Committee for Risk Assessment
RAC

Opinion
proposing harmonised classification and labelling
at Community level of
Perfluorooctanoic acid (PFOA)

ECHA/RAC/DOC No CLH-O-0000002227-78-01/F

Adopted
2 December 2011

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OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT COMMUNITY LEVEL

In accordance with Article 37 (4) of the Regulation (EC) No 1272/2008 (CLP Regulation), the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling of

Substance Name: ***Perfluorooctanoic acid (PFOA)***
EC Number: **206-397-9**
CAS Number: **335-67-1**

The proposal was submitted by ***Norway*** and received by RAC on ***7 January 2011***

Harmonised classification proposed by the dossier submitter:

	CLP Regulation (EC) No 1272/2008	Directive 67/548/EEC
Current entry in Annex VI of CLP Regulation (EC) No 1272/2008	-	-
Proposal by dossier submitter for consideration by RAC	Carc. 2, H351 Repr. 1B, H360D STOT RE 1, H372 STOT RE 2, H373 Acute Tox. 3, H331 Acute Tox. 3, H301 Eye Irrit. 2, H319	Carc. Cat 3; R40 Repr. Cat. 2: R61 T; R48/23 Xn; R48/22, R20/22, Xi; R36
Resulting harmonised classification (future entry in Annex VI of CLP Regulation) as proposed by dossier submitter	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	Carc. Cat 3; R40 Repr. Cat. 2: R61 T; R48/23 Xn; R48/22, R20/22, Xi; R36

PROCESS FOR ADOPTION OF THE OPINION

Norway has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/consultations/harmonised_cl/harmon_cl_prev_cons_en.asp*** on ***7 January 2011***. Parties concerned and MSCAs were invited to submit comments and contributions by ***21 February 2011***.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: ***Norbert Rupprich***
Co-rapporteur, appointed by RAC: ***Agnes Schulte***

The opinion takes into account the comments of MSCAs and parties concerned provided in accordance with Article 37 (4) of the CLP Regulation.

The RAC opinion on the proposed harmonised classification and labelling has been reached on ***2 December 2011,*** in accordance with Article 37 (4) of the CLP Regulation, giving parties concerned the opportunity to comment. Comments received are compiled in Annex 2.

The RAC Opinion was adopted by ***consensus***

OPINION OF RAC

The RAC adopted the opinion that ***Perfluorooctanoic acid (PFOA)*** should be classified and labelled as follows[1]:

[1] Note that not all hazard classes have been evaluated

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard state-ment Code(s)	Pictogram, Signal Word Code(s)	Hazard state ment Code(s)	Suppl. Hazard statement Code(s)		
	Perfluorooctanoic acid (PFOA)	206-397-9	335-67-1	Carc. 2 Repr. 1B Lact. STOT RE 1 (liver) Acute Tox. 4 Acute Tox. 4 Eye Dam. 1	H351 H360D H362 H372 H332 H302 H318	GHS07 GHS08 Danger	H351 H360D H362 H372 H332 H302 H318		-	-

Classification and labelling in accordance with the criteria of Directive 67/548/EEC

Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits	Notes
	Perfluorooctanoic acid (PFOA)	206-397-9	335-67-1	Carc. Cat. 3; R40 Repr. Cat. 2; R61 T; R48/23 Xn; R48/21/22, R20/22 Xi; R41	T, Xn R: 40-61-48/23-48/21/22-20/22-41-64 S: 53-45	-	-

SCIENTIFIC GROUNDS FOR THE OPINION

The opinion relates only to those hazard classes that have been reviewed in the proposal for harmonised classification and labelling, as submitted by *Norway*.

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 is 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 warrant 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.

Acute toxicity

1. Proposal of the dossier submitter

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

Inhalation
Following inhalation exposure of APFO a LC_{50} of 0.98 mg/l (4 hour exposure), and a LC_{50} > 18.6 mg/l (1 hour exposure) was reported. According to the Directive 67/548/EEC classification criteria APFO dossier submitter proposed classification 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 relevant LC_{50} values were considered to be between 1.0 mg/l < ATE ≤ 5.0 mg/l.

Dermal
Based on the data and 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.

2. Comments submitted by concerned parties

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 highlighted the borderline situation between classes.

Others did not specifically refer to the classification proposal.

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.

3. Outcome of the RAC assessment

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 category 4 (CLP), have 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 a LC_{50} of 0.98 mg/l (4 hour exposure) was identified at the borderline between category 3 and category 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 category. 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 category 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.

Irritation

1. Proposal of the dossier submitter

Skin
Dossier submitter concluded that data do not allow drawing a conclusion on the need for classification with regard to skin irritation.

Eye
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 classified as Eye Irrit. 2 (H319) (CLP).

2. Comments submitted by concerned parties

Skin
No specific comments received.

Eye
One Member State expressed agreement with the CLP classification as Eye Irrit. 2 (H319) and the DSD classification Xi; R36 as agreed by TC C& L.

3. Outcome of the RAC assessment

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 irritative 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 the 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.

Sensitisation

1. Proposal of the dossier submitter
Skin and respiratory system

No classification for skin sensitisation is proposed due to insufficient data (skin) or lack of data (respiratory tract).

2. Comments submitted by concerned parties
No relevant comments received.

3. Outcome of the RAC assessment
RAC agrees not to propose classification for this endpoint.

Repeated dose toxicity

1. Proposal of the dossier submitter
Oral
The dossier submitter considered effects on repeated dose toxicity was on the borderline between Xn; R48/22 and T; R48/25, but refers to the decision of the former TC C&L group who concluded on a classification according to Directive 67/548/EEC with Xn; R48/22 . The according proposal to CLP criteria is to classify 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:
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/litre a classification according to the Directive 67/548/EEC criteria with T; R 48/23. The according proposal to CLP criteria is STOT RE 1 (H372) since the guidance value for STOT RE 1 inhalation exposure is $C \leq 0.02$ mg/litre.

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

2. Comments submitted by concerned parties

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: "Causes damage to organs (liver) through prolonged or repeated exposure."

3. Outcome of the RAC assessment

With respect to the CLP Regulation, RAC agrees to propose classification as STOT RE 1 (H372): Causes damage to organs (liver) through prolonged or repeated exposure.
RAC agrees with the proposal on a classification according to the Directive 67/548/EEC criteria with T; R 48/23 for the inhalation route and with 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, 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 five 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 dosages 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 observations 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.

Mutagenicity

1. Proposal of the dossier submitter

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 Directive 67/548/EEC criteria or CLP criteria for mutagenicity is proposed.

2. Comments submitted by concerned parties

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

3. Outcome of the RAC assessment

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

Carcinogenicity

1. Proposal of the dossier submitter

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 with Carc. Cat. 3; R40 (according to the Directive 67/548/EEC criteria). For these tumors there are insufficient data on MOA to conclude that tumours are not relevant for humans.

2. Comments submitted by concerned parties

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

3. Outcome of the RAC assessment

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 1: 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% (10/47)	-/ 40%# (19/47)	-/ 43%# (21/49)			18% or 37% Sykes, 1987 19% Chandra et al., 1992
Pancreas						
Acinar cell adenoma	0% / ά	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 et al., 2010 hypothesised that APFO increases mitochondrial mass in rats and monkeys 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 hPPARα-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
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.

Dossier submitter proposed that the induction of pancreatic acinar cell tumours is 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 tumor 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.
In conclusion, RAC followed the proposal by the dossier submitter, namely that APFO should be classified according to the Directive 67/548/EEC criteria as Carc. Cat. 3; R40, and according to the CLP criteria as Carc. 2 (H351).

Reproductive toxicity/Fertility

1. Proposal of the dossier submitter

No classification on fertility was proposed based on the outcome of a 2-generation study (York 202, 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.

2. Comments submitted by concerned parties

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

3. Outcome of the RAC assessment

Based on the previously available date 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 hPPARα-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.

Reproductive toxicity/Developmental toxicity

1. Proposal of the dossier submitter

The dossier submitter proposed to classify APFO as Repr. 1B (H360D) according to the 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.

2. Comments submitted by concerned parties

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 had led to a debate on whether the offspring effects are related to maternal toxicity, the majority agreed on a classification as Repr. Cat. 2; R61. Several Member States supported classification on this endpoint as proposed by TC C&L.

3. Outcome of the RAC assessment

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 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 the 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 category 2 may be considered more appropriate than category 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 fetal 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 CLP criteria for reproductive toxicity (Section 3.7.2)

Human data do not sufficiently give evidence to conclude on whether Repr. 1A is appropriate. Repr. 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

APFO 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 (CLP: Lact., H 362: May cause harm to breast-fed children; DSD: R64 May cause harm to breastfed babies).

Additional information

The Background Document, attached as Annex 1, gives the detailed scientific grounds for the Opinion.

ANNEXES:

Annex 1 Background Document (BD)[1]

Annex 2 Comments received on the CLH report, response to comments provided by the dossier submitter and rapporteurs' comments (excl. confidential information)

[1] The Background Document (BD) supporting the opinion contains scientific justifications for the CLH proposal. The BD is based on the CLH report prepared by a dossier submitter. The original CLH report may need to be changed as a result of the comments and contributions received during the public consultation(s) and the comments by and discussions in the Committees.