregnant rats than in pregnant mice.*[*] In the study by Lau et al., 2006 the serum levels of APFO was measured in adult rats and mice receiving daily oral gave of APFO. In rats given 10 mg/kg bw/day for 20 days the serum levels of APFO were 111 µg/ml in males and 0.69 µg/ml in females, and in mice given 20 mg/kg bw/day for 17 days the serum levels were 199 µg/ml in males and 171 µg/ml in females. Furthermore, in pregnant

[*] Text from the original report modified by the rapporteurs

[*] Text added to the original report by the rapporteurs

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 to be classified as Repr. 1B (H360D).

RAC evaluation of reproductive toxicity/ developmental effects

Summary of the dossier submitter's proposal

The dossier submitter proposed to classify APFO as Repr. 1B (H360D) according to CLP criteria and Repr. Cat. 2; R61 according to DSD as concluded by TC C&L based on evidence for increased postnatal pup mortality, decreased pup body weight and delayed sexual maturation observed in several mice studies and the rat 2-generation study in the absence of marked maternal toxicity.

Comments received during public consultation

One Member State considered mouse studies more relevant than rat data, since the renal clearance is lower in mice than in rats and in humans. At TC C&L this point has led to a debate on whether the offspring effects are related to maternal toxicity, the majority agreed on classification as Repr. Cat. 2; R61. Several Member States supported classification on this endpoint as proposed by TC C&L.

Outcome of the RAC assessment - comparison with the criteria and justification

Human data

Available biomonitoring indicated that human serum concentrations were lower than those reported for the mice at 5 mg/kg APFO (max. about 50 µg/ml in dams (White et al., 2007) compared to 6.8 µg/ml (max arithmetic mean in workers, see Olsen studies) and median concentrations of 0.0026 µg/ml in maternal samples of a pilot study (Midasch et al., 2007)). Absence of effects are no proof that effects in animals were not relevant for humans, since internal concentrations were much lower and epidemiological studies were not targeted on the effects of interest and of insufficient size for effect detection.

Animal data

Critical for the proposal of Repr. 1B (according to the CLP criteria) and against a proposal of Repr. 2 are effects of developmental toxicity from animal studies that were observed at doses at which no (or no indications of marked) maternal toxicity has been observed.

Rat

Relevant effects indicating developmental toxicity were observed at doses without treatment-related effects on body/organ weights in dams of the F0 generation during lactation phase (mortalities and reduced growth) and caused delayed sexual maturation later on in the rat offspring of a 2-generation

study (York, 2002; Butenhoff et al., 2004). Effects on or via lactation have not been tested on in this species. No treatment-related effects were seen in the F2-generation.

Test substance administration to rats during the mid and late gestation period only (GD 6-15/18) did not cause adverse effects on rat offspring except a dose-related increase of rib variations in a study during GD 6-18. There were no developmental studies addressing effects of APFO in rats where treatment started in the early gestational phase.

Mouse

Without any sign of marked maternal toxicity, exposure during the gestational phase was effective in mice to cause developmental deficits; no malformations occurred. This was demonstrated by a number of studies; most recent studies were not present at TC C&L discussion in 2006.

Full litter resorptions

Most severe effects (whole litter loss in early pregnancy) were seen in the study of Wolf et al. (2007) when treatment with 5 mg/kg APFO started early at GD1.

Percentages of dams with full-litter resorptions significantly increased from 5 mg/kg onwards (26% at 5 mg/kg to 100% at 40 mg/kg) (Lau et al., 2006). Body weight gain started early (from GD5 onwards) to be significantly lower in dams at ≥20 mg/kg than in controls and was interpreted to indicate that full-litter resorption must have occurred in early pregnancy. It could be assumed that liver effects in dams at this early time of gestation are less pronounced than they may be at the end of gestation (as indicated by liver weight increase on GD18, no data on clinical pathology and microscopy). While maternal toxicity (reduced body weight gain) might be discussed to be linked to resorptions for the dams receiving 20 and 40 mg/kg, no effect on body weight was seen for the 5 mg/kg (26% full litter resorption) and 10 mg/kg (46% full litter resorption versus 7% in controls).

While these studies revealed (early) full litter resorptions, no such effect was seen up to 10 mg/kg PFOA in the developmental study of Yahia et al. (2010).

Other effects

Other developmental effects (reduced postnatal survival (≥5 mg/kg), severely compromised postnatal survival (≥20 mg/kg), delays in general growth (≥3 mg/kg), and development (delay of eye opening ≥5 mg/kg), as well as sex-specific alterations in pubertal maturation (separable prepuce indicating earlier onset of male puberty ≥1 mg/kg) were reported in the study of Lau et al. (2006).

Liver weight increases were seen in dams of all dose groups, but APFO treatment did not change the number of implantations. However, weight gain of dams indicating marked maternal toxicity was markedly reduced at 20 mg/kg bw/d or after correction for gravid uterine weight and liver weight only at 40 mg/kg bw/d (see RCOM doc). Significantly reduced postnatal survival could be discussed as secondary effects at ≥20 mg/kg bw/d. However dose-dependent increases in liver weight from 1 mg/kg onwards alone were not found to be plausibly linked to the adverse effects on pup growth and development in the study of Lau et al. (2006).

In utero exposure to 5 mg/kg APFO alone was sufficient to reduce pup growth and developmental delay in the pups (Wolf et al., 2007). Reduced postnatal survival in pups was seen at 5 mg/kg APFO if exposure in utero continued through the lactation period. No detrimental effect on maternal weight and number of live born pups was seen in groups receiving 3 and 5 mg APFO. 23 days after last treatment (on PND 22) there was a dose-dependent absolute and relative increase in liver weight in dams. Reduction of body weight of pups on PND 22 was dose-dependent and more severe after continued exposure via milk. This effect may be related to reduced milk production (some

indication from the study of White et al. (2007) that showed inhibition of the mammary gland differentiation before birth) or to direct effects of APFO on pups exposed via the milk only. While maternal weight gain was similar between groups of dams exposed to 5 mg/kg APFO and control dams in the White study, mean body weights and diminished (delayed) development of the mammary gland was seen in pups at PND 10 and 20. This means APFO affected the development of the mammary gland during pregnancy and affected development of the mammary gland in pups. In a follow up study (2009) Wolf demonstrated that delayed mammary gland development in pups at 5 mg/kg APFO also occurred under lactional-only dosing. Mean serum concentrations were reported to be similar in mice exposed in utero than in mice exposed via milk. Effects on mammary gland development could also be induced in mice after peripubertal treatment (at 21-50 days of age), however testing revealed some strain specifity (Yang et al., 2009).

In these studies no marked maternal toxicity has been observed and developmental effects could not be interpreted to be secondary to the maternal toxicity.

The delay in mammary development has been confirmed in the recently published mouse study in pups where the dams received doses of 0, 0.3, 1.0, and 3.0 mg/kg bw/d APFO from GD 1-17 (Maron et al., 2011). This effect persisted until PND 84. Offspring liver weights were significantly increased in all dose groups (no data on dam effects). In a second study mice were administered to 0, 0.01, 0.1, 1.0 mg/kg APFO bw/d in the late gestation phase only (GD 10-17). Stunted mammary epithelial growth was seen at PND 21 in the 0.01 mg/kg dose group, increased offspring liver weight was seen in the 1.0 mg/kg bw/d dose group indicating that the delay in mammary gland development is more sensitive than the liver effect in pups.

The RAC discussion focussed on the relevance of liver weight changes for developmental effects. Doses of APFO without any effect on body weight gain in dams (up to 5 mg/kg or even higher) should not be considered as marked maternal toxicity which according to the CLP guidance could justify no classification. Compared to the 28 day study in mice (Christophe and Marisa, 1977) where all mice at 300 ppm (15 mg/kg) died during the study and single premature deaths were seen at 30 (1.5 mg/kg) and 100 ppm, mortalities of dams in the Lau et al. study were not reported up to 40 mg/kg.

Guidance to CLP considers developmental effects even in the presence of maternal toxicity to be evidence of developmental toxicity unless it can be unequivocally demonstrated that these effects are secondary to maternal toxicity. In case a specific maternally mediated mechanism has been demonstrated, the guidance says that Cat 2 may be considered more appropriate than Cat 1. Developmental toxicity induced by repeated APFO administration were seen in a dose-related manner, also at doses without indication of marked maternal toxicity, appears not to be linked to maternal toxicity and no specific maternally mediated mechanism was identified.

Liver weight increase also at low doses without any effect on body weight gain and one might assumed that liver toxicity (if liver weight increase is interpreted as toxic effect) is the primary effect and developmental effects could be interpreted as secondary to liver toxicity. Unfortunately no other data are available from 2-generation and developmental studies on APFO to characterise liver weight increase (by microscopy or clinical pathology) with respect to its degenerative nature or as adaptive enzyme activation.

From a number of studies it was demonstrated that liver cell hypertrophy and related liver weight increase is the most sensitive effect and cytotoxicity was observed at higher doses. Hepatocellular hypertrophy and increased mitosis (no quantification available) were observed at all doses (no details on dose-dependency of incidences and severity); single cell necrosis and mild calcification were only seen at 10 mg/kg PFOA (Yahia et al., 2010). Corresponding effects at 10 mg/kg were

significantly increased liver transaminases (ALT, AST) and enzyme activities indicating membrane leakage (LDH, ALP). No microscopic degenerative abnormalities were reported for the dams' liver at 5 mg/kg, where foetal body weight and postnatal survival was already reduced. Assumed that at similar doses of APFO no marked liver cell toxicity had occurred, this indicates that developmental toxicity is not a consequence of liver toxicity.

The observation of increased cell proliferation at doses without overt liver toxicity in mice (Yahia et al, 2010) is consistent to the observation of Elcombe et al. (2010) of increased cell proliferation of liver cells at a non-cytotoxic dose in rats. This is considered to reflect the mitogenic nature of effect rather than a regenerative proliferation response at non-cytotoxic doses.

RAC recognises that there are signs of marked maternal toxicity at high doses. However liver weight increase alone could not be plausibly linked to developmental effects in pups. Dose-dependent increases in liver weight were seen in dams (and pups) most likely as a direct effect of APFO caused by liver cell hypertrophy with major contribution of PPARα-related peroxisome proliferation. Newer study clearly demonstrated that liver toxicity (single cell toxicity) started at higher doses than hypertrophic response. Therefore the observed developmental effects were not considered to be a secondary non-specific consequence of the maternal (liver) toxicity.

Studies in mice allow conclusion that gestational administration of APFO was sufficient to impair neonatal growth and development and that developmental toxicity was linked to the gestational phase of exposure.

Mechanistic studies using PPAR knock-out mice demonstrated that some effects (complete litter loss and liver weight increase in dams and pups) seem to be independent of PPARα expression (Abbott et al., 2007). Others such as increased postnatal pup mortality, reduction in pup body weight and postnatal growth and development (delayed eye opening) indicated interference/contribution of PPARα expression most likely as a direct effect of APFO (which is not mediated via liver cell response to PPARα). The observation that liver weight increases are similar in wild type dams and in PPARα-knock out dams and their respective offspring questioned the importance of PPARα expression for the liver effects. PPARα-related effects may contribute, but other modes of action must also be active.

In addition the relevance of PPARα expression for humans is well established for the liver, however much less is known for the relevance of PPARα-related effets in other organs and effects in the offspring and juvenile.

Comparison with the CLP criteria for reproductive Toxicity (Section 3.7.2)

Human data do not sufficiently give evidence to conclude on whether category 1A is appropriate. Category 2 would be appropriate if there is some, but less convincing evidence on adverse development effects. Overall there is no convincing evidence that developmental effects in pups are exclusively secondary to maternal (liver) toxicity.

For APFO there is clear evidence on developmental effects from perinatal studies in mice. Mechanistic considerations allow contribution of some effects to a PPARα-related mode of action. However other modes appear to be active and developmental effects could not be attributed to liver toxicity as a secondary mechanismn. Also the role of PPARα-related mode of action is not fully elucidated for the developmental effects. A contribution to some effects is assumed based on their lack of expression in knock-out mice.

Therefore RAC decided to follow the proposal of the dossier submitter that evidence is sufficiently convincing to classify for developmental effects as Repr. 1B (H360D) according to CLP criteria and

Repr. Cat 2; R61 according to DSD.

Criteria for hazard category for lactation effects

PFOA has also been found to be transferred to infants through breast-feeding. Although the criteria from human evidence and/or from results from two generation studies in animals do not provide effects in the offspring due to transfer in the mild or adverse effects on the quality of the milk, there is sufficient evidence from mouse studies with postnatal administration of APFO that indicated adverse effects (delayed/stunted mammary gland development in the offspring) which cause concern for the health of a breastfed child. Classification for effects on or via lactation is independent of whether or not a substance is also classified for reproductive toxicity.

In addition RAC agreed on an additional classification on lactation effects (H 362: May cause harm to breast-fed children and R64 May cause harm to breastfed babies).

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/mlL (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 specific exposure measures were used, and in this study no significant association for prostate cancer was observed (Alexander, 2001).	Olsen et al., 1998c; 1999; 2000; 2001a and c; 2003 a, b, e and f. DuPont2001a and b. Gilliland and Mandel, 1993; 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	3M Company, 1999a and b; Olsen et al., 2002 a, b and c; Olsen et al., 2003 d; Olsen et al., 2004a

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/mlL (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).	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 include 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 discussion and conclusion of the TC C&L group.

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.

Abbott BD, Wood CR, Watkins AM, Das KP and Lau CS. 2010. Peroxisome proliferator-activated receptors alpha, Beta, and gamma mRNA and protein expression in human fetal tissues. PPAR Res. 2010. pii: 690907.*

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.

Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL and Goldman LR. 2007. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environ Health Perspect. 115(11):1670-6.*

Berthiaume J, Wallace KB (2002) Perfluorooctanoate, perXourooctanesulfonate,and N-ethyl perXuorooctanesulfonamido ethanol; peroxisome proliferation and mitochondrial biogenesis. Toxicol Lett 129:23–32.*

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.

* Reference added to the original report by the rapporteurs or dossier submitter after public consultation

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.

Cheung C, Akiyam TE, Ward JM, Nicol CJ, Feigenbaum L, Vinson C, Gonzalez FJ. 2004. Diminished hepatocellular proliferation in mice humanized for the nuclear receptor peroxisome proliferator-activated receptor alpha. Cancer Res 64, 3849–3854.[*]

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.

[*] Reference added to the original report by the rapporteurs or dossier submitter after public consultation

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).

Cook JC, Klinefilter GR, Hardisty JF, Sharpe RM, Foster PM. 1999. Rodent Leydig cell tumorigenesis: A review of the physiology, pathology, mechanisms, and relevance to humans. Crit Rev Toxicol 19:169-261*

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.

* Reference added to the original report by the rapporteurs or dossier submitter after public consultation

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

Elcombe, CR, Elcombe BM, Foster JR, Farrar DG, Jung R, Chang S-C, Kennedy GL, Butenhoff JL (2010) Hepatocellular hypertrophy and cell proliferation in Sprague–Dawley rats following dietary exposure to ammonium perfluorooctanoate occurs through increased activation of the xenosensor nuclear receptors PPARα and CAR/PXR Arch Toxicol (2010) 84:787–798*

EPA (2003) Proposed OPPTS Science Policy:PPAR"-Mediated Hepatocarcinogenesis in Rodents andRelevance to Human Health Risk Assessments. Office Office of Prevention, Pesticides & Toxic Substances U.S. Environmental Protection AgencyWashington, D.C. 20460, November 5, 2003*

Fei C, McLaughlin JK, Tarone RE and Olsen J. 2007. Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. Environ Health Perspect. 115(11):1677-82.*

Freberg BI, Haug LS, Olsen R, Daae HL, Hersson M, Thomsen C, et al. 2010. Occupational exposure to airborne perfluorinated compounds during professional ski waxing. Environ. Sci. Technol. 44, 7723-7728.

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.

* Reference added to the original report by the rapporteurs or dossier submitter after public consultation

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.

Gonzalez FJ, Shah YM. 2008. PPARalpha: mechanism of species differences and hepatocarcinogenesis of peroxisome proliferators. Toxicology; 246(1):2-8.

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.

Haug LS, Thomsen C, Brantsaeter AL, Kvalem HE, Haugen M, Becher G, Alexander J, Meltzer HM, Knutsen HK. Diet and particularly seafood are major sources of perfluorinated compounds in humans. 2010. Environ Int. 36(7), 772-8.

Haug LS, Huber S, Becher G and Thomsen C. 2011. Characterisation of human exposure pathways to perfluorinated compounds - comparing exposure estimates with biomarkers of exposure. Environ. Int. 37, 687-693.

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.

Kim SK, Lee KT, Kang CS, Tao L, Kannan K, Kim KR, Kim CK, Lee JS, Park PS, Yoo YW, Ha JY, Shin YS and Lee JH. Distribution of perfluorochemicals between sera and milk from the same mothers and implications for prenatal and postnatal exposures. Environ Pollut. 159(1):169-74.*

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

Kudo N, Kawashima Y. 2003. Toxicity and toxicokinetics of perfluorooctanoic acid in humans and animals. J Toxicol Sciences 28:49-57.*

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.

Li Y, Ramdhan DH, Naito H, Yamagishi N, Ito Y, Hayashi Y, Yanagiba Y, Okamura A, Tamada H, Gonzalez FJ, Nakajima T. Ammonium perfluorooctanoate may cause testosterone reduction by adversely affecting testis in relation to PPARα. Toxicol Lett. 2011 Sep 10;205(3):265-72*

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

* Reference added to the original report by the rapporteurs or dossier submitter after public consultation

* Reference added to the original report by the rapporteurs or dossier submitter after public consultation

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

Liu RC, Hurtt ME, Cook JC, Biegel LB. 1996. Effect of the peroxisome proliferator, ammonium perfluorooctanoate (C8), on hepatic aromatase activity in adult male Crl:CD BR (CD) rats. Fund Appl Toxicol 30:220-228*

Macon MB, Villanueva LR, Tatum-Gibbs K, Zehr RD, Strynar MJ, Stanko JP, White SS, Helfant L, Fenton SE (2011) Prenatal Perfluorooctanoic Acid Exposure in CD-1 Mice: Low-Dose Developmental Effects and Internal Dosimetry. TOXICOLOGICAL SCIENCES 122(1), 134–145 (2011)*

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.

Monroy R, Morrison K, Teo K, Atkinson S, Kubwabo C, Stewart B and Foster WG. 2008. Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environ Res. 108(1):56-62.*

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.

* Reference added to the original report by the rapporteurs or dossier submitter after public consultation

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.

Nilsson H, Karrman A, Rotander A, Van BB, Lindstrom G, Westberg H. 2010a Octa. Inhalation exposure to fluorotelomer alcohols yield perfluorocarboxylates in human blood? Environ Sci Technol 44(19):7717-7722.

Nilsson H, Kärrman A, Westberg H, Rotander A, van Bavel B, Lindström G. 2010b. A Time Trend Study of Significantly Elevated Perfluorocarboxylate Levels in Humans after Using Fluorinated Ski Wax. Environ. Sci. Technol. 44, 2150-2155.

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.

Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL and Zobel LR. 2007. Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ Health Perspect. 115(9):1298-305.*

Olsen GW, Butenhoff JL and Zobel LR. 2009. Perfluoroalkyl chemicals and human fetal development: an epidemiologic review with clinical and toxicological perspectives. Reprod Toxicol. 27(3-4):212-30. Epub 2009 Feb 20. Review.*

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.

Palkar PS, Anderson CR, Ferry CH, Gonzalez FJ and Peters JM. 2010. Effect of prenatal peroxisome proliferator-activated receptor alpha (PPARalpha) agonism on postnatal development. Toxicology. 276(1):79-84.*

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.

* Reference added to the original report by the rapporteurs or dossier submitter after public consultation

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.

Ross J, Plummer SM, Rode A, Scheer N, Bower CC, Vogel O et al. 2010. Human Constitutive Androstane Receptor (CAR) and Pregnane X Receptor (PXR) Support the Hypertrophic but not the Hyperplastic Response to the Murine Nongenotoxic Hepatocarcinogens Phenobarbital and Chlordane In Vivo. Toxicological Sciences; 116(2):452-466.

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.

Shah YM, Morimura K, Yang Q, Tanabe T, Takagi M, Gonzalez FJ. 2007. Peroxisome proliferator-activated receptor alpha regulates a microRNA-mediated signaling cascade responsible for hepatocellular proliferation. Mol Cell Biol; 27(12):4238-4247.

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.

Tatum-Gibbs K, Wambaugh JF, Das KP, Zehr RD, Strynar MJ, Lindstrom AB, Delinsky A and Lau C. 2011. Comparative pharmacokinetics of perfluorononanoic acid in rat and mouse. Toxicology. 281(1-3):48-55.*

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.

Walters MW, .. Bjork A, Wallace KB (2009) Perfluorooctanoic acid stimulated mitochondrial biogenesis and gene transcription in rats. Toxicology 264:10-15.*

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).

Yahia, D, El-Nasser MA, Abedel-Latif M, Tsukuba C, Yoshida M, Sato I, Tsuda S (2010). Effects of perfluorooctanoic acid (PFOA) exposure to pregnant mice on reproduction. J Toxicol Sciences 35:527-533.*

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

* Reference added to the original report by the rapporteurs or dossier submitter after public consultation

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.

Zhao Y, Tan YS, Haslam SZ and Yang C. 2010. Perfluorooctanoic acid effects on steroid hormone and growth factor levels mediate stimulation of peripubertal mammary gland development in C57BL/6 mice. Toxicol Sci. 115 (1).[*]

[*] Reference added to the original report by the rapporteurs or dossier submitter after public consultation

APPENDIX 1

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

Perfluorooctanoic 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, Xn; R 20/22, R 48/22, Xi; R 36.

ECBI/18/06 ADD 1

Norway introduced its proposal for the classification of PFOA 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 Carc. Cat. 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.

Repeated 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)

Perfluorooctanoic 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.

<u>Conclusion :</u>

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.

References	Included in CLH-dossier	Reason for not including in CLH-dossier
Abbott, B. D., et al. (2007). Perfluorooctanoic acid (PFOA)-induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator activated receptor-alpha (PPARa). *Toxicol Sci* **98**, 571-81.	Yes	
Abbott, B. D. (2009). Review of the expression of peroxisome proliferator activated receptors alpha (PPARa), beta (PPARb), and gamma (PPARg) in rodent and human development. *Reproductive Toxicology* **27**.	Yes	Review discussing the importance of PPARα in mouse, rat and human. “With the exception of the disruptions in development that were discovered using genetically altered mice, little is known about the roles of the PPARs during development, however the expression patterns of PPARs during development suggest that PPARα, β and μ have important functions throughout development in many cell types and organs.” Dossier submitters view of the study: the PPARα mechanism is already discussed in the CLH dossier.
Andersen, C. S., et al. (2010). Prenatal exposures to perfluorinated chemicals and anthropometric measures in infancy. *Am J Epidemiol* **172**, 1230-1237.		The authors estimated the associations between maternal plasma levels of PFOS and PFOA and infants’ weight, length, and body mass index development during the first year of life. “In summary our study suggests that prenatal exposure to PFOS and PFOA may be inversely associated with weight and body mass index in boys during infancy. Furthermore, length did not seem to be associated with prenatal PFC exposure.” Dossier submitters view of the study: in general data from epidemiological studies will always be a combination of exposure to PFOA, PFOS and other PFCs, and exposure levels to especially PFOA would be too low to expect any effect. Other confounding factors will also affect the results.
Apelberg, B. J. (2006). Fetal Exposure to Perfluorinated Compounds: Distribution and determinants of exposure and relationships with weight and size at birth, Ed.^ Eds.), pp. 1-222. Johns Hopkins, Baltimore.		This is a dissertation for the degree of Doctor of Philosophy. Data are related to epidemiology. In general data from epidemiological studies will always be a combination of exposure to PFOA, PFOS and other PFCs, and exposure levels to especially PFOA would be too low to expect any effect. Other confounding factors will also affect the results.
Apelberg, B. J., et al. (2007). Cord serum concentrations of perfluorooctane sulfonate	Yes	

(PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. *Environ Health Perspect* **115**, 1670-1676.		
Braissant, O., et al. (1996). Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* **137**, 354-66.		Dossier submitters view of the study: the PPARα mechanism is already discussed in the CLH dossier.
Butenhoff, J. L., et al. (2004). The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. *Toxicology* **196**, 95-116.	Yes	
Christensen, K. Y., et al. (2011). Exposure to polyfluoroalkyl chemicals during pregnancy is not associated with offspring age at menarche in a contemporary British cohort. *Environ Int* **37**, 129-135.		“Conclusions: We compared exposure to PFC’s during pregnancy among mothers of girls who did and did not have earlier age at menarche in the ALSPAC cohort. PFC serum concentrations, both total and for individual compounds, varied by maternal characteristics .However, gestational PFC exposure during pregnancy did not appear to be associated with age at menarce in this cohort.” Dossier submitters view of the study: in general data from epidemiological studies will always be a combination of exposure to PFOA, PFOS and other PFCs, and exposure levels to especially PFOA would be too low to expect any effect. Other confounding factors will also affect the results.
Elcombe, C. R., et al. (2010). Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats following dietary exposure to ammonium perfluorooctanoate occurs through increased activation of the xenosensor nuclear receptors PPARa and CAR/PXR. *Arch Toxicol* **84**, 787-798.	Yes	“These data demonstrate an early hepatocellular proliferative response to APFO treatment and suggest that the hepatomegaly and tumors observed after chronic dietary exposure of S_D rats to APFO likely are due to a proliferative response to combined activation of PPARα ans CAR/PXR. This mode of action is unlikely to pose a human hepatocarcinogenic hazard.” “Thus, the work reported herein has confirmed that APFO-mediate hypertrophic changes in the liver are the result of increased peroxisomal proliferation, expansion of smooth ER proliferation, and increased cell proliferation.” Dossier submitters view of the study: liver tumors in rodents that are conclusively linked to peroxisome proliferation are proposed not to be of relevance for humans (CLP guidance, 3.6.2.3.2 (k)).
Fei, C., et al. (2007). Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. *Environ Health Perspect* **115**, 1677-1682.	Yes	

Fei, C., et al. (2008a). Prenatal exposure to PFOA and PFOS and maternally reported developmental milestones in infancy. *Environ Health Perspect* **116**, 1391-1395.		Epidemiological study including1400 pregnant women and their children from the Danish National Birth Cohort. Plasma concentrations of PFOA ranged from 4,65 ng/mL to 6,65 ng/mL. "We found no convincing associations between developmental milestones in early childhood and levels of PFOA or PFOS as measured in maternal plasma early in pregnancy." Dossier submitters view of the study: in general data from epidemiological studies will always be a combination of exposure to PFOA, PFOS and other PFCs, and exposure levels to especially PFOA would be too low to expect any effect. Other confounding factors will also affect the results.
Fei, C., et al. (2008b). Fetal growth indicators and perfluorinated chemicals: A study in the Danish National Birth Cohort. *Am J Epidemiol* **168**, 66-72.		Epidemiological study including 1400 pregnant women and their children from the Danish National Birth Cohort. Investigating if PFOA reduces organ growth. PFOA was measured in maternal blood samples taken early in pregnancy. Placental weight, birth length, and head and abdominal circumferences were measured shortly after birth. Maternal PFOA levels in early pregnancy were associated with smaller abdominal circumference and birth length. Findings suggest that fetal exposure to PFOA but not PFOS during organ development may affect the growth of organs and the skeleton. Mean PFOA level was 5,6 ng/ml. Dossier submitters view of the study: in general data from epidemiological studies will always be a combination of exposure to PFOA, PFOS and other PFCs, and exposure levels to especially PFOA would be too low to expect any effect. Other confounding factors will also affect the results.
Fei, C., et al. (2009). Maternal levels of perfluorinated chemicals and subfecundity. *Human Reproduction* **24**, 1200-1205.		PFOA and PFOS exposure at plasma levels seen in the general population may reduce fecundity; such exposure levels are common in developed countries. PFOA levels ranging from 4,6-6,7 ng/ml. Dossier submitters view of the study: in general data from epidemiological studies will always be a combination of exposure to PFOA, PFOS and other PFCs, and exposure levels to especially PFOA would be too low to expect any effect. Other confounding factors will also affect the results.
Fei, C., et al. (2010). Prenatal exposure to PFOA and PFOS and risk of hospitalization for		Dossier submitters view of the study: in general data from epidemiological studies will always be a

infectious diseases in early childhood. *Environmental Research* **110**, 773-777.		combination of exposure to PFOA, PFOS and other PFCs, and exposure levels to especially PFOA would be too low to expect any effect. Other confounding factors will also affect the results.
Fletcher (2010). Patterns of age of puberty among children in the Mid-Ohio Valley in relation to perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS). Briefing notes. www.C8sciencepanel.org.		Delays of puberty have been observed correlated with PFOS in boys and PFOA and PFOS in girls. Authors underlines that caution is needed in interpreting the results, and further work is planned.
Gonzalez, F. J. and Shah, Y. M. (2008). PPARalpha: mechanism of species differences and hepatocarcinogenesis of peroxisome proliferators. *Toxicology* **246**, 2-8.		Review of PPARα-mechanisms in general. Mainly related to drugs that are PPAR targets. Dossier submitters view of the study: the PPARα mechanism is already discussed in the CLH dossier.
Gortner, E. G. (1981). Oral teratology study of T-2998CoC in rats. Experiment Number 0681TR0110. Safety Evaluation Laboratory and Riker Laboratories, Inc., St. Paul, MN. USEPA Public Docket, AR-226-0463. 110216 Submission Norway CLP Page 13 of 14	Yes	
Gortner, E. G. (1982). Oral teratology study of T-3141CoC in rabbits. Experiment Number 0681TB0398. Safety Evaluation Laboratory and Riker Laboratories, Inc., St. Paul, MN. USEPA Public Docket AR-226-0465.	Yes	
Grice, M. M., et al. (2007). Self-reported medical conditions in perfluorooctanesulfonyl fluoride manufacturing workers. *J Occup Environ Med* **49**, 722-9.		Evaluates whether some cancers, other conditions, and pregnancy outcomes were related to occupational PFOS exposure. A self-administered questionnaire was used to look at the occurrence of both cancers and noncancerous conditions. The article does not report on serum levels of PFOS. Dossier submitters view of the study: PFOS is not relevant for the classification of PFOA/APFO.
Hamm, M. P., et al. (2010). Maternal exposure to perfluorinated acids and fetal growth. *Journal of Exposure Science and Environmental Epidemiology* **20** 589-597.		The results suggest that maternal PFA exposure had no substantial effect on fetal weight and length of gestation at the concentrations observed in this population. PFOA concentrations in serum ranged from <LOD to 18 ng/ml (median 1,5 ng/ml). Also PFOS and PFHxS were measured. Observed a reduction of birth weight of 12,4 g/ng/ml. Dossier submitters view of the study: in general data from epidemiological studies will always be a combination of exposure to PFOA, PFOS and other

		PFCs, and exposure levels to especially PFOA would be too low to expect any effect. Other confounding factors will also affect the results.
Hinderliter, P. M., et al. (2005). Perfluorooctanoate: Placental and lactational transport pharmacokinetics in rats. *Toxicology* **211**, 139-48.	Yes	
Hochberg, Y. and Lachenbruch, P. A. (1976). Two stage multiple comparison procedures based on the studentized range. *Commun Stat A* **5**, 1447-1453.		Statistical method.
Inoue, K., et al. (2004). Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. *Environ Health Perspect* **112**, 1204-7.		PFOA was detected only in maternal samples (range <0,5 to 2,3 ng/ml, 4 of 15). The article states that PFOA cannot cross the placental barrier to enter fetal circulation. Dossier submitters view of the study: In the study by Fenton et al (2009) included in the CLH dossier, PFOA was measures in the serum of pups following exposure in utero.
Klaunig, J. E., et al. (2003). PPARalpha agonist-induced rodent tumors: modes of action and human relevance. *Crit Rev Toxicol* **33**, 655-780.		Purpose of the report: - To describe the current understanding of the mode(s) of carcinogenic action of PPAR-α agonist-induced tumors - To determine if PPAR-α agonist-induced rodent tumors should (continue to) be considered relevant and applicable in human cancer hazard/risk assessments of substances belonging to this group of chemicals. Dossier submitters view of the study: the PPARα mechanism is already discussed in the CLH dossier. At the special expert meetin January 22-23. 2004 it was concluded that non genotoxic chemicals causing Leydig cell tumors in rats by pertubating the HPT axis should be classified in Carc cat 3 (DSD), carc cat 2 CLP.
Lake, B. G. (2009). Species differences in the hepatic effects of inducers of CYP2B and CYP4A subfamily forms: relationship to rodent liver tumour formation. *Xenobiotica* **39**, 582-96.		Review. Rodent CYP2B and CYP4A inducers do not pose a hepatocarcinogenic hazard for humans. Dossier submitters view of the study: liver tumors in rodents that are conclusively linked to peroxisome proliferation are proposed not to be of relevance for humans (CLP guidance, 3.6.2.3.2 (k)).
Lau, C., et al. (2004). The developmental toxicity of perfluoroalkyl acids and their derivatives. *Toxicol Appl Pharmacol* **198**, 231-41.		Review. Referers to several of the studies already included in the CLH dossier.

Lau, C., et al. (2005). Pregnancy loss associated with exposure to perfluorooctanoic acid in the mouse. *Birth Defects Research (Part A)* **73**, 358.		Abstract to poster. Seems to be related to Lau et al 2006 that is included in the CLH dossier.
Lau, C., et al. (2006). Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. *Toxicol Sci* **90**, 510-518.	Yes	
Lee, S. S., et al. (1995). Targeted disruption of the alpha isoform of the peroxisome proliferatoractivated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol Cell Biol* **15**, 3012-22.		Dossier submitters view of the study: the PPARα mechanism is already discussed in the CLH dossier.
Monroy, R., et al. (2008). Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. *Environ Res* **108**, 56-62.	Yes	
Nolan, L. A., et al. (2009). The relationship between birth weight, gestational age and perfluorooctanoic acid (PFOA)-contaminated public drinking water. *Reprod Toxicol* **27**, 231-238.		Markedly elevated PFOA exposure, as categorized by water service category is not associated with increased risk of lowered birth weight or gestational age. This study does not confirm earlier findings of an association between PFOA and lowered birth weight observed at normal population level. Dossier submitters view of the study: in general data from epidemiological studies will always be a combination of exposure to PFOA, PFOS and other PFCs, and exposure levels to especially PFOA would be too low to expect any effect. Other confounding factors will also affect the results.
Nolan, L. A., et al. (2010). Congenital anomalies, labor/delivery complications, maternal risk factors and their relationship with perfluorooctanoic acid (PFOA)-contaminated public drinking water. *Repro Toxicol* **29**, 147-155.		At the levels measured in the LHWA (Little Hocking Water Association), they conclude that PFOA is not associated with increased risk of congenital anomalies, most labour and delivery complications and maternal risk factors. Additional research is required to assess the observed associations between PFOA, anemia and dysfunctional labor. Dossier submitters view of the study: in general data from epidemiological studies will always be a combination of exposure to PFOA, PFOS and other PFCs, and exposure levels to especially PFOA would be too low to expect any effect. Other confounding factors will also affect the results.
Olsen, G. W., et al. (2004). Serum concentrations of perfluorooctanesulfonate and other fluorochemicals in an elderly population from Seattle, Washington. *Chemosphere* **54**, 1599-611.	Yes	

Olsen, G. W., et al. (2009). Perfluoroalkyl chemicals and human fetal development: an epidemiologic review with clinical and toxicological perspectives. *Reprod Toxicol* **27**, 212-30.	Yes	
Rosen, M. B., et al. (2009). Does exposure to perfluoroalkyl acids present a risk to human health? *Toxicol Sci* **111**, 1-3.		Dossier submitters view of the study: this is a comment to other studies on risk to human health following exposure to perfluoroalkyl acids. The PPARα mechanism is already discussed in the CLH dossier.
Ross, J., et al. (2010). Human constitutive androstane receptor (CAR) and pregnane X receptor (PXR) support the hypertrophic but not the hyperplastic response to the murine nongentoxic hepatocarcinogens phenobarbital and chlordane *in vivo*. *Toxicol Sci* **116**, 452-466. 110216 Submission Norway CLP Page 14 of 14		Mechanistic study. Not directly related to PFOA.
Staples, R. E., et al. (1984). The embryo-fetal toxicity and teratogenic potential of ammoniumperfluorooctanoate (APFO) in the rat. *Fundam Appl Toxicol* **4**, 429-40.	Yes	
Steenland, K., et al. (2010). Epidemiologic Evidence on the Health Effects of Perfluorooctanoic Acid (PFOA). *Environ Health Perspect* **118**, 1100-1108.		"Epidemiologic evidence remains limited, and to date data are insufficient to draw firm conclusions regarding the role of PFOA for any of the diseases of concern." Dossier submitters view of the study: in general data from epidemiological studies will always be a combination of exposure to PFOA, PFOS and other PFCs, and exposure levels to especially PFOA would be too low to expect any effect. Other confounding factors will also affect the results.
Stein, C. R., et al. (2009). Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome. *Am J Epidemiol* **170**, 837-846.		This study identified modest association of PFOA with preeclampsia and birth defects and of PFOS with preeclampsia and low birth weight, but associations were small, limited in precision, and based on self-reported outcomes. Dossier submitters view of the study: in general data from epidemiological studies will always be a combination of exposure to PFOA, PFOS and other PFCs, and exposure levels to especially PFOA would be too low to expect any effect. Other confounding factors will also affect the results.
Washino, N., et al. (2009). Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. *Environ Health Perspect* **117**, 660-667.		"The results indicate that in utero exposure to relatively low levels of PFOS was negatively correlated with birth weight. PFOA levels did not correlate with birth weight." Dossier submitters view of the study: in general data from epidemiological studies will always be a

		combination of exposure to PFOA, PFOS and other PFCs, and exposure levels to especially PFOA would be too low to expect any effect. Other confounding factors will also affect the results.
Wolf, C. J., et al. (2007). Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures. *Toxicol Sci* **95**, 462-73.	Yes	
Yahia, D., et al. (2010). Effects of perfluorooctanoic acid (PFOA) exposure to pregnant mice on reproduction. *The Journal of Toxicological Sciences* **35**, 527-533.	Yes	"Pregnant ICR mice were given 1, 5 and 10 mg/kg PFOA daily by gavage from gestational day (GD) 0 to 17 and 18 for prenatal and postnatal evaluations, respectively. Five to nine dams per group were sacrificed on GD 18 for prenatal evaluation; other 10 dams were left to give birth. No maternal death was observed. The liver weight increased dose-dependently, with hepatocellular hypertrophy, necrosis, increased mitosis and mild calcification at 10 mg/kg. PFOA at 10 mg/kg increased serum enzyme activities with hypoproteinemia and hypolipidemia. PFOA treatment reduced the fetal body weight at 5 and 10 mg/kg. Teratological evaluation showed delayed ossification of the sternum and phalanges and delayed eruption of incisors at 10 mg/kg, but did not show intracranial blood vessel dilatation. Postnatal evaluation revealed that PFOA reduced the neonatal survival rate at 5 and 10 mg/kg. At 5 mg/kg pups were born alive and active and 16% died within 4 days observation, while all died within 6 hr after birth at 10 mg/kg without showing intracranial blood vessel dilatation." They compared if mechanisms for fetal death were similar for PFOA and PFOS and concluded that the cause of neonatal death by PFOA may be different from PFOS.