COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

COT STATEMENT ON THE TOLERABLE DAILY INTAKE FOR PERFLUOROOCTANOIC ACID

Introduction

1. The Food Standards Agency has commissioned research to determine the concentrations of perfluorooctanoic acid (PFOA) in the 2004 Total Diet Study (TDS) samples. The Committee was invited to assess the toxicology of PFOA in order to advise on any health implications arising from the results of the survey.

Background

2. The unexpected discovery of fluorinated organic compounds of anthropogenic origin, identified as predominantly perfluorooctane sulfonate (PFOS), in biological and environmental samples [1,2] has resulted in the toxicology of structurally similar perfluorocarbons being investigated.

3. Perfluorooctanoic acid (PFOA) and its salts are fully fluorinated organic compounds produced synthetically or through degradation of some PFOS-related substances [3] and fluorotelomer alcohols [4]. PFOA is primarily used as an emulsifier in industrial applications, for example in the production of fluoropolymers such as polytetrafluoroethylene (PTFE). PFOA may also be found at low levels in some fluorotelomers, as an unintended by-product of the manufacturing process. Fluorotelomer derivatives are ingredients of fire-fighting foams and coatings, and are intermediates in the manufacture of stain-, oil-, and water-resistant additives for some textiles, coatings and food contact papers.

4. PFOA has not been evaluated by the Scientific Committee on Food (SCF) or the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The US Environmental Protection Agency (EPA) is currently revising its draft risk assessment of the potential human health effects associated with exposure to PFOA and its salts following peer-review by the EPA Science Advisory Board.

Evidence considered in this evaluation

5. The COT has not previously evaluated PFOA or its salts. From an initial assessment of the relevant information it was considered essential to have advice from the Committees on Mutagenicity (COM) and Carcinogenicity (COC) regarding the genotoxicity of PFOA and whether it was appropriate to assume the existence of a threshold for carcinogenicity. The recommendations provided by the COM and COC are summarised in this statement.

6. The evaluation of PFOA considered toxicological data in the published literature and unpublished final reports. Access to the unpublished reports was through the US EPA Office of Pollution Prevention and Toxics (OPPT) Administrative Record AR-226.

Chemical information

7. The high ionization potential and low polarizability of fluorine lead to weak inter- and intra-molecular interactions. This is reflected by the extremely low surface tension of the perfluoroalkyl acids. Their partitioning behaviour is unique; when mixed with water and hydrocarbons, three immiscible phases are formed, indicating that perfluoroalkyl acids are hydrophobic and oleophobic in nature. Consequently, these compounds are ideal surfactants. The strength of the carbon-fluorine bonds makes PFOA and its salts highly stable and, therefore, persistent in the environment.

8. The structure of PFOA ($C_8HF_{15}O_2$, CAS registry number 335-67-1) is shown in Figure 1. The typical structure has a linear chain of eight carbon atoms, but dependent on the manufacturing process branched chain PFOA may also be produced. The electrochemical fluorination process generally gives a product with up to 30% branched PFOA, whereas production by oxidation of perfluorooctyl iodide leads to 100% linear PFOA. Ammonium perfluorooctanoate is of most widespread use and is commonly referred to in the literature as APFO, C8 or FC-143.

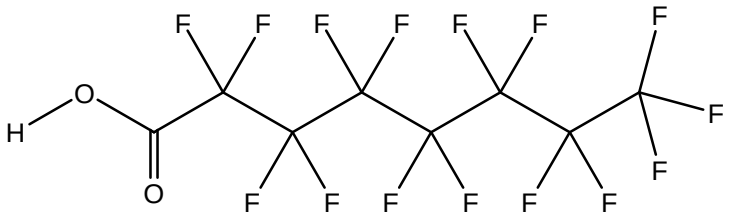

Figure 1 – Structure of perfluorooctanoic acid (PFOA)

9. In water the free acid will completely dissociate to perfluorooctanoate. Water solubility is published as 3.4 g/L, and a 1 g/L solution has a pH of 2.6. An octanol/water partition coefficient cannot be determined for PFOA due to the fact that, rather than being soluble, PFOA forms microdispersion micelles.

Toxicological profile

10. The majority of the toxicological studies have been conducted using ammonium perfluorooctanoate and in most cases the test material was a mixture of the ammonium salts of several perfluorinated acids as manufacturing residues. The typical composition profile is 93-97% ammonium perfluorooctanoate, 1-3% ammonium perfluoropentanoate, 1-3% ammonium perfluoroheptanoate and 1-3% ammonium perfluorohexanoate.

Toxicokinetics – rodents

11. PFOA is well absorbed by rats following oral dosing. Male rats absorbed 93% of an 11 mg/kg bw gavage dose of ^{14}C-PFOA within 24 hours [5]. In this study the ^{14}C total elimination half-life was estimated at 4.8 days (115 hours), whereas, other studies have estimated that the elimination half-life for PFOA in male rats is in the range of 138 to 350 hours [6,7,8]. There is a clear sex-related difference in clearance of PFOA in rats. In female rats, PFOA is more efficiently cleared from the body, primarily via rapid excretion in the urine with a plasma half-life of approximately 10 hours [9,10]. Following oral gavage of female rats with 2 mg PFOA, the quantity of non-ionic fluorine recovered in the urine was 89% of the administered dose within 96 hours.

12. Distribution studies, using administration of PFOA via gavage, and intravenous and intraperitoneal injection, have shown that PFOA does not partition to the lipid fraction or adipose tissue, but is primarily found in the liver, plasma, and kidney [6].

13. Following PFOA administration by gavage, Kemper [8] determined tissue concentrations at the time of maximum plasma concentration (T_{max}) and when plasma concentration had fallen to 50% maximum (i.e. at 11 and 171 hours post-dosing for males and at 1.3 and 4 hours post-dosing for females). In males, tissue concentrations remained constant or decreased with time, in all but the liver where PFOA levels increased. The fraction of the dose present in all female rat tissues remained constant or decreased with time. Kidney to blood PFOA concentration ratios at T_{max} were approximately 2 at all dose levels in females and remained constant with time.

14. PFOA crosses the placenta. The concentration of PFOA in fetal plasma on gestation day (GD) 21, following continuous maternal exposure from GD 4 was approximately half the steady state concentration in maternal plasma. PFOA was also detected in the milk of rats, at levels approximately 10% those of maternal plasma concentrations [11]

15. PFOA undergoes enterohepatic circulation. By disrupting enterohepatic circulation (with cholestyramine treatment) in male rats, the level of PFOA eliminated in faeces increased by approximately 9.6-fold [12]. A concomitant decrease in the proportion of the PFOA dose excreted in the urine was seen (from 67% to 41% over 14 days).

16. There is evidence that PFOA is not metabolised in the rat.

17. In the 24 hours following treatment with a single i.v. dose (mean doses: 16.7 and 13.1 mg/kg bw for female and male CD rats, respectively) of ^{14}C-PFOA, female rats had excreted essentially all the administered dose via the urine, whilst the males had only excreted 20% of the dose [9]. In total, over the course of 36 days the male rats excreted 83% of the total dose via the urine and 5.4% via the faeces.

18. Studies investigating the sex-related difference in elimination of PFOA have shown that the female rat possesses an active secretory mechanism which rapidly eliminates PFOA from the body. Administration of probenecid (an inhibitor of the renal active secretion system for organic acids) reduced the PFOA/inulin clearance ratio in female rats from 15 to 0.46 [13]. PFOA clearance was reduced from 5.8 to 0.11 mL/min/100 g bw, a level similar to male rat PFOA clearance which was virtually unaffected by probenecid administration.

19. In male rats, testosterone has been shown to exert a suppressive effect on renal excretion of PFOA [14]. Castration of male rats increased the elimination of PFOA in the urine. Kudo *et al.*, [7] demonstrated that organic anion transporter 2 (OAT2) mRNA levels in male rats were only 13% those in female rats. Castration or oestradiol treatment increased the levels of OAT2 mRNA whereas treatment of castrated rats with testosterone reduced them. Evidence was also obtained for the involvement of OAT3 in PFOA excretion.

20. Comparison of area under concentration-time curve in plasma for oral and intravenous doses of PFOA (1 mg/kg bw) in Sprague-Dawley rats indicated that oral bioavailability is approximately 100% [8]. Plasma elimination curves for PFOA following gavage at doses of 0.1, 1, 5, and 25 mg/kg bw were log-linear with respect to time in male rats, while elimination kinetics were biphasic in the 5 and 25 mg/kg bw female dose groups. Estimated plasma elimination half-lives were approximately 277 hours in males and 3.4 hours in females, using non-compartmental pharmacokinetic models. In contrast, Kudo *et al.*, [7] using a two-compartment open model, found the terminal elimination half-lives in Wistar rats to be 137 and 1.9 hours in males and females, respectively. Females appeared to exhibit biphasic elimination. The main component was rapid (half-life of 1.9 hours), with a slower minor component

21. Hormonal changes associated with pregnancy have been shown not to alter the rate of elimination of PFOA [15].

22. An unpublished comparative study with male and female rats, mice, hamsters and rabbits [16] dosed by oral gavage (10 mg/kg bw ^{14}C-PFOA) and sacrificed 5-7 days later, showed significant differences in PFOA elimination between the species. Male hamsters and both sexes of rabbits excrete PFOA as rapidly as female rats, however, the female hamster cleared the chemical more slowly, having excreted only 58% of the administered dose in 5 days. Male and female mice retained between 50-70% of the administered dose 5 days after dosing.

23. Lau *et al.* [17] compared body burdens of PFOA between rat and mouse after subchronic exposure. A clear sex-related difference in PFOA accumulation was confirmed in the rat; a serum level of 111 µg PFOA/mL was reached in male rats 24 h after the last of 20 daily 10 mg/kg bw/day doses, while only 0.7 µg PFOA/mL was detectable in female rat serum. A steady state level of PFOA in mice was reached in one week of exposure. PFOA concentrations in serum following 7 daily 20 mg/kg bw/day doses were approximately 180 µg PFOA/mL for male and female mice.

Toxicokinetics – dog

24. The renal clearance rate of PFOA in beagles (3/sex) following an i.v. administration (30 mg/kg bw) was approximately 0.03 mL/min/kg bw [18]. Probenecid significantly reduced clearance rates in both sexes, indicating an active excretion mechanism. Plasma half-life was 473 hours in male dogs and 202 hours in female dogs.

Toxicokinetics – non-human primates

25. The toxicokinetics of PFOA following a single i.v. dose (10 mg/kg bw) have been assessed in cynomolgus monkeys (3 monkeys/sex) [19]. Body weights were unaffected between days 1 and 28. PFOA serum concentrations 0.5 hours post-dosing were similar in males and females. By day 123, male PFOA serum levels were at or slightly above 0.02 µg/mL (the limit of quantitation), and female serum concentrations were between 0.89 and 4.7 µg/mL. There were no obvious sex differences in the urinary excretion of PFOA, which was slow (less than 20% of the administered dose was excreted in the urine over the first 48 hours). However, although the number of monkeys in this study was limited, estimated half-life and total body clearance values indicated elimination in males may occur at a slightly faster rate than females. The average terminal elimination phase half-lives were approximately 21 and 33 days for males and females, respectively.

26. In a six month oral capsule dosing study of PFOA male cynomolgus monkeys (4-6/group) were administered 0, 3, 10 or 30/20 (reduced on day 22 from 30 to 20) mg/kg bw/day [19]. During the first week of dosing monkeys in the 30 mg/kg bw/day group showed general signs of toxicity, including low food consumption, significant loss of body weight, and 4 of the 6 monkeys also had few or no faeces. Dosing was suspended on Day 12 and reinitiated at 20 mg/kg bw/day on Day 22. Steady state was reached within four weeks in serum, urine and faeces. Serum PFOA followed first-order elimination kinetics following the last dose, with a half-life of approximately 20 days. Urine was the primary elimination route. The i.v. study would predict that steady state in the daily oral dosing study would not be reached until at least eight weeks of daily oral dosing. The reasons for the apparent achievement of steady state before this time are not known.

Toxicokinetics – humans

27. Human toxicokinetic data on PFOA are limited in number and conflicting.

28. A number of studies have assessed the levels of PFOA in blood of occupationally and non-occupationally exposed populations. Serum PFOA levels in 3M workers have been measured since 1993. In the Cottage Grove, Minnesota production plant, PFOA serum levels were highest amongst the 3M plants (geometric mean = 1.7 µg/mL, range, 0.07-33 µg/mL) [20].

29. Ubel *et al.*, [21] reported an approximate half-life of PFOA of 18 months based on one PFOA worker. A report from 3M on nine fluorochemical production plant retirees suggested a serum half-life of 4.4 years, with a range of 1.5 to 14 years [22]. This study suffered from significant limitations, such as small sample size, reference material purity unchecked, and unreplicated serum measurements.

30. In a study of Japanese maternal and cord blood samples [23], PFOA was detected in only 3 of the 15 maternal blood samples (range 0.0005-0.003 µg/mL) and not in any fetal blood samples (limit of detection <0.0001 µg/mL).

31. In contrast to the large active renal excretion of PFOA in female rats [7], renal clearance of PFOA is almost negligible in both sexes in humans [24].

32. PFOA has also been found in the serum of children, adults and the elderly in the general population [25,26]. In the US, serum concentrations follow a log-normal distribution with geometric mean concentrations of 0.004-0.005 µg/mL, and over 90% of serum samples had quantifiable levels of PFOA. The upper bound of the 95th percentile estimate of the population, below which the 95th percentile serum concentration of the samples falls with 95% confidence, was 0.014 µg/mL.

33. An assessment of the prevalence of organic perfluorochemicals in the blood of Swedish mothers and sons concluded that PFOA levels in the general population of Sweden and the US are similar [27]. In whole blood samples (*n* = 66) PFOA concentrations ranged from 0.0005 to 0.0124 µg/mL with an arithmetic mean of 0.0027 µg/mL. There was no significant difference between males and females.

34. In 473 human blood/serum/plasma samples collected in various countries worldwide (USA, Colombia, Brazil, Belgium, Italy, Poland, India, Malaysia, and Korea) PFOA was seen at a low frequency [23]. Samples (*n* = 50) from Italy did not have quantifiable levels of PFOA, whereas PFOA was quantifiable in all samples from Poland (*n* = 25). Two Korean females in particular showed PFOA levels greater than 0.1 µg/mL.

35. Concentrations of PFOA in sera sampled from a small number (*n* = 23-60) of female residents of three Japanese cities have increased by 14-fold over the last 25 years [28]. In 2003, the geometric mean concentrations of PFOA in sera, from both sexes, ranged from 0.0028 to 0.012 µg/mL, with significantly higher levels reported in males.

36. Olsen *et al.*, [26] compared PFOA levels in samples taken from the same subjects, that were part of two large community-based cohorts established in Maryland, US, in 1974 and 1989 and reported that concentrations were two-fold

higher in 1989. However, the trend may not have continued, since more recent samples (taken in 2001) appeared similar to the 1989 levels.

Acute and sub-acute toxicity

Rodents

37. There is reasonable consistency between several acute oral LD_{50} studies, which indicate PFOA is moderately toxic.

38. Oral studies with PFOA indicate an oral LD_{50} in rats ranging between 430 and 680 mg/kg bw [29]. There are no reported differences in the sensitivity of castrated or ovariectomised versus intact rats (male or female) to PFOA [30]. Newborn rats (<2 days old) (LD_{50} ~250 mg/kg bw) were more sensitive to PFOA than weanlings and adult animals [31]. Pre-treatment of rats with phenobarbital [29] (an enzyme inducer, in particular of CYP2B1 and CYP2B2) or proadifen hydrochloride (a cytochrome P450 inhibitor) did not alter the LD_{50} of PFOA.

39. PFOA administered to ChR-CD mice for 28 days [32] resulted in dose-related reductions in mean body weight and in muscular weakness after 9, 6 and 4 days at 18, 60 and 200 mg/kg bw/day, respectively. Absolute and relative liver weights were also increased in both sexes in a dose-related manner. Treatment-related changes in the liver included hepatic enlargement and/or discoloration of one or more liver lobes. Histopathological examination revealed panlobular hypertrophy accompanied by focal to multifocal cytoplasmic lipid vacuoles of variable size.

40. In a similar 28-day study [33] with ChR-CD rats PFOA induced absolute liver weight increases in both sexes. The severity and degree of tissue involvement were more pronounced in males than females. Panlobular, multifocal to diffuse, hypertrophy was observed, with focal to multifocal cytoplasmic enlargement of hepatocytes in the centrilobular and midzonal areas. The hypertrophy was associated with acidophilic degeneration and necrosis of scattered hepatocytes with no lobular distribution.

41. Goldenthal *et al.* [34] administered ChR-CD rats (5/sex/dose group) dietary levels of 0, 10, 30, 100, 300 and 1000 ppm PFOA for 90 days, equivalent to dietary intakes of 0.6, 1.7, 5.6, 18, and 64 mg/kg bw/day in males and 0.7, 2.3, 7.7, 22.4 and 76 mg/kg bw/day in females. In male rats body weight gain was reduced at 18 and 64 mg/kg bw/day. Relative kidney weights were significantly increased in males at 5.6 mg/kg bw/day and above, which was dose-related. Absolute and relative liver weights were increased in males of the 18 and 64 mg/kg bw/day groups and also 76 mg/kg bw/day treated females. Males in the 1.7 mg/kg bw/day dose group also had increased absolute liver weights. Hepatocellular hypertrophy (focal to multifocal in the centrilobular to midzonal regions) was observed in males of the 5.6 mg/kg bw/day and higher dose groups, with hepatocellular necrosis in the 1.7 mg/kg bw/day and above dose groups. Based on liver effects in this study, the no observed adverse effect level (NOAEL) was 0.56 mg/kg bw/day in males, and in females the NOAEL was 22 mg/kg bw/day.

42. A 13-week study performed in male ChR-CD rats (45-55/group) at dietary intakes equivalent to 0.06, 0.64, 1.94, and 6.4 mg PFOA/kg bw/day reported no treatment-related clinical signs [35,36]. Effects on the liver (increased hepatic palmitoyl CoA oxidase levels, increased relative liver weights and hepatocellular hypertrophy) were observed in the 0.64, 1.94 and 6.4 mg/kg bw/day dosed animals. In the 0.64 mg/kg bw/day group liver effects were statistically significant at 4 weeks of dosing, but not at 7 or 13 weeks. Hypertrophy at 0.64 mg/kg bw/day was described in the pathology report as minimal and "characterised by an accentuated centrilobular pattern in which the hepatocytes appear to have a more homogenous cytoplasm and the cell borders are more rounded giving the cells a more 'plump' appearance. Except for this 'enlarged' appearance the cells are otherwise 'normal'."

43. The unpublished report [35] concluded that the no observed adverse effect level was 6.4 mg/kg bw/day, the highest tested dose, and the no effect level was 0.06 mg/kg bw/day based on increases in absolute and relative liver weights. In the published report [36] the authors do not establish a NOAEL, but refer to a no effect level of 0.06 mg/kg bw/day with doses of 0.64 mg/kg bw/day and higher producing adaptive and reversible liver changes.

44. The COT modelled the absolute and relative liver weight data using US EPA Benchmark Dose Software to estimate a benchmark dose for a 10% response[†] (BMD_{10}) and its lower confidence limit ($BMDL_{10}$) for these effects. BMD_{10} and $BMDL_{10}$ for absolute liver weights were 0.60 and 0.40 mg/kg bw/day (week 4), 0.66 and 0.29 mg/kg bw/day (week 7) and 0.89 and 0.44 mg/kg bw/day (week 13), respectively. For liver weight relative to body weight, the BMD_{10} and $BMDL_{10}$ were 0.50 and 0.36 mg/kg bw/day (week 4), 0.58 and 0.33 mg/kg bw/day (week 7) and 0.84 and 0.54 mg/kg bw/day (week 13), respectively. In the 1.94 mg/kg bw/day dosed animals the hepatocyte hypertrophy was grade 2 (mild) or higher in 2 of 15 animals. BMD modelling of grade 2 or higher hepatocellular hypertrophy estimated the $BMDL_{10}$ at 0.95 mg/kg bw/day. The duration of exposure did not appear to increase severity of the hypertrophy and liver effects were reversed following an 8 week recovery period.

Non-human primates

45. In a 90-day study in rhesus monkeys [39] (2/sex/group) doses of 0, 3, 10, 30 and 100 mg/kg bw/day PFOA were administered by gavage. By week 5 all monkeys in the highest dose group had died and by week 13 three monkeys from the 30 mg/kg/day group had died. Absolute and relative heart weights of females from the 10 mg/kg bw/day group were significantly decreased as were absolute brain weights of females. No associated morphological changes were observed. No treatment-

[†] The benchmark dose (BMD) approach [37,38] aims to provide an approach to dose-response assessment that is more quantitative than the current NOAEL process. This approach constructs mathematical models to fit all data points in the dose-response study and uses the best fitting model to interpolate an estimate of the dose that corresponds to a particular level of response (a benchmark response), often 10%. A measure of uncertainty is also calculated, and the lower confidence limit on the benchmark dose is called the BMDL. This accounts for the uncertainty in the estimate of the dose-response that is due to characteristics of the experimental design such as sample size. The BMDL can be used as the point of departure for derivation of a health-based guidance value or a margin of exposure.
When the COT has performed benchmark dose modelling as part of this assessment the US Environmental Protection Agency's Benchmark Dose Software (2000) was used.

related lesions were seen in the organs of animals from the 3 and 10 mg/kg bw/day groups. The surviving 30 mg/kg bw/day dose group male had moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen. The LOAEL was 3 mg/kg bw/day on the basis of soft stools or moderate to marked diarrhoea or frothy emesis.

46. The study in male cynomolgus monkeys, described in paragraph 26 above, also reported effects of 0, 3, 10 and 30/20 mg/kg bw/day PFOA for 26 weeks [40]. Dose-dependent increases in absolute liver weight associated with mitochondrial proliferation occurred in all PFOA-treated groups, although histopathological evidence of liver toxicity was not seen at 3 or 10 mg/kg bw/day. A liver-to-body weight ratio percentage of 2.4 for a 3 mg/kg bw/day monkey found in moribund condition was comparable to that of the high-dose monkeys. The moribund 3 mg/kg bw/day monkey was sacrificed on day 137, however, a complete review of the in-life monkey history including review of the clinical and microscopic pathology failed to identify the cause of this monkey's extreme poor health. These findings were non-specific and, largely, were not consistent with the findings observed for monkeys in the high dose group. There was considerable variation in PFOA concentrations measured in serum and liver with no linear relationship with dose detected. Two control and 10 mg/kg bw/day monkeys were designated as recovery group monkeys and over the course of a 90-day recovery period, PFOA concentrations returned to pre-treatment levels. Markers of tumour formation in the liver, pancreas and Leydig cells, i.e. replicative DNA synthesis in the liver, and serum cholecystokinin (CCK), alkaline phosphatase, bilirubin, bile acids, oestradiol, oestriol and testosterone concentrations were also assessed. There was a two-fold increase in hepatic palmitoyl CoA oxidase activity in the high-dose group and the other markers were unaffected. The study authors acknowledge significant limitations in this study, but suggested that it demonstrates a "*dramatic demarcation in dose-response between a relatively mild response (liver weight increase at the 3 and 10 mg/kg bw/day doses) and serious toxicity (dramatic weight loss and one death at the 30/20 mg/kg bw/day dose)*". As the cause of the 3 mg/kg bw/day monkey's moribund state was not established it was not possible to identify a NOAEL.

Mutagenicity and carcinogenicity

47. The COM considered the mutagenicity of PFOA in May 2005. PFOA has no apparent structural alerts for mutagenicity and the evidence from animal studies is that absorbed material is not metabolised.

48. Members concluded that the plate incorporation bacterial mutagenicity tests using strains of *Salmonella typhimurium* and *Eschericia coli* using sodium [41] or ammonium [42] perfluorooctanoate were adequate and gave negative results. The *in vitro* hprt assay in Chinese hamster ovary (CHO) cells using ammonium perfluorooctanoate [43] also gave negative results.

49. PFOA (sodium salt) at high concentrations induced a reproducible response in the *in vitro* chromosomal aberration assay in CHO cells in the presence of metabolic activation [44]. No evidence for chromosomal aberrations was documented in the absence of exogenous metabolic activation. It was not clear to what extent the

positive results reported were due to cytotoxicity. Ammonium perfluorooctanoate also increased chromosomal aberrations in CHO cells in the presence of exogenous metabolic activation [45]. However, for this study there was clear evidence of cytotoxicity at the same dose level. The COM concluded that the positive results were likely to represent a cytotoxic response.

50. Sodium perfluorooctanoate in the absence and presence of metabolic activation had not induced chromosomal aberrations in cultured human whole blood lymphocytes when tested up to doses that were cytotoxic [46].

51. No evidence for a mutagenic effect was found in mouse bone marrow micronucleus assays testing single oral gavage doses of up to 5000 mg/kg bw sodium perfluorooctanoate [47] or 1990 mg/kg bw ammonium perfluorooctanoate [48]. The COM considered the *in vivo* bone marrow mouse micronucleus studies had been adequately conducted. However, it was noted that there was no direct measure of exposure of the bone marrow in the test materials.

52. Overall, the COM concluded that PFOA was not mutagenic, however, a plausible *in vitro* mechanism for the positive response in the *in vitro* chromosomal aberration assay in CHO cells was required to reassure COM about this conclusion.

53. The carcinogenicity of PFOA has been investigated in two dietary exposure studies in Sprague-Dawley rats [49,50]. Ammonium perfluorooctanoate was administered to Sprague-Dawley rats at levels of 0, 30 ppm (mean achieved dose levels 1.3 and 1.6 mg/kg bw/day in males and females, respectively) or 300ppm (mean achieved dose levels 14.2 and 16.1 mg/kg bw/day in males and females, respectively) in the diet for 104 weeks [49]. Dose-related non-neoplastic liver effects included megalocytosis, cystoid degeneration and portal mononuclear infiltration. Red blood cell counts, haemoglobin and haematocrit values were minimally decreased in the high-dose male rats compared to control values. There were statistically significant decreases in the following parameters: erythrocytes at 6, 12 and 18 months; haemoglobin at 3 and 18 months; and haematocrit at 3, 12 and 18 months. In high dose females, erythrocyte count, haemoglobin and haematocrit were statistically significantly decreased at 12 months. Mean leucocyte counts were increased in the low-dose male group compared to control values, throughout the first year, but not in the high dose group. Statistically significant increases were observed: in lymphocyte counts at 3 months in the high- and low-dose groups, and at 6 and 18 months in the low-dose group; and in neutrophil counts at 12 months in both groups. No statistically significant haematological changes were evident in low dose males and females at 24 months. On the basis of increases in liver weight and hepatic changes in males, and reduced body weight gain and haematological changes in females the NOAEL was 1.3 mg/kg bw/day in males and 1.6 mg/kg bw/day in females. BMD modelling of induced hepatocytic megalocytosis in male rats, by COT, estimated the BMD_{10} and $BMDL_{10}$ at 1.1 and 0.74 mg/kg bw/day, respectively. A significant increase in female mammary fibroadenomas was considered not to be significant by the study authors when compared to historical control data. PFOA also induced an apparent dose-related increase in Leydig cell adenomas, which was not significant compared to historical control incidence. COC members were concerned to note the occurrence of at least two viral infections in the rats used in this study. This limited the value of the results.

54. Biegel *et al.*, [50] investigated a single high dietary dose of PFOA (300 ppm for 24 months; mean achieved dose level 13.6 mg/kg bw/day) in male rats and reported increased incidences of hepatocellular adenomas, Leydig cell adenomas and pancreatic acinar cell adenomas. Serum estradiol concentrations were significantly increased in treated animals. COC noted that this study was not designed to identify a NOEL. In light of the pancreatic acinar cell adenoma findings the pancreas slides from the earlier study [49] were reassessed and the occurrence of proliferative lesions of the pancreatic acini was confirmed [51].

55. A hypothesis had been put forward which proposed that PFOA induces liver, Leydig cell and pancreatic acinar cell tumours via PPAR-alpha activation and that because of lack of relevance of this mode of action in human carcinogenicity, PFOA is unlikely to induce such tumours in humans [52]. COC agreed with the proposal by Klaunig *et al.* that activation of aromatase and subsequent increases in serum estradiol levels were suggestive that a mode of action (MOA) could be proposed for the Leydig cell tumours. However, studies in PPARα-null mice had shown PFOA-induced liver effects [53], which is not consistent with the proposed MOA for liver tumours [52]. COC did not consider it possible to propose MOAs for the liver and pancreatic tumours reported [50].

56. COC concluded that for the purpose of the risk assessment, it would be acceptable to use a threshold approach, and to select an appropriate NOAEL for a precursor event for the most sensitive tumour and that an uncertainty factor of 100 would be appropriate for this endpoint.

57. In two linked, retrospective cohort studies of mortality in an occupationally exposed population [54,55], small increases were reported in death from cancer of the large intestine and from cancer of the prostate in employees with over 1 year definite exposure to PFOA. Standardised mortality ratios were zero, i.e. there were no cases reported, for tumour types observed in the two year rodent carcinogenicity study. COC considered that none of the effects reported were significant for risk assessment.

Developmental and reproductive toxicity

58. A number of prenatal developmental toxicity studies with PFOA have been conducted in rats, mice and rabbits.

59. Time-mated Sprague-Dawley rats (22/dose group) were administered 0, 0.05, 1.5, 5 and 150 mg/kg bw/day PFOA by gavage on GD 6-15 [56]. Animals were sacrificed on GD 20. The only statistically significant sign of maternal toxicity was a reduction in mean maternal body weights (150 mg/kg bw/day dose group). Administration of PFOA during gestation did not affect the ovaries or reproductive tract of the dams. Based on signs of maternal toxicity the NOAEL was 5 mg/kg bw/day. The NOAEL indicated for developmental toxicity was 150 mg/kg bw/day, the highest dose tested.

60. Gortner [57] administered four groups of pregnant New Zealand rabbits (18/dose group) doses of 0, 1.5, 5, and 50 mg/kg bw/day PFOA by gavage on GD 6-18. Fetuses were examined for gross abnormalities and placed in a 37°C incubator for a 24 hour survival check. A transient, but statistically significant, reduction in maternal body weight gain was noted on GD 6-9, but body weights returned to control levels on GD 12-29. The authors concluded that because this was the only sign of maternal toxicity, the NOAEL was 50 mg/kg bw/day. A dose-related increase in a skeletal variation (extra ribs or 13th rib) was the only sign of developmental toxicity. Incidence was 16, 20, 30 and 38% in 0, 1.5, 5, and 50 mg/kg bw/day dose groups and this reached statistical significance in the high dose group. The authors concluded that the NOAEL was 5 mg/kg bw/day on the basis of the skeletal variation observations.

61. Staples *et al.*, [58] administered PFOA (0 and 100 mg/kg bw/day) to pregnant rats in two oral dosing studies for GD 6-15. Study one sacrificed dams at GD 21 and study two allowed parturition and sacrificed pups on postnatal day (PND) 35. Three out of the twenty five dams in study one died in the 100 mg/kg bw/day PFOA dose group (one on GD 11 and two on GD12). Food consumption and body weights were reduced in treated animals compared to controls. No adverse effects were noted in any of the reproductive parameters assessed. Fetal weights and incidences of malformation were similar in the control and treated animals. Study two noted similar clinical signs in dams as in study one and no significant effects of PFOA treatment on reproductive performance or in the pups.

62. In a two-generation rat study PFOA was administered by oral gavage (0, 1, 3, 10 and 30 mg/kg bw/day) to the F_0-generation rats, beginning at 6 weeks of age and at least 10 weeks before cohabitation [59]. F_1-generation rats were treated at the same dosage levels as their respective sires and dams beginning at weaning (lactation day (LD) 22). F_0-generation males were 106-110 days of age at sacrifice and F_1-generation males were 109-120 days of age at sacrifice.

63. At terminal sacrifice F_0-generation male rats showed liver and kidney weight increases at all doses, i.e. for these effects it was not possible to establish a NOAEL and decreased body weights at 3 mg/kg bw/day and above. F_1-generation male rats, also at terminal sacrifice, showed significantly decreased body weights and increased liver weights at all doses. The absolute weights of the left and/or right kidneys were significantly increased in the 1 and 3 mg/kg bw/day dose groups and significantly decreased in the 30 mg/kg bw/day dose group compared to controls. F_0-generation females showed decreases in relative liver weight at 10 mg/kg bw/day, and decreases in absolute and relative kidney weights at 30 mg/kg bw/day. BMD modelling by the COT of the absolute liver weight data in the F_0-generation male rats estimated that the BMD_{10} and $BMDL_{10}$ were below the tested dose range at 0.68 and 0.31 mg/kg bw/day, respectively. For F_1-generation male rats BMD_{10} and $BMDL_{10}$ for absolute liver weight data were estimated to be 0.78 and 0.31 mg/kg bw/day, respectively. Body weights were assessed in directly-dosed rats during different periods of sexual development. Findings showed a greater sensitivity of sexually mature male rats to PFOA-induced bodyweight effects compared to sexually immature rats. Statistically significant decreases in bodyweight were present only at 30 mg/kg bw/day during the juvenile period (from PND 21 to 35) and peripubertal period (PND 36-60) but were present in all dose

groups by the last three weeks of dosing in sexually mature male rats. The authors concluded that this may be related to differences in testosterone levels during different development phases. Thus, lower serum testosterone levels in male rats during the juvenile and peripubertal periods of sexual development may be associated with PFOA elimination kinetics similar to that of the female rat, i.e. more rapid renal clearance and shorter serum half-life, as demonstrated by numerous studies [7,14,60].

64. Reproductive endpoints were not affected in either generation. The 30 mg/kg bw/day F_1-generation pups had decreased body weight at birth and a reduced viability, however, F_2-generation pups at the same dosage levels, although somewhat lighter, did not show a loss in viability. Preputial separation and vaginal opening were somewhat delayed at 30 mg/kg bw/day in the F_1- and F_2-generation rats but this had no apparent consequences with regard to reproductive performance of F_1-generation rats. The NOAELs were; 30 mg/kg bw/day for reproductive function of the F_0- and F_1-generation, 10 mg/kg bw/day for F_1-generation pup mortality, birth weight, and sexual maturation, and a NOAEL could not be determined for male body weight and organ weight changes, as effects were observed in the lowest tested dose group (1 mg/kg bw/day). The NOAEL for F_2-generation rats was 30 mg/kg bw/day, the highest tested dose.

65. A recently published study investigated the developmental toxicity of PFOA (0, 1, 3, 5, 10, 20, and 40 mg/kg bw/day by oral gavage daily from GD 1-17) in timed-mated CD-1 mice [17]. Maternal absolute liver weight at term (*n* = 9-45 per dose group) was statistically significantly increased at all dose levels, and BMD_5 and $BMDL_5$ were estimated, by the study authors using the US EPA Benchmark Dose Software, at 0.20 and 0.17 mg/kg bw/day, respectively. In contrast to findings in the rat [59], in mice Lau and colleagues observed statistically significant increases in the incidence of full-litter resorptions and neonatal mortality at 5 mg/kg bw/day and above. No significant increase in malformations was noted in any treatment group. The incidence of live birth was significantly lowered by PFOA: approximately 70% for the 10 and 20 mg/kg bw/day groups compared to 96% for controls. Neonatal survival at postnatal day 23 was significantly compromised at 5 mg/kg bw/day and above. The BMD_5 and $BMDL_5$ for this effect were estimated at 2.84 and 1.09 mg/kg bw/day, respectively. Dose-dependent growth deficits were detected in all PFOA-treated litters except the 1 mg/kg group. Significant delays in eye-opening (up to 2-3 days) were noted at 5 mg/kg and higher dosages. Accelerated sexual maturation was observed in male offspring (preputial separation), but not in females. The authors of this study hypothesise that the species difference in terms of developmental toxicity of PFOA in rats and mice is, in part, due to the differential pharmacokinetic disposition of the chemical. In addition they propose that the lack of a sex-related difference in the pharmacokinetics of PFOA in humans, non-human primates and mice suggests that findings in mice maybe more appropriate for the purposes of species extrapolation in the human health risk assessment. However, the COT noted that it would also be necessary to consider relative sensitivity alongside sex- and species-related differences in pharmacokinetics before concluding studies in one species are more appropriate than in another species. At the request of the COT, the study authors agreed to repeat the BMD modelling in order to provide estimates of BMD_{10} and $BMDL_{10}$ for maternal liver weight at term,

which appears to be the most sensitive endpoint in this study. The BMD_{10} and $BMDL_{10}$ were estimated to be 0.52 and 0.46 mg/kg bw/day, respectively.

Mechanistic studies

66. Cook *et al.* [61], carried out a 14-day gavage study to investigate possible mechanisms of induction of the Leydig cell adenomas, reported in the 2-year feeding study [49]. Adult male CD rats were administered 0, 1, 10, 25, or 50 mg/kg bw/day PFOA by gavage for 14 days, with a second control group pair-fed to the 50 mg/kg bw/day group. A dose-dependent decrease in body and relative accessory sex organ (ASO) weights was seen, with the relative ASO weights of the 50 mg/kg/day group significantly less than those of the pair-fed controls. Serum oestradiol levels were elevated in the 10, 25, and 50 mg/kg bw/day dose groups, and levels in the 50 mg/kg bw/day group were 2.7-fold greater than in pair-fed controls. A statistically significant downward trend with dose was seen in serum testosterone levels when compared to *ad libitum* controls. Animals administered PFOA for 14 days were also challenged with human chorionic gonadotropin (hCG), gonadotropin-releasing hormone (GnRH), or naloxone (which antagonises the inhibitory effects of endogenous opioids on GnRH release) one day prior to termination. Results suggested decreases in testosterone levels following PFOA exposure were due to an effect at the level of the testis. The elevated oestradiol levels in treated rats were hypothesised as being responsible for the decreased relative ASO weight and serum testosterone levels seen in this study as well as the increased incidence of Leydig cell adenomas in the 2-year feeding study with PFOA.

67. In a mixture of *in vivo*, *ex vivo* and *in vitro* studies Biegel *et al.*, [62] investigated the mechanism for PFOA induction of Leydig cell tumours. In the *in vivo* and *ex vivo* studies, male CD rats were treated with 0 and 25 mg/kg bw/day PFOA for 14 days by gavage. Findings in the *in vivo* study were statistically significant increases in the serum and testicular interstitial fluid oestradiol concentrations in the treated group. Testicular interstitial TGFα levels were also raised in dosed animals.

68. Leydig cells were isolated from the testes of PFOA-treated and untreated rats for the *ex vivo* and *in vitro* studies, respectively. Treatment of Leydig cells *in vitro* with PFOA (100-1000 µM for 5 hours) followed by hCG stimulation resulted in a dose-dependent decrease in testosterone production. In contrast the *ex vivo* studies, which stimulated Leydig cells from PFOA-treated rats with hCG, demonstrated an increase in testosterone production.

69. Three immunotoxicity studies of PFOA have been conducted in mice [53,63,64]. PFOA in the diet (200 ppm in food, approximately equivalent to 30 mg/kg bw/day) of male C57Bl/6 mice for 2, 5, 7 or 10 days resulted in significant atrophy of the spleen and thymus [63]. The time-course of the thymic and splenic atrophy resembled that of liver weight increases and of peroxisome proliferation. PFOA treatment decreased the number of thymocytes and splenocytes by >90% and about 50%, respectively. Accumulation of thymocytes in the G_0/G_1 phase (assessed by flow cytometric analysis) indicated that thymocyte proliferation had been significantly inhibited by PFOA treatment.

70. Dose-dependency of PFOA effects was tested in male C57Bl/6 mice administered PFOA in the diet (10, 30, 100, 200 and 500 ppm in the diet, approximately equivalent to 1.5, 4.5, 15, 30 and 75 mg/kg bw/day) [65]. Significant increases in peroxisome proliferation (measured as induction of acyl-CoA oxidase) were observed at all doses. Liver weight increased with all doses, reaching statistical significance at doses of 30 mg/kg bw/day and above. Thymus and spleen weights were significantly decreased at 15, 30 and 75 mg/kg bw/day. PFOA-induced atrophy of the thymus was more severe than atrophy of the spleen. Following a 5 or 10 day recovery period after treatment with 30 mg/kg bw/day the weights of spleen and thymus, respectively, recovered to control values whereas liver weight had not returned to control values. Following withdrawal of PFOA no changes were noted in splenocyte or thymocyte numbers during the first 2 days, but cell numbers returned to normal between days 5 and 10. The study authors considered that the effects in the thymus were due to inhibition of cell proliferation.

71. Specific humoral immune responses in male C57Bl/6 mice, administered 200 ppm PFOA in the diet (equivalent to approximately 30 mg/kg bw/day) for 10 days, were assessed using a plaque forming cell assay and serum antibody titre assay [64]. PFOA treatment prevented increased plaque formation and serum IgG and IgM titres in response to immunisation with horse red blood cells. PFOA also exerted immunosuppressive effects on lipopolysaccharide- and concanavalin A-stimulated proliferation of splenic lymphocytes.

72. PPARα-null mice, which do not exhibit peroxisome proliferation or hepatomegaly and hepatocarcinogenesis after exposure to peroxisome proliferators, did not show significant changes in body or spleen weight or the number of splenocytes after administration of 30 mg/kg bw/day for 7 days [53,63,64]. The decrease in thymus weight and cellularity observed in wild-type mice was attenuated, but not totally eliminated, in PPARα-null mice. Significantly the increases in liver weight observed in wild-type mice was virtually unaltered in null mice exposed to PFOA, indicating that this may not be a PPARα mediated effect. However, hepatic peroxisome proliferation was not observed in PFOA-treated PPARα-null mice.

COT evaluation

73. The COT considered it appropriate to take a threshold approach to establishing a tolerable intake for PFOA, in accordance with the advice of COM and COC. This is based upon predominantly negative genotoxicity in standard *in vitro* and *in vivo* assays and equivocal evidence for carcinogenicity. The positive response from the *in vitro* chromosomal aberration assay was considered to most likely represent a cytotoxic response.

74. COC considered that it was not possible to propose a PPARα agonist mode of action for the liver and pancreas tumours induced by PFOA. Therefore, relevance of these tumours to humans could not be discounted. For the purpose of the risk assessment, the COC concluded that selection of a NOAEL for a precursor event for the most sensitive tumour (liver or pancreas) as the critical effect level would be appropriate for the derivation of a safety limit.

75. There is considerable uncertainty regarding the pharmacokinetics of PFOA in rats and humans, especially in relation to human half-life data. Studies have provided some insight into possible mechanisms for the sex-related difference in PFOA elimination in rats. However, the rapid elimination of PFOA by female rats suggests that developmental/reproductive toxicity studies in this species may not be particularly informative for the risk assessment of PFOA for human health.

76. Due to the long half-life of PFOA in humans, estimated on the basis of the available data, the risk assessment for PFOA could be based on a comparison of the internal dose of PFOA from animals, for a specific endpoint, with the internal dose in humans. This approach is somewhat analogous to using a margin of exposure, calculated as the ratio of the NOAEL or LOAEL for a specific endpoint to the estimated human exposure level. However, the toxicokinetics of PFOA in rodents and humans are not yet fully understood. The sex-related difference in half-life of PFOA in rats was particularly noted as a source of uncertainty, as the active renal clearance in female rats is specific to that species. Therefore, the use of internal doses for the risk assessment was not considered appropriate on the basis of available data.

77. Considering the non-hepatic toxicity of PFOA, the lowest LOAEL indicated in the database was 1 mg/kg bw/day for kidney weight increases in the F_0- and F_1-generation males in the two-generation rat reproductive study [59]. Haematological effects were observed in male rats at interim sacrifices, but not at the terminal sacrifice, of a two year carcinogenicity study [49] at the lowest tested dose of 1.3 mg/kg bw/day. In female rats the NOAEL for haematological changes was 1.6 mg/kg bw/day (the lowest tested dose).

78. The database indicates that hepatic effects in rodents may occur at lower doses than non-hepatic effects. The lowest no observed effect level (NOEL) was 0.06 mg/kg bw/day for increased liver weight seen at 0.64 mg/kg bw/day in a 13-week dietary study in rats [35,36]. COT estimated that $BMDL_{10}$s for increased absolute liver weight at week 4, 7 and 13 sacrifices were 0.4, 0.3 and 0.44 mg/kg bw/day, respectively. A $BMDL_{10}$ of 0.74 mg/kg bw/day was estimated for hepatocytic megalocytosis in male rats of the two-year carcinogenicity study [49]. A LOAEL of 1 mg/kg bw/day was identified for increased liver weight, and focal to multifocal hepatic necrosis in the F_0- and F_1-generational male rats in the two-generation reproductive study [59], and $BMDL_{10}$s were 0.31 mg/kg bw/day in both generations. Increased maternal liver weight was also reported in the reproductive toxicity study in mice [17]. The $BMDL_5$ estimated, by the study authors, for increased maternal absolute liver weight was 0.17 mg/kg bw/day (almost one order of magnitude below the lowest tested dose). A benchmark response rate of 10% is more commonly used in order to be within the observed dose range. At the request of the COT, the authors of this study remodelled the data to estimate BMD_{10} and $BMDL_{10}$ for maternal liver weight at term. These were 0.52 and 0.46 mg/kg bw/day, respectively.

79. For deriving a tolerable daily intake (TDI) a dose level of 0.3 mg/kg bw/day was selected as a suitable point of departure expected to be without adverse effect on the basis of a number of endpoints of PFOA toxicity.

80. An uncertainty factor of 100 was applied to allow for inter- and intra-species variation. Therefore, the TDI indicated for PFOA is 3 µg/kg bw/day.

Exposure assessment

81. The Food Standards Agency has completed an analysis of composite food groups samples from the 2004 Total Diet Study (TDS) for a range of fluorinated chemicals, including PFOA and PFOS [66]. The TDS models the typical UK diet and is fully described in Food Survey Information Sheet 38/03 [67].

82. PFOA was only detected at a concentration above the limit of detection in the potatoes food group.

83. The estimated average and high level adult intakes of PFOA from the whole diet in 2004 were 0.001-0.07 µg/kg bw/day and 0.003-0.1 µg/kg bw/day (range of lower to upper bound figures)[†], respectively. Estimated high level dietary intake for toddlers was 0.01-0.3 µg/kg bw/day (range of lower to upper bound figures). These estimated intakes of PFOA from the diet are below the TDI recommended by the COT.

Conclusions

84. We recommend a TDI of 3 µg/kg bw/day be established, based on the range of effects on the liver, kidney, haematological and immune systems. We consider that the TDI is adequate to protect against other potential effects, such as cancer.

85. We note the results of the Food Standards Agency analysis of composite food group samples from the 2004 Total Diet Study (TDS) that estimated high level adult dietary intakes of PFOA are lower than the recommended TDI. The estimated intakes are not of concern regarding human health.

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[†] Upper bound concentrations assume that PFOA is present at the reporting limit for those food groups in which PFOA is present at concentrations below the reporting limit (limit of detection), and therefore could be an overestimate of the true concentrations. By contrast, lower bound concentrations assume that PFOA is absent for those food groups in which PFOA is present at concentrations below the limit of detection, and will therefore be an underestimate of the true concentrations. The range between the lower and upper bound values demonstrates the uncertainty in these exposure estimates and the true values will lie somewhere between the upper and lower bounds.

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