Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Nonadecafluorodecanoic acid (PFDA) [1] and its ammonium (PFD-A) [2] and sodium (PFD-S) [3] salts
Nonadecafluorodecanoic acid [1], ammonium nonadecafluorodecanoate [2], sodium nonadecafluorodecanoate [3]

EC Numbers: 206-400-3 [1], 221-470-5 [2], - [3]
CAS Numbers: 335-76-2 [1], 3108-42-7 [2], 3830-45-3 [3]

CLH-O-0000001412-86-92/F

Adopted

4 December 2015

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CLH-O-0000001412-86-92/F

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

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

Chemicals name:	**Nonadecafluorodecanoic acid (PFDA) [1] and its ammonium (PFD-A) [2] and sodium (PFD-S) [3] salts**
EC numbers:	**206-400-3 [1], 221-470-5 [2], - [3]**
CAS numbers:	**335-76-2 [1], 3108-42-7 [2], 3830-45-3 [3]**

The proposal was submitted by **Sweden** and received by RAC on **29 May 2015**.

In this opinion, all classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonized System (GHS).

PROCESS FOR ADOPTION OF THE OPINION

Sweden 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/harmonised-classification-and-labelling-consultation/*** on **16 June 2015** . Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **31 July 2015**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Stéphanie Copin**

Co-rapporteur, appointed by RAC: **Radu Branisteanu**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation; the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonized classification and labelling was reached on **4 December 2015** and was adopted by **consensus**.

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 statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	**No current Annex VI entry**										
Dossier submitters proposal	xxx-xxx-xx-x	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluorodecanoic and its ammonium and sodium salts	206-400-3		Carc. 2 Repr. 1B Lact.	H351 H360Df H362	GHS08 Dgr	H351 H360Df H362	-	-	-
RAC opinion	xxx-xxx-xx-x	nonadecafluorodecanoic acid [1], ammonium nonadecafluorodecanoate [2], sodium nonadecafluorodecanoate [3]	206-400-3 [1]; 221-470-5 [2]; - [3]	335-76-2 [1]; 3108-42-7 [2]; 3830-45-3 [3]	Carc. 2 Repr. 1B Lact.	H351 H360Df H362	GHS08 Dgr	H351 H360Df H362	-	-	-
Resulting Annex VI entry if agreed by COM	xxx-xxx-xx-x	nonadecafluorodecanoic acid [1], ammonium nonadecafluorodecanoate [2], sodium nonadecafluorodecanoate [3]	206-400-3 [1]; 221-470-5 [2]; - [3]	335-76-2 [1]; 3108-42-7 [2]; 3830-45-3 [3]	Carc. 2 Repr. 1B Lact.	H351 H360Df H362	GHS08 Dgr	H351 H360Df H362	-	-	-

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Based on the data on nonadecaflourodecanoic acid (PFDA) on its own, there is not sufficient evidence to conclude on harmonised classification. However, data are available from a closely related analogous chemical PFOA and its ammonium salt APFO (see below) and it was considered that data from these analogous substances can be used to fill the observed data-gaps.

It is considered that :

- 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Nonadecafluorodecanoic acid (or perfluorodecanoic acid: PFDA) with its sodium (PFD-S) and ammonium (PFD-A) salts;

and

- 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid (PFOA) with its ammonium salt, ammoniumpentadecafluorooctanoate (APFO);

are close structural analogues.

Indeed, both PFDA (-C10) and PFOA (-C8) are in the same chemical class of perfluorinated carboxylic acids (PFCA), consisting of a linear carbon chain that is entirely substituted by strong bonds to fluorine atoms. They share a common functional group (a perfluorinated carbon backbone) with only the carbon chain length differing (2 carbons– associated with 4 fluorines more for PFDA). In addition, both PFDA and PFOA are strong acids which are expected to dissociate at physiological pH. They are expected to be available to organisms and to exert systemic toxicity in the form of their corresponding carboxylate anion (PFD and PFO, respectively) and are therefore considered to be toxicologically equivalent, e.g. exerting similar toxic effects, although their potency may differ.

Furthermore, PFDA (and its salts) and PFOA/APFO possess physicochemical and toxicokinetic properties which are similar or in the same range. The dossier submitter (DS) provided a table (Table 10 of the CLH report) summarising the trend in physicochemical properties and the structural similarities as well as similarities in health effects.

Physicochemical properties and toxicokinetics

Regarding the physicochemical properties of PFDA, the dissociation constant is predicted to be similar among the compounds in this chemical class, with the chain length having only a minimal impact on the pKa values, and the calculated pKa values for PFDA and PFOA are indeed similar (-0.22 and -0.21, respectively, when using SPARC or -5.2 and -4.2, respectively, when using ChemId software). PFDA differs slightly, with decreased water solubility and increased octanol/water partition coefficient, as would be expected with its longer perfluorinated carbon chain length.

Concerning toxicokinetics, PFOA is absorbed after oral, inhalation and dermal exposure. Although no studies are available for PFDA, oral absorption can be assumed based on liver toxicity reported after oral exposure in one study in mice by Harris and Birnbaum (1989); no further data are available. Oral absorption of PFDA has been further substantiated by its detection in human blood. Both substances have a similar organ distribution based on available data: the highest levels were found in liver, blood (plasma) and kidneys after intra-peritoneal (i.p.) exposure to PFDA, and in blood and liver followed by kidneys (as well as lungs, skin) after oral exposure to PFOA. Both PFDA and PFOA have been detected in human breast milk. None of these substances are metabolised, which is an expected common trend for this group due to the strong carbon-fluoride bond (and therefore resistance to thermal/chemical/biological degradation).

In rats, a major gender difference in the rate of elimination was observed for PFOA, as well as perfluorononaoic acid (PFNA; a perfluorocarboxylic acid with carbon chain length C9), but not for PFDA (half-lives were reported by Ohmori *et al.*, 2003, after a single intra-venous dose: 5.63 days in males and 0.08 days in females for PFOA, 29.5 days in males and 2.44 days in females for PFNA and 39.9 days in males and 58.6 days in females for PFDA). However this gender difference was not reported (for PFOA or PFNA) in mice or humans (no data is available for PFDA in the CLH report). Fecal elimination was demonstrated to be a major route for PFDA in contrast to PFCAs with shorter carbon chain such as PFOA (Kudo *et al.*, 2001), and, based on the longer half-life, PFDA was more slowly eliminated than PFOA in rats. These differences in elimination rate would further reinforce the concern for PFDA (slower elimination rate) but do not preclude these substances from being considered similar based on the general trend of their physico-chemical profile and considering that these substances are absorbed and not metabolised.

Finally, the DS provided data on PFCAs with carbon chain length C11-C12 to support the weight of evidence assessment (as described in the section on Reproductive toxicity). RAC has previously used a similar read-across approach from PFOA to PFNA, which has just one carbon less than PFDA (C10) and one carbon more than PFOA/APFO (C8).

Taking into account the above considerations, RAC agrees with the DS to read-across data on effects between the PFO (C8) and PDF (C10) anions, with supportive data from the PFN (C9) anion.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

No OECD test guideline studies on the carcinogenic properties of PFDA are available, and only two limited studies investigating the potential of PFDA to promote tumors were included.

Borges *et al.* (1993) investigated the tumour promoting activity of PFDA in two-stage hepatocarcinogenesis in rats. Twenty-four hours after partial hepatectomy, female Sprague-Dawley (SD) rats were given an initiating dose of 10 mg/kg diethylnitrosamine by gavage and then the rats were divided into five groups that received monthly i.p. injections of 0.0, 0.05, 0.50 or 5.0 mg/kg PFDA in corn oil for 9 or 18 months. Control groups were placed on diets that contained either 0.01% ciprofibrate or 0.05% phenobarbital. PFDA increased the activity of the peroxisomal enzyme fatty acyl-CoA oxidase at the highest dose. PFDA treatment did not increase the tumour incidence or the number of altered hepatic foci at 9 or 18 months (although the mean volume of foci was increased at 9 months). The results of this investigation indicated that PFDA is not a promoter of liver tumours.

In contrast, PFDA was shown to be a liver tumour promoter in the rainbow trout in the study of Benninghof *et al.* (2012). The authors investigated the promoting activity of various perfluoroalkyl compounds, including PFDA, in the rainbow trout. This animal model is known to be insensitive to peroxisome proliferation (like humans) and the aim of this study was therefore to investigate a mode of action for tumour promotion, namely estrogen signaling similar to 17β-oestradiol (E2). A two-stage chemical carcinogenesis model was employed in trout to evaluate the role of PFDA as well as PFOA, PFNA, perfluorooctane sulfonate (PFOS), and 8:2 fluorotelomer alcohol (8:2 FtOH) as complete carcinogens or promoters of aflatoxin B(1) (AFB(1))- and/or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced liver cancer. Positive controls were E2 and the classic peroxisome proliferator, clofibrate (CLOF). The initial treatment for PFDA was 2000 ppm but due to an unexpected number of early mortalities, this was reduced to 200 ppm (5 mg/kg). Incidence of liver tumours (6.8 fold), multiplicity, and size of liver tumours in trout fed diets containing PFDA were significantly higher compared with AFB(1)-initiated animals fed a control diet. PFDA was the most potent promoting agent tested in the study: 200 ppm PFDA increased liver tumour incidence to a greater extent (26% higher) than did a 10-fold

higher diet concentration of PFOA. According to statistical analyses (Pearson correlation analyses, unsupervised hierarchical clustering, and principal components analyses), the hepatic gene expression profiles for E2 and PFOA, PFNA, PFOs and PFDA were highly similar overall, although distinct patterns of gene expression were evident for each treatment, particularly for PFNA. Overall, these data on the rainbow trout suggest that PFDA, in common with the other tested PFAAs, can promote liver cancer in this animal model (with a mode of action that would therefore be independent of peroxisome proliferation but linked to an estrogenic signaling, similar to 17β-oestradiol based on gene-transcription profiling)

Given that the information on PFDA itself and its salts are very limited, the DS used the data and the RAC assessment from the analogue PFOA and its ammonium salt APFO to evaluate the carcinogenic properties of PFDA. This approach has been followed by RAC (cf. "Assessment and comparison with criteria" below).

In conclusion, the DS proposed to classify PFDA and its sodium and ammonium salts as Carc. 2; H351 (Suspected of causing cancer) based on read-across of this hazardous property from APFO/PFOA.

Comments received during public consultation

For this hazard endpoint, two member states (MS) supported classification of PFDA and its sodium and ammonium salts as Carc. 2, H351 (Suspected of causing cancer) based on read-across from APFO/PFOA with support from two non-guideline compliant studies on tumourigenesis.

Assessment and comparison with the classification criteria

The information on PFDA itself is not sufficient for a conclusion on classification. However, RAC agrees with the DS that the classification for PFDA can be based on read-across from data for APFO/PFOA.

The RAC assessment from the Opinion Document for APFO (ECHA 2012) provides a summary of the neoplastic and non-neoplastic lesions from carcinogenicity studies of APFO in rats.
The ammonium salt of PFOA (APFO) has been tested in two carcinogenicity studies (Sibinski, 1987; Biegel *et al*., 2001; cf. RAC opinion for APFO) that showed increased incidences of liver adenomas, Leydig cell adenomas and pancreatic acinar cell tumours in male SD rats. In addition, increased rates of mammary fibroadenomas were seen in female rats, albeit due to the high incidence in the control female group, evidence for a carcinogenic potential of APFO in female rats is equivocal. For the liver adenomas, it was considered that much of the response to APFO could be attributed to PPARα and induction of PPARα regulated genes (peroxisome proliferation). It is noted in the CLP Guidance (Section 3.6.2.3.2) that "liver tumours in rodents conclusively linked to peroxisome proliferation" are considered not relevant for humans. However, it was also concluded that other modes of action cannot fully be excluded because ,as stated in the RAC opinion for APFO: "*there is still some degree of uncertainties with the significance of other nuclear receptor activation on tumour growth"*.

Increased incidences of pancreatic acinar tumours were seen in male rats in two carcinogenicity studies and in one of the studies both the incidence of tumours and corresponding hyperplasia were significantly increased. Also, in the RAC opinion for APFO, RAC agreed that there was insufficient evidence to link the significant increases in Leydig cell adenomas to PPARα. Overall, the information from animal studies were considered to provide some evidence of carcinogenicity of APFO and relevance to humans could not be ruled out, therefore APFO (as well as PFOA) were classified in category 2 for carcinogenicity.

RAC agrees with the DS and concludes that PFDA and its sodium (PFD-S) and ammonium (PFD-A) salts should be classified for their potential to cause cancer as **Carc. 2; H351 (Suspected of causing cancer)**.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The DS proposed to classify PFDA (-C10) and its sodium and ammonium salts as Repr. 1B; H360Df (May damage the unborn child, and Suspected of damaging fertility) and Lact.; H362 (May cause harm to breast-fed children), based on the limited available data on PFDA itself and read-across of reproductive hazard properties from the structural analogue APFO/PFOA (assessed by RAC in 2011) and PFNA (C9 analogue of PFOA which was assessed by RAC in 2014) was also used. Data on longer chain PFCA (C11-C12) were also included to provide additional support to the proposal.

Comments received during public consultation

Two MSs supported the classification proposal of PFDA and its sodium and ammonium salts as as Repr. 1B; H360Df (May damage the unborn child and Suspected of damaging fertility.) and Lact.; H362 (May cause harm to breast-fed children) as proposed by the DS.

One of these MSs also highlighted an additional human study (Jensen *et al.*, 2015) providing further support of PFDA as a reproductive toxicant (significant association between serum levels of PFDA and miscarriages in pregnant women (2800 participants in Denmark), with higher serum levels in women with spontaneous miscarriages than in women giving birth .
One MS provided additional information regarding human studies that could have been included in the CLH report, but did not express agreement or disagreement with the proposal.
Studies in which PFDA was measured in breast milk included the following:

- In the study of Kim *et al*. (2011), levels of perfluoroalkyl acids (PFAAs) in maternal serum, umbilical cord and breast milk in the general population of South Korea (small samples, n=20) were measured. PFDA, as well as PFNA, were not detected in breast milk, while PFOA was detected (41 pg/mL, LOD of 8 pg/mL). The DS acknowledged in the RCOM that PFDA and PFNA were not detected above the limit of detection (LOD) in breast milk but also emphasised that from that study, it could be noted that PFDA was detected in umbilical cord serum (0.12 ng/mL) in addition to maternal serum (0.36 ng/mL) and that a correlation could be made between concentrations in maternal serum and cord serum for PFDA as well as for PFOA and PFNA.
- In the study of Kärrman *et al*. (2007), PFAA were measured in breast milk and maternal serum from primiparous Swedish women. PFDA was detected in blood (serum 0.53 ng/mL) but to a lesser extent than PFNA (0.80 ng/mL) or PFOA (3.8 ng/mL). PFDA was not detected in breast milk when PFOA and PFNA were detected. The DS responded in the RCOM that PFNA was detected in 2 out of 12 samples and PFOA in 1 out of 12 samples and at very low levels.

The DS further underlined in the RCOM that while PFDA was not detected in the breast milk in these 2 studies (Kim *et al.*, 2011; Kärrman *et al.*, 2007), this is in contrast with the results of the Fujii (2012) study (quoted in the CLH report) where PFDA was detected. In addition, 2 other studies conducted in China and referenced in the Fujii (2012) study also reported PFDA in breast milk (So *et al.*, 2006 and Liu *et al.*, 2010). According to the DS, it seems that levels of PFDA in breast milk vary depending on the regions: samples of breast milk from specific regions in Japan and China had higher levels of PFDA than samples of breast milk from Sweden, South Korea or Spain.

Other human studies provided during public consultation on reproductive toxicity provided additional information:

- Louis *et al.* (2012, 2013) did not report an association between PFDA serum levels and endometriosis in US women (n=190) nor did they report an effect on couple fecundity (n=501). However, as highlighted by the DS in the RCOM, the same author in a very recent publication (2015, after the CLH report data collection had been completed) reported that PFDA (as well as other compounds such as PFOA, PFOS and PFNA) were associated with a lower percentage of sperm with coiled tails (n=501). These findings are in line with animal studies pointing to effects on semen endpoints.

- Joensen *et al*. (2009) did not observe a difference in mean serum levels of PFAA (including PFDA) in 105 men with high or low testosterone levels. No association was shown between testosterone levels and level of PFDA, as was also the case for PFOA or PFNA. Moreover, effects on the semen quality were reported in that preliminary study. In addition, this study was used for classification of PFNA and therefore reported in the CLH report (although not cited).
- Vestergaard *et al.* (2012) reported that serum levels of PFAA did not differ among the women who became pregnant and those that did not in a group of 430 couples.
- Christensen *et al.* (2011) investigated the association between maternal serum concentrations of PFC and the age of the offspring girls' menarche. All levels of PFDA were below the detection limit (0.2 ng/mL) and therefore no conclusion can be made on the reprotoxicity of PFDA.
- The study in mice of Johansson (2008) compared PFOA and PFDA for neurobehavioural effects: PFDA did not affect body weight, clinical signs or behavioural parameters in contrast to PFOA or PFOS but the DS rightly emphasized that classification of PFOA is not based on such effects and the implication of these data for the appropriateness of the read-across is unclear.

Assessment and comparison with the classification criteria

RAC, after reviewing the additional studies from the public consultation, acknowledged that, from the extensive literature on perfluorinated compounds, some studies can provide further support while others did not examine a sufficiently large number of people to conclude, or provided a negative association. RAC also considered that the analysis by the DS was thorough and carefully developed, paying attention to the weight of evidence (both positive and negative findings). The CLH report together with the information from the public consultation contained an appropriate dataset for concluding on classification for PFDA.

Fertility

(a) Available data on PFDA itself consists of mechanistic studies; no study on adverse effects on fertility and sexual function of PFDA is available.

<u>One intra-peritoneal study investigating androgenic status of PFDA in rats</u>

In the study of Bookstaff *et al.* (1990), investigating androgenic status in rats, animals were treated with a single i.p. dose of PFDA at 0, 20, 40 and 80 mg/kg. Three or six rats from each dosing group were castrated 2 hours after treatment and a testosterone-containing capsule was inserted subcutanesously (no information on the number of animals in each treatment group). Two controls were used: *ad libitum*-fed controls (ALC) and pair-fed controls (PFC). A decrease in bodyweight by 72% when compared to ALC controls was reported at the high dose of 80 mg/kg, this decrease was less than 10% when compared to PFC controls. This decrease correlated with reduced food consumption: a decrease of 44% when compared to ALC controls was reported. At 40 mg/kg, body weight was lower than controls by 16%.

A statistically significant decrease in plasma testosterone concentration and its metabolite 5-alpha-dihydrotestosterone were reported at 40 and 80 mg/kg, although these decreases were not dose-related: 25% and 32% at 40 mg/kg and 12% and 18% at 80 mg/kg, respectively (when compared to both ALC and PFC controls). No effect on plasma testosterone concentration was observed in castrated (and implanted) rats.
A slight but statistically significant decrease in testis weight (8% estimated from graphical representation) was reported at the high dose of 80 mg/kg but no information was provided whether is absolute or relative and the bodyweight was decreased by 72% in comparison to controls at this dose level. In addition, it was not associated with histological changes. However, a decrease in the weight of seminal vesicles and ventral prostates was also reported, in a dose-related manner, 42% and 49%, respectively, at 80 mg/kg when compared to ALC controls. No information is provided whether is a decrease in absolute or relative weight but these decreases were associated with changes at microscopic examination with marked atrophy of the epithelia of the seminal vesicle and ventral prostate. The epithelial height in seminal vesicles was

50% less than in controls (both ALC and PFC) at 80 mg/kg. In the ventral prostate, 60% of the glandular acini were lined by low cuboidal epithelium versus 20% in controls (both ALC and PFC). In castrated animals, these changes were not reported: ventral prostate weights were not different from controls and seminal vesicle weights were different from PFC controls only at 20 and 40 mg/kg (not at 80 mg/kg).

Since the observed effects correlated with the decrease in plasma testosterone in intact rats and, in contrast, in castrated rats no effects were reported in the absence of alterations in plasma testosterone concentration, it was concluded that the effects on seminal vesicles and prostates were secondary to plasma androgen concentration.

In addition, the *ex vivo* experiment with animals with decapsulated testes from PFDA-treated rats demonstrated a decreased testosterone secretion after stimulation with LH analogue human chorionic gonadotropin at 100 mIU/mL, therefore suggesting that PFDA decreased the testicular responsiveness to LH stimulation.

Two acute intra-peritoneal mechanistic studies with PFDA – testes effects

Olson and Andersen (1983) investigated the acute toxic effect of PFDA in tissue fatty acids in male Fisher rats after a single i.p. injection of 50 mg/kg. ALC and PFC were used. PFDA caused a decreased food intake on the first day (intake was close to 0 on day 7 to 14). This was associated with decreased body weight with almost 50% weight loss (from 207 to 109 g), the decrease being >20% from day 6. The bodyweight loss was in a similar range in the PFC group (from 207 to 131 g). After day 8, mean testes weights were significantly lower than in both vehicle and PFC controls (1.7 g vs. 2.8 g in controls and 2.2 g in PFC). Adrenal, heart and liver weight were also lower than controls in this study. PFDA-exposed animals exhibited increased fractions of palmitic and oleic acids and decreased fractions of stearic and arachidonic acids.

George and Andersen (1986) investigated the toxic effects of PFDA in male Fisher rats after a single i.p. injection of 50 mg/kg. Rats were killed and examined 4, 8, 12, 16 or 30 days after injection (6 rats/group at least). Pair-fed controls were used. Treatment with PFDA caused decreased food intake within the first days (close to 0 over days 4 to 12). Associated decreased body weight gains with weight loss was observed until day 13 in both the treated and control groups, but the weight loss was greater in the PFDA treated group, with bodyweight of 100 g vs. 70 g at day 16. Bodyweight remained about the same until day 18-20, after which rats gradually started to gain weight. Atrophy and degeneration of the seminiferous tubules in the testes was observed from day 16 and persisted up to day 30. No quantification was available but the findings were significant according to the authors of the study. Inflammation and ulceration of the stomach, thymic atrophy as well as liver effects (increased weight, cellular swelling, associated with inflammatory cell infiltration and signs of necrosis) were also reported in that study.

One in vitro study – antiandrogenic effect of PFDA

In an *in vitro* mechanistic study, Kjeldsen *et al.* (2013) investigated the interference of PFDA (and other perfluorinated compounds) with steroid hormone receptor functions on the Chinese hamster ovary cell-line. No estrogenic or antiestrogenic effects were observed, with or without co-treatment with E2 for PFDA (weak effects for PFOA and PFOS). As regards the androgenic receptor, upon co-treatment with DHT, PFDA elicited significant concentration-dependent antagonist effects on DHT-induced androgenic receptor activity, similar to PFNA, PFOA and PFOS with an IC_{50} of 6.10^{-6} M (IC_{50} for PFOA and PFNA were $1.1.10^{-5}$ M and $5.2.10^{-5}$ M, respectively). In addition, PFDA weakly decreased the aromatase activity at a high test concentration of 1.10^{-5} M but cytotoxicity was noted from 1.10^{-4} M, and possibly beginning already at 10^{-5} M.

(b) Given that the information on PFDA itself and its salts is very limited, RAC agreed with the dossier submitter to use the data from the analogue PFOA and its ammonium salt APFO to assess the reproductive toxicity of PFDA. The results of the 2-generation study in rats (York, 2002; Butenhoff *et al*., 2004) as well as the conclusion of the RAC opinion on classification (2011) were provided in the CLH report.

No effects on mating and *fertility parameters* were reported in the F0 and F1 generation exposed to up to and including the highest dose level of 30 mg/kg bw/d APFO in the diet. The only effects observed were increased relative weights of the epididymides and seminal vesicles but these were considered by RAC as probably linked to body weight loss: 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 bw/d. However, due to a statistically significant reduction in body weight at the same dose level, the organ-to-body weight ratios were either normal or increased.

An increased incidence in Leydig cell tumours and vascular mineralisation in testes of rats in the chronic 2-year study in rats (Sibinski *et al.*, 1987) was observed but was not considered by RAC to be indicative for effects on fertility: at the 2-year sacrifice, vascular mineralisation 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 adenomas in the high-dose 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 been a result of endocrine changes, because a reduced aromatase activity and a sustained increase in serum oestradiol were reported in the study by Biegel *et al.* (2001).

An additional study on testosterone levels and male reproductive organ effects of APFO was published after submission of the CLH dossier on APFO and was assessed by RAC (Li *et al.*, 2011): in male mice, oral APFO-treatment (0, 1 and 5 mg/kg bw/d) for 6 weeks of both wild-type (WT), null- or humanised 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/d) 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).
RAC concluded in 2011 that this study provided evidence on impaired fertility through sperm abnormalities and reduced testosterone levels but was not (yet) sufficient to overcome the negative evidence from the 2-generation study and repeated dose toxicity study. No classification was concluded but reconsideration of the endpoint was also recommended.

(c) Data from the analogue PFNA and its ammonium and sodium salts, documented in the RAC opinion on PFNA (from 2014), were also used to assess the reproductive toxicity of PFDA.
In the RAC assessment of PFNA, in addition to the results of the Li *et al.* (2011) study cited above, the mechanistic study of Feng *et al.* (2009), in which male SD rats were exposed by gavage to PFNA at doses of 0, 1, 3 and 5 mg/kg bw/d for 14 days was considered. According to the RAC opinion, neither the Li *et al*. (2009) study nor the Feng *et al*. (2009) study, due to the aims of the studies and methodologies used, demonstrated that APFO or PFNA produces an adverse effect on sexual function and fertility, such as reduction of mating or fertility indexes or sperm counts. However, they demonstrated that APFO and PFNA may affect morphology of sperm, alter level of sex hormones (testosterone and oestradiol) and biochemical processes essential for sperm production or sexual behavior.

In addition, an oral 2-generation reproductive toxicity study using S-111-S-WB in rats (Stump *et al*., 2008) was also assessed. S-111-S-WB (fatty acids C6–C18, perfluoro, ammonium salts, CAS No. 72968-38-8) is a mixture of perfluorinated fatty acid ammonium salts of different carbon chain lengths that is used a surfactant in polymer manufacturing. The major component of S-111-S-WB is PFNA, although detailed information on content of various constituents was not provided. S-111-S-WB was administered daily via oral gavage to 30 Crl:CD(SD) rats/sex/group at doses of 0.025, 0.125 and 0.6 mg/kg bw/d over two generations to assess its potential for reproductive toxicity.
Reproductive performance, mean litter size, pup survival and pup weights were unaffected. No test substance-related effects were observed in the F0 and F1 generations on male and female fertility index, estrous cycle length, mean testicular sperm numbers and sperm production rate at any dose. Slightly lower, but statistically significant, mean sperm motility (95.3% of the control

value) and progressive motility (94.4% of the control value) was noted for F0 males, but not in F1 males, in the 0.6 mg/kg bw/d group when compared to the control group values.

Sperm concentration (10^6/g) in the left epididymis in F0 males was reduced in the 0.025 and 0.6 mg/kg bw/d groups to 86.4% and 86.5% of control values, respectively, but sperm concentration in the left epididymis was not reduced in the 0.125 mg/kg bw/d group. In the F1 male generation, sperm concentration (10^6/g) in the left epididymis was not affected by S-111-S-WB treatment. No pathological changes were observed in histopathological examinations of the testes of F0 and F1 male rats.

General toxicity was also reported. Lower mean body weights were observed in the 0.6 mg/kg bw/d group in F0 and F1 males. Higher absolute and relative liver weights were noted in F0 and F1 males in the 0.125 and 0.6 mg/kg bw/d groups, and in F0 and F1 females in the 0.6 mg/kg bw/d group. Hepatocellular hypertrophy was observed in F0 and F1 males in the 0.025, 0.125 and 0.6 mg/kg bw/d groups and in F0 females of the 0.6 mg/kg bw/d group. The foci of hepatocellular necrosis with associated subacute inflammation were observed in F0 and F1 males of the 0.025, 0.125 and 0.6 mg/kg bw/d group. Higher kidney weights were observed for parental males and females in the 0.125 and 0.6 mg/kg bw/d groups. Hypertrophy of renal tubule cells for F0 males and females were seen in the 0.6 mg/kg bw/d group correlated with increases in mean absolute and relative kidney weights. Total S-111-S-WB concentration in the serum of male and female pups was 1.2-1.4-fold higher than in the dams 2 h following administration to the dams on lactation day 13.

RAC considered that the results of the 2-generation study with S-111-S-WB, containing a mixture of perfluoroalkyl acids, primarily of longer carbon chain length than PFOA, with PFNA as a major component, did not provide sufficient evidence of alterations of fertility due to exposure to this mixture at dose levels of 0.125 and 0.6 mg/kg bw/d. The exposure at these doses elicited clear systemic toxicity due to hepatotoxicity and nephrotoxicity of the mixture, particularly in male rats. Statistically significant, although not dose-related, and quantitatively minor (5-14%) reductions in sperm motility and sperm count in the epididymis of F0 males, but not in F1 males, without histopatological changes in the testes, demonstrated potential for testicular toxicity from exposure to S-111-S-WB. However, these minor alterations in sperm quality could be related to systemic toxicity due to liver and kidney dysfunction.

Finally, RAC assessed the available data from humans in the study of Nordström Joensen *et al*. (2009), in which a group of 105 young adult men reporting for military draft in Denmark were examined to discover a possible association between the levels in serum of PFAA and testicular function. The serum level of 10 different PFAA with carbon chain length from C6 to C13 was examined. Out of all the PFAAs examined, the highest concentrations were found for perfluorooctane sulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), PFOA and PFNA (medians of 24.5, 6.6, 4.9, and 0.8 ng/mL, respectively). The high serum concentrations of PFAAs were significantly associated with reduced numbers of normal spermatozoa. In addition, sperm concentration, total sperm count, and sperm motility showed some tendency toward lower levels in men with high PFAA levels, although not at statistically significant levels. The authors noted that the results from this preliminary study should be corroborated in larger studies.

RAC concluded that classification of PFNA, PFN-S and PFN-A as Repr. 2; H361f (suspected of damaging fertility) was warranted. In the opinion of RAC, the existing evidence was not sufficient to classify PFNA, PFN-S and PFN-A as Repr.1B; H360F (May damage fertility), because the effect on the sperm count was observed only in the F0 generation, but not in F1 males exposed to a mixture of perfluorinated fatty acid ammonium salts of different carbon chain lengths in a 2-generation study (Stump *et al*., 2008) and the epididymal sperm count was not affected in WT, Pparα-null and PPARα-humanized mice exposed orally to APFO for 6 weeks (Li *et al*., 2011). The fact that PFOA and APFO were not classified for sexual function and fertility (due to negative results of a 2-generation study with APFO [York, 2002; Butenhoff *et al*., 2004] and the lack of supporting evidence from repeated dose toxicity studies, which gave no indication of disturbances of fertility) in the RAC opinion (December, 2011) was also considered.

(d) To strengthen the weight of evidence, in addition to the data for the analogues PFOA/APFO and PFNA, the DS provided data from longer perluoroalkyl acids (PFCAs), C11 (perfluoroundecanoic acid, PFUnDA) and C12 (perfluorododecanoic acid, PFDoDA), i.e. two recent studies which reported effects, mainly on male reproductive organs.
In a screening test for reproduction/developmental toxicity conducted according to OECD TG 422, in rats dosed by gavage at doses of 0.1 to 1.0 mg/kg bw/d of PFUnDA (Takahashi *et al.*, 2014), no histopathological effects on reproductive organs were observed. Yet, a slight but significant (11%) decrease in testes weights was reported at the high dose of 1 mg/kg bw. Minimal spermatic granuloma in the epididymis was detected in 1/5 animals and mild spermatic granuloma at the low dose of 0.1 mg/kg bw in 1/1 males was also reported.
A similar screening test for reproduction/developmental toxicity was conducted according to OECD TG 422 on PFDoDA (Kato *et al*., 2014) at doses of 0.1, 0.5 and 2.5 mg/kg bw/d via gavage. The findings on reproductive toxicity in this study were mainly reported at the top dose of 2.5 mg/kg bw, which was associated with general toxicity: body weight was decreased, with mean body weight being approximately 30% lower than controls (for males at recovery day 14 and in females at GD 20-21).

In the male reproductive organs, various histopathological changes, including decreased (not statistically significant) spermatid (slight change in 2/7 animals) and spermatozoa counts (slight change 2/7; moderate change 1/7; severe change 1/7); slight spermatic granuloma (2/7) and cell debris in the lumen of the epididymis (slight to moderate change 3/7); and slight to moderate glandular epithelium atrophy of the prostate (4/7), seminal vesicles (4/7), and coagulating gland (4/7) were observed after exposure to 2.5 mg/kg bw/d for 42 days. The absolute testis weight was 15% lower than in controls (not statistically significant) at the end of the recovery period at the top dose of 2.5 mg/kg bw.
In that study, toxicity in females was also reported in 7 out of 12 females that died during late pregnancy. Haemorrhage at the implantation site and/or congestion of the endometrium were detected in the uterus of all the 7 females that died during the gestation period. Haemorrhage at the implantation site was also detected in one female that delivered stillborn pups. Continuous diestrous was observed in the females of the 2.5 mg/kg bw/d group that were not mated (satellite group) during the administration period (length of estrous cycle could not be determined). During the recovery period, 1 out of 5 females in the same group had normal estrous cycles. Female rats that were assigned to the dosing groups to be mated displayed normal estrous cycles and length during the premating period at all doses. Four out of 12 female rats (in addition to those 7 rats who died during late pregnancy) receiving 2.5 mg/kg bw/d did not deliver live pups, i.e. only one dam delivered pups normally (14 alive, 2 dead).

Alterations in gene and protein expression in the testes of rats exposed to PFDoDA have been investigated by Shi *et al.* in a number of studies with shorter (14 days) or longer (110 days) periods of treatment and the results indicated that PFDoDA disrupts testicular steroidogenesis and expression of related genes in male rats.

At the highest dose levels tested (5 or 10 mg/kg/bw), where excessive general toxicity were evident (markedly reduced body weight), PFDoDA was reported to induce an apoptotic effect in cells in rat testes: Leydig cells, Sertoli cells and spermatogonic cells were displaying apoptotic morphological features after 14 days treatment of PFDoDA (Shi *et al.*, 2007). The testicular mRNA expression of several genes involved in cholesterol transport and steroid biosynthesis were significantly reduced at the same dose levels. However, it is unclear to what extent this deteriorated expression is relevant considering the concomitant apoptotic cell death in the tissue.

Exposure to PFDoDA for 110 days resulted in a dose-dependent decrease in serum testosterone levels and levels were statistically significant markedly decreased ($p<0.05$) at 0.2 mg PFDoA/kg bw/d (56% of control levels) and 0.5 mg PFDoA/kg bw/d (40% of control levels) (Shi *et al*., 2009a). PFDoA exposure resulted in significantly decreased ($p<0.05$) protein levels in testes: steroidogenic acute regulatory protein (62.6%, 50.6% and 53% of control levels at 0.05, 0.2 and 0.5 mg/kg, respectively) and decreased cholesterol side-chain cleavage enzyme at 0.5 mg/kg bw/d (Shi *et al*., 2009a). Also, in female rats, genes responsible for cholesterol transport and steroidogenesis were reported to be affected. The ovarian expression of steroidogenic acute regulatory protein and cholesterol side-chain cleavage enzyme was significantly decreased

($p<0.05$) at 3 mg/kg bw/d (72% and 62% of control levels, respectively). 17-beta-hydroxysteroid dehydrogenase was increased ($p<0.05$) from 0.5 mg/kg bw/d. Furthermore, PFDoDA significantly decreased oestradiol levels (60% of control levels) and increased cholesterol levels ($p<0.05$) at 3 mg/kg bw/d. These expressional changes in genes show that PFDoDA may play a role in the reduction of testosterone.

Conclusion on fertility

RAC agrees with the DS that that the classification for PFDA can also be based on read-across from data for APFO/PFOA and PFNA using the analogue approach.

In the RAC opinion ((2011) on classification of PFOA/APFO, no classification was considered warranted, mostly based on negative results of a 2-generation study with APFO, and the lack of supporting evidence from repeated dose toxicity studies. No relevant effects in male and female animals were reported from the 2-year carcinogenicity study in rats: this study revealed only treatment related testis tumours, which were not related to fertility effects.

However, RAC also noted the recently published study of Li *et al.* (2011) in mice, indicating a potential for adverse effect on the male mouse reproductive system (study conducted on APFO with the aim of elucidating the mechanism on lowering testosterone levels). RAC noted the evidence on impaired fertility through sperm abnormalities and reduced testosterone levels from the Li *et al.* (2011) study but concluded that they were not (yet) sufficient to override the negative evidence from the 2-generation and repeated dose toxicity studies but reconsideration of the endpoint (fertility) was recommended.

For the classification of PFNA in 2014, RAC reconsidered the data and included the results of the study of Li *et al*. (2011) on APFO, which is an analogue compound to both APFO/PFOA and PFDA. RAC then concluded that PFNA should be classified in category 2 for fertility based on the weight of evidence from the following data:

- minor effects (small reductions in sperm motility and sperm count in epididymis of F0 males, but not in F1 males) without reductions in mating or fertility indexes with the mixture S-111-S-WB which has PFNA as major constituent, in a 2-generation study (Stump *et al*., 2008);
- increased serum testosterone levels, decreased serum oestradiol levels and increased frequency of spermatogenic cells with apoptotic features in rats exposed by gavage to 5 mg PFNA/kg bw/d (Feng *et al*., 2009);
- reduced plasma testosterone concentrations, increased frequency of abnormalities in sperm morphology and vacuolated cells in the seminiferous tubules of 129/sv WT (mPPARα) mice and hPPARα mice exposed orally to APFO for 6 weeks, although these effects could be mediated in part by liver peroxisome proliferation, since they were not observed in similarly exposed PPARα-null mice (Li *et al*., 2011); and
- the supporting preliminary human data.

Although the effects on testosterone concentration and sperm abnormalitites observed in the Li *et al.* (2011) study could be related in part with liver peroxisome proliferation, as they were not observed in similarly exposed PPARα-null mice, these results were included in the weight of evidence evaluation.

In addition, two recent studies conducted on longer alkyl PFCA (C11 and C12) also reported effects on male reproductive organs (testicular toxicity, decreased testosterone levels).
Data on the substance PFDA itself may be limited to mechanistic studies but they did report alterations for male parameters with decrease testis weight, atrophy or degeneration of seminiferous tubules in the testes, decreased weight of seminal vesicles and ventral prostates with marked atrophy of the epithelia, decreased plasma testosterone after i.p. exposure as well as *in vitro* antagonism on androgenic receptor activity upon co-treatment with DHT, in a similar way to PFOA/APFO and PFNA (sperm abnormalities, effects on male reproductive organs, altered testosterone levels).

In conclusion, RAC agrees with DS that overall, the data provide some evidence of adverse effects on sexual function and fertility for PFDA and that classification of PFDA as **Repr. 2; H361f (Suspected of damaging fertility)** is warranted.

Development

(a) Very limited data was available for PFDA. In humans, one cross-sectional study aimed to determine if specific blood levels of perfluoroalkylated substances are associated with impaired response inhibition in children (Gump *et al*., 2011). The mean content of PFDA in blood samples from 83 children was 0.26 ng/mL (the concentrations for PFOS, PFOA and PFNA were 9.90, 3.23 and 0.82 ng/mL, respectively). The blood levels were analysed in relation to the "differential reinforcement of low rates of responding" (DRL) task. This task rewards delays between responses, i.e., longer inter-response times (IRTs) and therefore constitutes a measure of (impulsive) response inhibition.The authors considered the hypothesis that increasing blood levels of perfluoroalkylated chemicals are associated with increasing impulsivity in children (as measured using the DRL task). The task was conducted on computer and the IRT between each button press was recorded. Rapid responding is reflected by short IRTs and delayed responding is reflected by longer IRTs. As a result, the tested PFAAs were associated with impaired response inhibition in this group of children, and IRTs for PFDA were in a similar range as for the other tested PFAAs.

In addition, as raised during public consultation, in a cohort study with 2008 participants in Denmark, the authors (Jensen *et al.*, 2015) reported an increased risk of miscarriages in women exposed: a significant association was shown between serum levels of PFDA and miscarriages. Higher levels of PFAAs were reported in women with spontaneous miscarriages compared to women giving birth and an increased risk by a factor of 16 was calculated.

In the prenatal developmental toxicity study (no guideline, non-GLP) with PFDA (Harris and Birnbaum, 1989) in rodents, groups of 10-14 females C57BL/6N mice were dosed orally once per day by gavage during gestation,

- either 4 consecutive days (GD 10-13) at 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16 or 32 mg/kg bw/d;
- or 10 consecutive days (GD 6-15) at 0.03, 0.3, 1.0, 3.0, 6.4 or 12.8 mg/kg bw/d.

At 12.8 mg/kg bw/d, among the dams dosed over GD 6-15, 3/10 dams died on GD 18 (30% mortality), which is considered as excessive toxicity. At 6.4 mg/kg bw/d, a small decrease in net body weight change over GD 6-18 was reported compared to controls (0.4 g; 1.8% increase vs. 4.9 g; 22% increase) (-2.4g body weight loss was reported at the dose of 12.8 mg/kg). Maternal relative liver weight was significantly increased from 1.0 mg/kg bw/d (7.9, 10.3, 13.8, 15.2 g at 1.0, 3.0, 6.4, and 12.8 mg/kg bw/d, respectively, vs. 6.7 g in controls), but this was not associated with adverse histopathological effects in the liver and was not considered as evidence of maternal toxicity.

Increased resorptions per litter were observed at 6.4 mg/kg (19.1%, not statistically significant) and 12.8 mg/kg (41.7%) with full litter resorptions in 1/13 dams at 6.4 mg/kg bw/d and 3/7 dams at 12.8 mg/kg bw/d. The number of live foetuses per litter was decreased from 6.4 mg/kg bw/d, although not statistically significant compared to control at 6.4 mg/kg bw/d (5.8 and 4.6 at 6.4 and and 12.8 mg/kg bw/d, respectively, vs. 7.2 in controls). Foetal body weight per litter was decreased in a dose dependent manner from 1.0 mg/kg bw/d and up; -4.3%, -6%, -23% and -50% at 1.0, 3.0, 6.4 and 12.8 mg/kg bw/d, respectively.

(b) Given that the information on PFDA itself and its salts is very limited, RAC agrees with the dossier submitter to use the data from the analogue PFOA and its ammonium salt APFO to assess the adverse effects on development of PFDA. Relevant effects indicating developmental toxicity were observed at doses without marked maternal toxicity. APFO/APFO were assessed by RAC in 2011.

In rats, in the 2-generation study (York, 2002; Butenhoff *et al*., 2004), relevant adverse effects on development with pup mortalities and reduced growth were reported during lactation and caused delayed sexual maturation in the rat offspring.

In mice, exposure during gestation caused developmental effects (but no malformations occurred) without signs of marked maternal toxicity and this was demonstrated in a number of studies. The most severe effects were whole litter loss in early pregnancy at 5 mg/kg bw/d when treatment was started on GD1 (Wolf *et al.*, 2007) and the percentage of dams with full litter resorptions increased from 5 mg/kg bw/d (Lau *et al.*, 2006). Other developmental effects included reduced pup viability and pup body weight gain (growth), delayed development (delayed onset of eye opening) and puberty (delayed development of the mammary gland (White *et al*. (2007, 2009) and Yang *et al*. (2009)) were also observed.

An increase in liver weights was observed in dams treated with APFO, and RAC, in its opinion of 2011, discussed the relevance of liver weight changes for developmental effects.
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 caused by liver cell hypertrophy as a direct effect of APFO, with a major contribution from PPARα-related peroxisome proliferation. The newer study clearly demonstrated that liver toxicity (single cell toxicity) started at higher doses than the hypertrophic response. Therefore the observed developmental effects were not considered to be a secondary non-specific consequence of the maternal (liver) toxicity. Overall it was considered that there is no convincing evidence that the developmental effects in pups were exclusively secondary to maternal (liver) toxicity.

Mechanistic studies used PPAR knock-out mice to investigate the role of PPAR in the developmental effects. Abbott *et al.* (2007) studied the influence of PPARα on PFOA-induced developmental toxicity using WT and PPARα knockout (KO) mice (129S1/SvlmJ). This study indicated that the incidence of complete litter loss appeared to be independent of PPARα expression, while in contrast several of the developmental effects in mice were influenced by PPARα (post-natal pup mortality and reduction in postnatal weight gain and development with delayed eye opening); however, other modes of action must also be active and contribute, given that the increases in liver weight were similar in WT and KO mice.
In addition, the relevance of PPAR expression for humans is well established for the liver but much less is known about the relevance of PPAR-related effects in the offspring and juveniles.

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. This study underlined that the mechanisms of PPARα-associated developmental toxicity of PFOA is far from clear and that the relevance to humans can not be disregarded.

In humans, available biomonitoring indicated that human serum concentrations were lower than those reported for 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 the studies by Olsen *et al*. quoted in the CLH report for PFOA) and median concentrations of 0.0026 µg/mL in maternal samples of a pilot study (Midasch *et al*., 2007)). However, the absence of effects are not proof that effects in animals are not relevant for humans, since internal concentrations were much lower and epidemiological studies were not targeted on the effects of interest and were of insufficient size for effects to be detected.

Therefore, RAC concluded in 2011 that for APFO, the human data do not give sufficient evidence to conclude on category 1A, but that there is clear evidence on developmental effects from perinatal studies in mice. RAC considered that there was not convincing evidence that developmental effects in pups were exclusively secondary to maternal (liver) toxicity. Mechanistic considerations indicate a possible contribution (for some effects) of a PPARα-related mode of action (based on their lack of expression in knock-out mice) but other modes of action appear to be active and the role of PPARα-related mode of action has not been fully elucidated for the developmental effects. It was concluded that the available evidence is sufficiently convincing to classify APFO for developmental effects as Repr. 1B; H360D (May damage the unborn child) according to the CLP criteria.

(c) Data from the analogue chemical PFNA and its ammonium and sodium salts, based on the RAC opinion on PFNA from 2014, was also used to generate information on the adverse effects of PFDA on development.

There were two developmental studies in mice for PFNA (Lau *et al.*, 2009, Wolf *et al.*, 2010). RAC concluded that the available information indicates that exposure to PFNA during gestation reduces pup viability and pup body weight gain, delays puberty as well as the onset of eye opening, increases both dam and pup liver weight (absolute and relative) and causes full litter resorptions at higher doses.

It is noted that one of the mechanisms implicated in the toxicity of the PFNA is the activation of PPARα (Wolf *et al*., 2010). PPARα is a nuclear receptor that plays a role in regulating lipid and glucose homeostasis, cell proliferation and differentiation, and inflammation. However, the role of PPARα in mediating developmental toxicity effects in humans cannot be excluded.

Taking into account that exposure to PFNA in mice during gestation results in the findings described above, as well as that the developmental toxicity findings with PFNA in mice are qualitatively and quantitatively similar to the developmental toxicity findings with PFOA (reduced pup viability, full litter resorption and delay in the onset of eye opening), it was concluded to classify PFNA in category 1B for adverse effects on development.

(d) In addition to the data for the analogues PFOA/APFO and PFNA, the DS provided data on longer PFCAs with chain lengths C11 (PFUnDA) and C12 (PFDoDA) that were studied in reproduction/developmental toxicity screening tests (OECD TG 422; dosing beginning 14 days before mating and ending on day 4 of lactation) in rats (Takahashi *et al*., 2014; Kato *et al*., 2014).

Based on findings in a 14-day dose range-finding study where 9/10 animals (males and females) died after administration (oral gavage) of PFUnDA at 20 mg/kg bw/d, doses of 0.1, 0.3, and 1.0 mg/kg bw/d were selected for further studies. Treatment with PFUnDA resulted in statistically significantly reduced ($p \leq 0.01$) rat pup body weights at birth (13.4% in male pups and 12.5% in female pups, $p \leq 0.01$) at 1 mg/kg, and body weight gain was still reduced to the same extent at 4 days after birth (19.1% in male pups and 16% in female pups, $p \leq 0.01$) in the absence of statistically significant effects on maternal body weight (Takahashi *et al*., 2014). No other significant changes in reproductive or developmental parameters were reported.

The same research group also studied PFDoDA in a similar manner (Kato *et al.,* 2014) at doses of 0.1, 0.5 and 2.5 mg/kg bw/d. No reproductive or developmental parameters were affected at 0.1 or 0.5 mg/kg bw/d. No effect on rat pup body weights was noted (only in one litter in the high dose group; body weight in these pups were not taken into account). The delivery index at 0.5 mg/kg bw/d was slightly but not statistically significantly decreased: 89.7% compared to 94.3% in control. Body weight in the dams was significantly lower than controls at 2.5 mg/kg bw/d throughout the gestation period (approx. 30% less than control, $p<0.01$ at GD 20-21). At this dose level of 2.5 mg/kg bw/d, 7 of 12 female rats died during late pregnancy while four other females in this group did not deliver live pups (Kato *et al*., 2014), with only one dam left that delivered normally (14 alive, 2 stillborns) and therefore no statistical evaluation of the results could be conducted.

Conclusion on development

RAC agrees with the DS that that the classification for PFDA can be based on read-across from data for APFO/PFOA and PFNA.

There was limited but useful information on PFDA itself indicating that, similar to its structural analog APFO, it can induce effects on development. In humans, there is one cross-sectional study that aimed to determine if specific blood levels of perfluoroalkylated substances are associated with impaired behavioural response inhibition in children (Gump *et al*., 2011). The results for PFDA were in a similar range to the other tested compounds.

A Danish study (Jensen *et al.*, 2015) reported an increased risk of miscarriages in women with high serum levels of PFDA. In a non-guideline prenatal developmental study (Harris and Birnaum, 1989), exposure to PFDA in mice during gestation induced full litter resorptions/loss at high doses, seen together with maternal toxicity, as well as a decreased number of live foetuses and reduced foetal weight. These findings reported after exposure to PFDA are therefore similar to those reported for APFO, which induced full litter resorptions and decreased pup weight at birth, and which were not considered to be secondary to maternal toxicity (significant increase in relative liver weight in dams and pups). For PFDA, unlike APFO, the observed effects on development co-occurred with maternal toxicity (increased relative liver weight and decreased body weight gain and mortality at the high dose), but they still provided indications of the similarity of the adverse effects with PFOA/APFO. Indeed, APFO and PFOA induced developmental effects in the absence of marked maternal toxicity: complete litter loss in early pregnancy as well as other developmental effects with increased postnatal pup mortality and developmental delays in general growth (decrease pup body weight) and development (delayed eye opening) as well as sex-specific alterations in pubertal maturation (separable prepuce indicating earlier onset of male puberty) observed in several studies in mice and the 2-generation rat study (this led to their classification as Repro 1B). Increased liver weight occurred in dams but the developmental effects were not considered secondary to maternal toxicity.

Regarding post-natal developmental effects, no study investigating this was available for PFDA so no comparison on this aspect is possible. In addition, the closely related substance PFNA caused developmental effects in mice, which (both qualitatively and quantitatively) were similar to the developmental toxicity of PFOA (reduced pup viability, full litter resorption and delay in the onset of eye opening). Available data from PFNA was considered as further support for evidence of similarity of developmental effects of PFCA and thus of an adverse effect on development for PFDA.

Therefore, considering that:

- PFDA and APFO/PFOA have very similar structure and physico-chemical as well as toxicokinetic properties, justifying a read-across approach of developmental data from APFO/PFOA (C8);
- this read-across is further substantiated by additional data from another close analogue compound PFNA (C9); and
- the available information so far on PFDA (C10) itself (full litter resorption at high doses, reduced foetal weight at birth in a non guideline study with maternal toxicity) indicate that it may affect development with similar effects to those seen with PFOA/APFO,

RAC agrees with the DS that the data provide clear evidence of adverse effects on the development of the offspring not secondary to maternal toxicity, and without mechanistic evidence indicating lack of relevance to humans, and therefore PFDA and its ammonium and sodium salts should be classified as **Repr. 1B; H360D (May damage the unborn child)**.

Lactation

(a) PFDA was detected in human breast milk in several studies. In the study of Fujii *et al.* (2012), human breast milk from a small group of non-randomly selected volunteers (90 women from Japan, Korea and China; 30 samples from each country) were analyzed for the content of perfluorinated carboxylic acids. PFDA was detected in 67% (20/30) of the samples from Japan, and in 13% (4/30) of the samples from Korea and China. Detection limit for PFDA was 15 pg/mL. Detected values ranged from <15 to 29 pg/mL in Korea and China (the mean was <15 pg/mL), and from <15 to 65 pg/mL in Japan (the mean was 21.3 pg/mL). For comparison, the mean values for PFOA were 51.6 (China), 64.5 (Korea) and 93.5 pg/mL (Japan); the mean values for PFNA were 15.3 (China), 14.7 (Korea) and 32.1 pg/mL (Japan).

In the study of Tao *et al.* (2008), 9 perfluorinated compounds were analyzed in human breast milk of 45 women from USA (Massachusetts). PFDA was detected in 4 out of the 45 samples, ranging from <7.72 to 11.1 pg/mL.

PFDA was below the limit of quantification in most breast milk samples in the study of Llorca *et al*. (2010) which analysed breast milk of 20 women from Spain (Barcelona). In the study of Llorca, as

in some other studies quoted during public consultation (Kim *et al.*, 2011; Kaarman *et al.*, 2007), PFDA was not detected in breast milk. However, the levels in breast milk are in general lower than in the two other matrices (mother serum and cord blood) and since in blood the levels of PFDA were lower than those of PFOA, the levels in milk might be below the limit of detection in these studies. Indeed, for example, in the study of Kim *et al.* (2011) quoted during public consultation, the level of PFOA was of 1.6 ng/mL in blood and 0.041 ng/mL in milk. PFDA was not detected in breast milk and the blood level was 0.36 ng/mL; a calculation assuming a similar ratio to that for PFOA (1:40) would lead to an expected calculated concentration in breast milk of 0.009 ng/mL for PFDA, which means below the limit of detection (0.018 ng/mL). In the Kärrman *et al.* (2007) study, the general ratio between blood and milk for perfluoronated chemicals was indicated to be 1:100. In addition, in the study of Kärrman (2007), even PFOA and PFNA were detected in only a few samples. Therefore, the negative findings in breast milk in some studies do not contradict the conclusion that PFDA exposure can occur through lactation. Despite these lower levels in breast milk, animal studies with postnatal administration of PFOA indicated clear evidence of adverse effects in the offspring due to transfer in the milk or adverse effect on the quality of the milk. Similar findings were reported for the closely related compound PFNA, which was assessed by RAC in 2014.

PFDA has also been found at detectable levels in the serum of adults, including pregnant women, and in children (Freberg *et al.*, 2010; Nilsson *et al.*, 2010; Berg *et al.*, 2014; Morck *et al.*, 2014; Tao *et al.*, 2008), and as emphasized by the DS during public consultation, PFDA was also found in cord blood (0.12 ng/mL) in addition to maternal serum (0.36 ng/mL) as was PFOA (1.6 and 1.1 ng/mL in serum and cord blood, respectively), although to a lesser extent (Kim *et al.*, 2011*)*.

(b) Given that the information on PFDA itself and its salts is limited, RAC agrees with the DS to use the data from the analogue chemical PFOA and its ammonium salt APFO to generate information on effects on lactation of PFDA. APFO/APFO were assessed (and classified as Lact.) by RAC in 2011.
Studies by Wolf *et al*.(2007), White *et al*. (2007 and 2009), Macon *et al*. (2011), and Yang *et al*. (2009) demonstrated that PFOA can induce effects on or via lactation. Wolf *et al*. (2007) reported that effects on mouse pup survival from birth to weaning were affected in litters that were exposed to PFOA both *in utero* and *via* lactation (although *in utero* exposure, in the absence of lactational exposure, was sufficient to produce postnatal body weight deficits and developmental delay in the pups). Exposure of PFOA during late foetal and early neonatal life in mice was reported by White *et al.* (2007) to delay development of the mammary gland, which was evident in pups at PND 10 and 20. The same group further corroborated these findings in mice by showing that delayed mammary gland development in pups also occurred after dosing only during lactation (White *et al*., 2009) and that the delay in mammary development was persistent (up to PND 84) and was the most sensitive endpoint for developmental toxicity of PFOA (Macon *et al*., 2011). Furthermore, in humans, PFOA have been shown to be readily transferred to infants through breast-feeding and the PFOA exposure for these infants is considerably higher than for adults.

(c) Data from the analogue chemical PFNA and its ammonium and sodium salts, through the RAC opinion on PFNA from 2014, is also used to generate information on the effects on lactation of PFDA.

In the study of Wolf *et al*. (2010), PFNA was detected in the serum of all animals. Serum levels in pups at weaning were similar (WT) or higher (KO mice) than that of their mothers exposed during GD1-18. These data indicated a substantial transfer of PFNA with mother's milk, related with adverse effect on pups survival and development (the WT mice strain only). These findings were considered similar to the ones observed with APFO in the study of Wolf *et al.* (2007) showing that pup survival from birth to weaning was only affected if the pups that had been exposed *in utero* and via lactation (whereas exposure of the dams to APFO during gestation was sufficient to produce postnatal body weight deficits and developmental delay in the pups).

PFNA has been detected in serum, cord blood and human breast milk.
Based on the read-across, using data from its analog APFO/PFOA as well as data on PFNA itself, RAC considered that classification of PFNA for lactation was justified.

Conclusion on lactation

RAC agrees with the DS that the classification for PFDA can based on read-across from data for APFO/PFOA and PFNA.

PFDA was detected in human breast milk in several studies representing samples from different countries.

The analogue substance PFOA/APFO has been found to be transferred to infants through breast-feeding. Although the results from human evidence and/or from 2-generation studies in animals did not provide effects in the offspring due to transfer in the milk or adverse effects on the quality of the milk, evidence from the mouse studies with postnatal administration of APFO was considered sufficient to indicate adverse effects (delayed/stunted mammary gland development in the offspring) which cause concern for the health of a breastfed child and consequently APFO was classified as Lact.; H362, according to CLP.

The analog PFNA also showed transfer in breast milk and altered pup survival in a similar way to APFO/PFOA and was detected in human breast milk, leading to its classification as Lact.; H362.

In conclusion, available data on the source chemical APFO/PFOA with similar toxicokinetic properties (adverse effects after exposure through lactation in rodents, detection in human breast milk) and the detection of PFDA in breast milk indicate that there is a likelihood that PFDA is present in potentially toxic levels in breast milk and that it may cause concern for the health of breast-fed children. According to CLP Annex I, classification of substances for effects on or via lactation can be assigned based on:
(a) human evidence indicating a hazard to babies during the lactation period; and/or
(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or
(c) *absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.*

Therefore, based on read-across from data for PFOA/APFO and with supporting data from PFDA itself, RAC agrees with the DS that PFDA should be classified as **Lact.; H362 (May cause harm to breast-fed children)** according to CLP.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and by RAC (excluding confidential information).