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

COT STATEMENT ON THE TOLERABLE DAILY INTAKE FOR PERFLUOROOCTANE SULFONATE

Introduction

1. Perfluorooctane sulfonate (PFOS) has the potential to enter the food chain and could have a negative health impact on humans. The Food Standards Agency commissioned analysis of the 2004 Total Diet Study samples for PFOS and the Committee was invited to consider the toxicology of PFOS and the results of the analysis.

Background

2. Perfluorooctane sulfonate is a member of the large chemical class of fluorochemicals referred to as perfluorinated alkyl compounds. All perfluorinated substances are of anthropogenic origin. These fluorochemicals have excellent surfactant properties and are widely used in the manufacture of plastics, electronics, textile, and consumer material in the apparel, leather, and upholstery industries [1]. The term PFOS covers its anionic, acid and salt forms, and the PFOS-moiety (the $C_8F_{17}SO_2$ group) is incorporated into a variety of compounds (referred to as PFOS-related substances) that have the potential to degrade subsequently to PFOS either metabolically or through environmental processes. PFOS is widely distributed on a global scale and has been identified in various food chains [2].

3. The major US manufacturer 3M announced in 2000 the voluntary cessation of production of PFOS and chemically-related substances due to reports of persistence and widespread exposure of wildlife and humans. Subsequent limited availability of PFOS-related substances and action within relevant industry sectors to decrease dependence on these substances have led to a significant reduction in the use of PFOS across the EU since 2002.

4. A hazard assessment for PFOS has been produced under the Existing Chemicals Programme of the Organisation for Economic Co-operation and Development (OECD) [3]. Given the widespread occurrence of PFOS the OECD evaluation recommended that national or regional exposure information gathering and risk assessment may need to be considered. The Environment Agency for England and Wales consequently reviewed the environmental risks of PFOS use and concluded that PFOS meets the criteria for classification as a Persistent,

Bioaccumulative and Toxic (PBT) substance [4]. In June 2005 the Swedish Environment Ministry announced that it will propose a ban for PFOS to the United Nations under the Stockholm Convention. Sweden also filed a national ban on PFOS to the European Commission.

Evidence considered in this evaluation

5. The COT has not previously evaluated PFOS. 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 PFOS 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 considered published literature and unpublished final reports of toxicology studies largely conducted by, or on behalf of, 3M.

7. Specialist teratology advice was sought from Professor Aldert Piersma (National Institute for Public Health and the Environment, The Netherlands) and his conclusions regarding the reported teratology findings are gratefully acknowledged.

Chemical information

8. The high ionization potential and low polarizability of fluorine lead to weak inter- and intra-molecular interactions that are reflected by the extremely low surface tension of the perfluoroalkyl acids. Their partitioning behaviour is also unique; when they are mixed with water and hydrocarbons, three immiscible phases are formed, indicating the hydrophobic and oleophobic nature of these chemicals. Consequently, these compounds are ideal surfactants. Due to the strength of the carbon-fluorine bonds, these compounds are highly stable leading to their persistence and bioaccumulative properties [2].

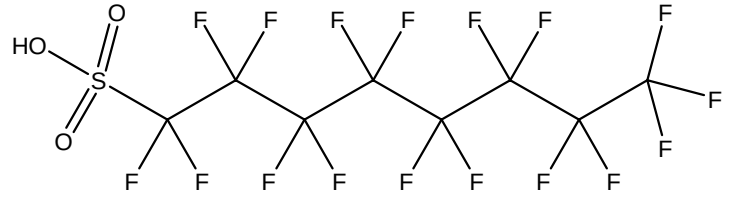

Figure 1 – Structure of perfluorooctane sulfonic acid (PFOS acid).

9. The perfluorooctane sulfonate anion (PFOS), does not have a CAS number, but that of the perfluorooctane sulfonic acid (Figure 1, $C_8F_{17}SO_3H$, molecular weight: 500) is 1763-23-1.

10. The Environment Agency has published a draft list of 96 PFOS-related substances which have the potential to degrade to PFOS [4]. Current information strongly supports a conclusion that PFOS and its salts cannot be broken down further chemically [3]. However, only limited data are available on the toxicology of the PFOS-related substances, such as 2-(*N*-ethylperfluorooctanesulfonamido)ethyl alcohol (*N*-EtFOSE, Figure 2).

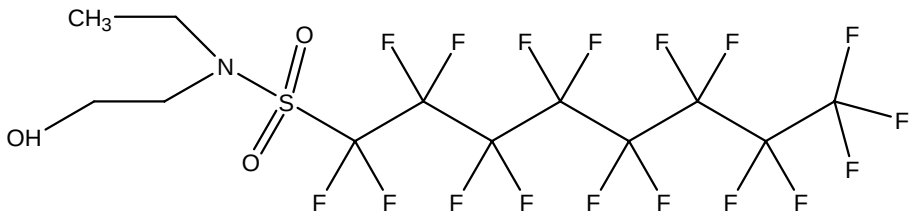

Figure 2 - Structure of *N*-ethylperfluorooctanesulfonamido-ethanol (*N*-EtFOSE).

11. PFOS is manufactured by a process known as Simons Electro-Chemical Fluorination (ECF). 3M reports a final product from ECF of approximately 70% linear PFOS and 30% branched impurities, including odd and even chain lengths. Although not specifically reported by 3M, manufacture of PFOS-related substances by ECF is assumed to result in the same proportion of linear and branched products.

12. Two determinations of the water solubility of PFOS have been reported. The average results were 519 mg/L at 20 ± 0.5°C, and 680 mg/L at 24-25°C. The surface active properties of the substance make a direct determination of the octanol-water partition coefficient impossible. In a preliminary study reported by 3M an inseparable emulsion was formed. 3M determined the solubility of PFOS in octanol as 56 mg/L.

Toxicological profile

13. The majority of the toxicology studies of PFOS have been conducted with its potassium salt (approximately 70% linear PFOS and 30% branched impurities), a white crystalline powder at normal temperature and pressure. No data are available on the relative toxicity of the non-linear contaminants of the test chemical.

Toxicokinetics – rat

14. Toxicokinetic data for potassium perfluorooctane sulfonate are available from five studies in the rat. These show that rather than bioconcentrating in the lipid fraction, PFOS tends to bind to plasma proteins.

15. Over 95% of an oral dose (4.2 mg/kg bw) of ^{14}C-PFOS was absorbed within 24 hours by male rats (8 weeks old) [5]. The redistribution half-life from plasma was 179 hours (7.5 days).

16. In two repeat dose studies to investigate the toxicokinetics of PFOS over the course of gestation, non-radiolabelled PFOS was administered by oral gavage to F_0 female rats. In the first study, PFOS was administered daily for 42 days prior to mating and continued through gestation day (GD) 20 at dose levels of 0, 0.1, 0.4, 1.6, and 3.2 mg/kg bw/day [6]. In GD 21 fetuses, serum PFOS levels were comparable to those of dams, but the fetal liver PFOS levels were considerably lower than in dams. In the second study female rats were dosed daily for 43 days prior to mating and through until confirmation of mating at three dose-levels, 0, 0.1, and 1.6 mg/kg bw/day PFOS [7]. As with the earlier study there was a dose-related increase in the levels of PFOS in the liver and serum, with much higher levels present in the liver than in the serum of both dams and pups.

17. Tissue distribution and extent and route of excretion of ^{14}C-PFOS were investigated in 8 week old male rats treated with a single dose of 4.2 mg/kg bw (i.v. tail vein) [8]. By post-dosing day 89, mean urinary excretion was 30% of administered ^{14}C, compared with 13% of administered ^{14}C excreted via faeces. Only liver and plasma contained a substantial percentage of the dose at 89 days post dosing, 25% and 2.8%, respectively. Elimination of only 42.8% of the dose through urine and faeces after 89 days indicated that the terminal half-life of elimination from the body was probably >89 days in the male rat.

18. PFOS undergoes considerable enterohepatic recirculation in the rat [9]. A 9.5-fold greater elimination of PFOS via faeces was observed in rats with disrupted enterohepatic circulation (induced by cholestyramine treatment) than for animals with normal enterohepatic circulation (mean percentage of dose eliminated via faeces in control rats was 8%).

Toxicokinetics – non-human primates

19. The pharmacokinetics and urinary excretion of PFOS, following a single i.v. bolus dose of 2 mg/kg bw, have been reported for male and female cynomolgus monkeys [10]. The serum concentration versus time data were subjected to non-compartmental pharmacokinetic analysis and the authors estimated that the serum terminal half-life of PFOS ranged from 122 to 146 days (mean: 132 days) in male monkeys and from 88 to 138 days (mean 110 days) in female monkeys. The study authors further concluded that these results provided no clear indication that the pharmacokinetics of PFOS were different in male and female monkeys.

20. In a six month study of PFOS toxicity, cynomolgus monkeys (4-6/sex/group) received 0, 0.03, 0.15 and 0.75 mg/kg bw/day by intragastric intubation of a capsule dose for at least 26 weeks [11,12]. Two monkeys/sex/group in the control, 0.15 and 0.75 mg/kg bw/day dose groups were monitored for one year following the end of treatment. Six months after cessation of treatment the control and 0.75 mg/kg bw/day dose group monkeys underwent partial hepatectomy. Serum PFOS concentrations showed a linear increase with time in both the low- and mid-dose groups and a non-linear increase in the high-dose group. Serum PFOS concentrations in the high-dose group monkeys appeared to plateau at approximately 20 weeks. The serum PFOS elimination curves, during the recovery

phase, appeared to be multiphasic in the high dose group and linear in the mid-dose group. During the first 23 weeks of the recovery phase serum concentrations in the high dose group decreased at a faster rate (half-life for elimination approximately 120 and 150 days in male and female monkeys, respectively) than in the mid-dose group (approximate half-life for elimination 180 days). However, towards the end of the one-year recovery period the slopes of the two recovery group elimination curves were similar (elimination half-life of approximately 180 days). This study did not show evidence for differences in pharmacokinetics between male and female monkeys.

Toxicokinetics – human

21. In 1976, Taves *et al.*, [13] reported that human serum samples contained non-ionic (organic) fluorine from perfluorocarbons. Preliminary evidence in 1979 from a 3M fluoropolymer production facility, showed that total serum organic fluorine levels for five employees were 4.1-11.8 parts per million (ppm), and that 55-80% of that was PFOS [14]. More recently PFOS was detected in 50% of non-occupationally exposed human blood donor samples in India and 100% of human blood samples in Poland, Italy, Belgium, USA and Japan [15].

22. There is some inconsistency with regard to the half-life of PFOS in humans. One study following 3 retired 3M workers for five and a half years suggested a mean elimination half-life of 1 428 days (approximately 4 years) [16].

23. The first report from an ongoing study following 27 retirees from a 3M production plant derived an elimination half-life of 139-640 days [17]. A mean serum half-life for PFOS of 8.7 years (S.D. = 6.1; range 2.3 – 21.3) was reported more recently [18]. The investigators have listed a number of limitations, and a number of attempts have been made to minimise the experimental error using selected subjects. No effort was made to determine, or control for, retiree re-exposure or endogenous metabolism of other perfluorinated chemicals to PFOS, both potentially leading to artificially long half-life estimations.

24. An analysis of PFOS concentrations in Kyoto City residents identified a sex-related pharmacokinetic difference [19]. Pre-menopausal females had higher serum PFOS concentrations than post-menopausal females and males. At an approximate age of 60 years, serum concentrations in post-menopausal females decreased to the level in males. Elimination in urine was approximately one-fifth of total PFOS elimination, assuming a one-compartment model.

25. PFOS can cross the human placenta [20]. PFOS concentrations in Japanese maternal blood samples were 4.9-18 ng/mL, whereas those in fetal samples were 1.6-5.3 ng/mL. The mean ratio of cord to maternal blood PFOS concentrations was 0.32 (range 0.23-0.41), indicating that PFOS may bind to a different extent in the fetal circulation.

26. A number of studies have assessed the levels of PFOS in blood of non-occupationally exposed humans. However, there have been no reports of levels of PFOS in UK subjects. The largest PFOS biomonitoring study of adults in the United

States [21] (645 Red Cross blood donors aged 20 - 69), reported a geometric mean serum concentration of 36 ng/mL (ppb). Given the consistency of the data in this large study with that of smaller studies in US and European populations, the authors hypothesised that the average serum PFOS concentrations in non-occupationally exposed populations may range from 30 to 40 ppb with 95% of a population's serum PFOS concentrations below 100 ng/mL.

27. A comparison of PFOS levels in 59 paired samples collected in 1974 (serum) and 1989 (plasma) from volunteer participants of a large community health study indicated serum concentrations of PFOS were statistically significantly higher in 1989 than 1974 (median concentrations of 34.7 ng/mL and 29.5 ng/mL, respectively, representing a 25% increase) [22]. The same study reported only a 9% increase in serum PFOS concentrations from 1974 to 1989 in non-paired samples adjusted for age and sex (120 samples/year) and this was not statistically significant. The levels of PFOS in 1989 were comparable to the levels in the Red Cross blood donor study [21].

28. In blood samples collected from the United States, Colombia, Brazil, Belgium, Italy, Poland, India, Malaysia and Korea PFOS was the predominant perfluorochemical detected [15]. The highest concentrations were in samples from the U.S. and Poland (> 30 ng/mL). Levels were lowest in India (<3 ng/mL) and the others were in the range of 3 – 29 ng/mL. No age- or sex-related differences were found.

29. The primary binding proteins in human plasma have been identified by incubating PFOS with seven separate human-derived plasma protein fractions at two different protein fraction concentrations (10% and 100% physiological concentrations) [23]. The percentage of PFOS bound to each human plasma protein at 100% physiological concentrations was 99.8% for albumin, 95.6% for beta-lipoprotein, 59.4% for alpha-globulin, 24.1% for gamma globulin, and <0.1% for each of fibrinogen, alpha-2-macroglobulin, and transferrin.

Acute and sub-acute toxicity

30. The oral LD_{50} in rat is 230 and 270 mg/kg bw (160-340 and 200-370 mg/kg bw, 95% confidence limits) for males and females, respectively [24].

31. Five sub-acute studies of PFOS have been conducted: two dietary studies in rats (a 90-day study [25] and a combined 4- and 14-week study [26]), two 90-day gavage studies in rhesus monkeys [27,28] and a 26 week study in cynomolgus monkeys [11,12].

Rat

32. In the 90-day study [25], Sprague-Dawley rats (5/sex/group) were administered potassium PFOS in the diet (mean achieved doses; 0, 2, 6, 18, 60, and 200 mg/kg bw/day). All the animals in the 18, 60, and 200 mg/kg bw/day dose groups died. Increased relative and absolute liver weights were reported at 2 and 6 mg/kg bw/day.

33. The second study [26], describes data from interim sacrifices at 4 and 14 weeks of a 2-year cancer bioassay. PFOS (potassium salt) was administered in the diet (mean achieved doses; 0, 0.05, 0.20, 0.42, and 1.6 mg/kg bw/day at 4 weeks, and 0, 0.04, 0.14, 0.37, and 1.40 mg/kg bw/day at 14 weeks) to Sprague-Dawley rats (5/sex/group) for 4 or 14 weeks.

34. Statistically significant effects were reported for the 1.6 mg/kg bw/day dose group at 4 weeks and the 1.4 mg/kg bw/day dose group at 14 weeks. At 4 weeks relative liver weights were significantly increased but absolute liver weights were unchanged. Male rats had lower serum glucose levels and females had elevated aspartate aminotransferase (AST) levels. Palmitoyl CoA oxidase activity in liver was 2-fold higher than in controls.

35. At 14 weeks in the 1.4 mg/kg bw/day dose group, absolute and relative liver weights were significantly higher in males and relative liver weight was significantly higher in females. Concentrations of PFOS in the livers were comparable between the sexes, but PFOS levels in serum were 31-42% higher in females than males. Compared with controls, males showed moderately lower serum cholesterol concentrations, mildly raised alanine aminotransferase (ALT) values and both sexes had mildly raised urea nitrogen values. Palmitoyl CoA oxidase activity in liver was not significantly different from controls. Centrilobular hepatocytic hypertrophy and midzonal to centrilobular vacuolisation were seen in males of the 0.37 mg/kg bw/day and 1.4 mg/kg bw/day dose groups and females of the 1.4 mg/kg bw/day group.

36. Serum and liver PFOS concentrations were used to provide a means of estimating internal doses that can be associated with effects and NOAELs. The mean serum PFOS concentration associated with the NOAEL (0.37 mg/kg bw/day, on the basis of liver weight changes at 14 weeks) was 44 µg/mL in males and 67 µg/mL in females. These doses corresponded to PFOS levels in the liver of 360 µg/g and 670 µg/g in males and females, respectively. A re-analysis of the data derived the lower 95% confidence interval of the benchmark dose[†] at the 10% response level ($BMDL_{10}$) for relative liver weights, the most sensitive endpoint in this study, of 0.20 mg/kg bw/day for males and females.

Non-human primate

37. Two 90-day subchronic studies in rhesus monkeys provide few reliable quantitative data. In the first study [27], animals (2/sex/group) were treated by gavage with PFOS at 0, 10, 30, 100, and 300 mg/kg bw/day. All treated animals died by day 20. Similar signs of toxicity were shown by all dose groups including decreased activity, emesis with some diarrhoea, general body trembling, twitching and

[†] The benchmark dose (BMD) approach [29,30] aims to provide an approach to dose-response assessment that is more quantitative than the 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.

convulsions. Necropsy showed yellowish-brown discoloration of the liver (no microscopic lesions on histological examination) in the 100 and 300 mg/kg bw/day groups. Congestion, haemorrhage and lipid depletion of the adrenal cortex were noted in all treatment groups.

38. Goldenthal *et al.*, [28] reported on a 90-day subchronic rhesus monkey study of 2 animals/sex/group dosed at 0, 0.5, 1.5, and 4.5 mg/kg bw/day via gavage.

39. All monkeys in the highest dose group (4.5 mg/kg bw/day) died or were sacrificed *in extremis* between weeks 5 and 7 of the study, having exhibited signs of gastrointestinal tract toxicity. After 30 days of treatment, there was a significant decrease in serum cholesterol and a 50% drop in serum alkaline phosphatase activity. There were no differences in mean organ weights compared to controls. In all treated animals there was marked diffuse lipid depletion in the adrenals. Both females and one male had moderate diffuse atrophy of the pancreatic exocrine cells with reduced size and loss of zymogen granules. Both males and one female had moderate diffuse atrophy of serous alveolar cells of the submandibulary salivary gland marked by decreased cell size and loss of cytoplasmic granules.

40. The 1.5 and 0.5 mg/kg bw/day dose groups survived until the end of the study and necropsy showed no treatment related lesions. However, both groups showed signs of gastrointestinal tract effects (soft stools and diarrhoea).

41. Cynomolgus monkeys (6/sex/group) were treated with 0, 0.03 (4/sex/group), 0.15, and 0.75 mg/kg bw/day PFOS by intragastric intubation of a capsule dose for at least 26 weeks [11,12]. Two monkeys/sex/group in the control, 0.15 and 0.75 mg/kg bw/day dose groups were monitored for one year following the end of treatment.

42. Two male animals in the high dose group died or were killed *in extremis* before the end of the dosing period, with indications of pulmonary necrosis or hyperkalemia.

43. Females in the high dose group had significantly increased absolute liver weights and males and females in this group had increased relative liver weights. Serum PFOS concentrations showed a linear increase with time in the low- and mid-dose groups but the serum PFOS concentration in the high-dose group was non-linear over time and appeared to plateau. Average liver to serum PFOS concentration ratios were not dose-related and ranged from 0.9:1 to 2.7:1.

44. High-dose group males had lower haemoglobin levels, which was considered to be a treatment-related effect. Serum total cholesterol values were significantly reduced in both sexes of the low- and high-dose groups. HDL cholesterol values were significantly lower for males in the low-dose group, females in the mid-dose group and for both sexes in the high-dose group. Due to the apparent lack of a dose response, the observed decrease in HDL cholesterol values in males given 0.03 mg/kg bw/day was considered, by the authors, unlikely to be a compound-related adverse effect. The significance of the decrease in HDL cholesterol values in 0.15 mg/kg bw/day dosed females was considered difficult to interpret, given the small number of study animals, lack of pre-study and interim HDL values and lack of proportionate changes in total cholesterol.

45. There was a statistically significant increase (50%) in hepatic palmitoyl CoA oxidase activity in the female 0.75 mg/kg bw/day dose group. In the 0.75 mg/kg bw/day dose group some animals presented with centrilobular vacuolisation, hypertrophy and mild biliary stasis.

46. Serum samples collected on days 50, 40 and 27 prior to treatment and days 37, 62, 91, 182 and 184 (necropsy) of treatment were analysed by standard radioimmunoassay (RIA) methods for cortisol, testosterone, estradiol, estrone, estriol, total triiodothyronine (T_3), total thyroxine (T_4), free T_3 and free T_4. Thyroid stimulating hormone (TSH) was measured by a double antibody RIA developed for determination of TSH in non-human primates that used human TSH standards, polyclonal rabbit antihuman TSH antibodies and radiolabelled human TSH. In 0.15 and 0.75 mg/kg bw/day dosed males at 26 weeks, TSH values were increased and total T_3 values were decreased. In the unpublished study report [11] the study authors concluded that the NOAEL was, therefore, 0.03 mg/kg bw/day. Analysis of thyroid hormone values was subsequently repeated by an independent laboratory on some of the archived serum samples taken at necropsy (day 184) using equilibrium dialysis followed by RIA for free T_4 and by standardised chemiluminometric immunoassays for the measurement of T_3, T_4 and TSH and reported by Seacat *et al.* [12] The reductions in T_3 and increases in TSH values in the 0.15 mg/kg bw/day dose group were not statistically significant in the second set of analyses. In both analyses, no dose-related changes were detected in total and free T_4 values.

47. All effects appeared completely reversible on withdrawal of treatment. Taking account of the re-analysis of male thyroid hormone values and acknowledging the uncertainty concerning the significance of lowered HDL observed in females given 0.15 mg/kg bw/day, the authors of the published report considered the study NOAEL was 0.15 mg/kg bw/day [12].

48. The COT considered the application of Benchmark Dose modelling to the analytical results for TSH and total T_3 values from the two laboratories and concluded that the data were insufficiently robust for BMD modelling to be applied with confidence. Therefore, although probably conservative, the Committee considered that a NOAEL in this study of 0.03 mg/kg bw/day was indicated on the basis of the totality of the data from the analysis of thyroid hormone values.

Mutagenicity and carcinogenicity

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

50. Members concluded that the *in vitro* plate incorporation test using five strains of *Salmonella typhimurium* and the D4 strain of *Saccharomyces cerevisiae* gave negative results [32]. The reverse mutation assay using *Eschericia coli* gave negative results [33]. For the *in vitro* chromosomal aberration assay in human lymphocytes [34], the Committee noted the difficulty in formulating adequate suspensions of PFOS but

agreed that this study had yielded negative results. The *in vitro* UDS assay in rat liver primary hepatocytes also gave negative results [35].

51. PFOS has also been tested in the mouse bone-marrow micronucleus test [36]. Members noted that only 1000 micronuclei had been evaluated at each dose level and that there was difficulty in adequately formulating PFOS for oral dosing. However, overall the study was considered to be acceptable and provided negative results.

52. The COM agreed that the studies undertaken with PFOS were acceptable and that PFOS should be regarded as not mutagenic.

53. The carcinogenicity and epidemiology studies relating to PFOS [37] (and a carcinogenicity study of the PFOS-related substance *N*-EtFOSE [38]) were considered by the COC in July 2005.

54. One dietary carcinogenicity study in Sprague-Dawley rats was available in which PFOS was administered in the diet for 104 weeks [37]. Interim sacrifices were made at 4, 14 (reported in [26]) and 52 weeks. Survival was considered to be adequate in this study. Non-neoplastic effects reported in the liver included increased absolute and relative liver weight, hepatocellular cystic degeneration and hepatocellular hypertrophy (often associated with vacuolation). No signs of hepatotoxicity were evident 52 weeks after cessation of a 52 week high-dose treatment. The NOAEL for non-neoplastic liver pathology was 2 ppm, i.e. a mean achieved dose of 0.16 and 0.14 mg/kg bw/day for males and females, respectively. This was based on the consideration that the low incidence of liver hypertrophy (3/17 and 1/9 in males and females, respectively at 2 ppm compared with 0/11 and 0/25 for males and females in the control group) associated with a lack of any effect on liver weight at this dose did not represent an adverse effect.

55. The incidence of hepatocellular adenomas was significantly increased at 20 ppm (mean achieved dose of 1.43 and 1.50 mg/kg bw/day for males and females, respectively). There was a single hepatocellular carcinoma in the female high dose (20 ppm) group. The incidence of thyroid follicular cell adenoma was significantly increased in the male high-dose recovery group, but not in the male and female high dose groups fed PFOS for 104 weeks.

56. A dietary carcinogenicity study in Sprague Dawley rats was also available in which *N*-ethylperfluorooctanesulfonamido ethanol (*N*-EtFOSE) was administered in the diet for 104 weeks [38]. No significant treatment-related effects were observed on 2-year survival rates, although survival in all groups including the controls was relatively poor. There was evidence of hepatocellular hypertrophy in high dose animals (mean achieved dose of 5.9 and 4.2 mg/kg bw/day for males and females, respectively). The incidence of hepatocellular adenomas was slightly higher in high dose male and female groups than in controls. This difference was statistically significant in the high-dose males. A single hepatocellular carcinoma was observed in a high dose female.

57. Two limited human epidemiological studies (a retrospective mortality study and an ‘episodes of care’ analysis) have been conducted in occupationally exposed

populations. Cohorts were relatively small and also relatively young. In the retrospective cohort mortality study, when restricted to workers with at least one year of employment and high exposure to PFOS, standardised mortality ratios (SMR) were below one for all causes of death and all malignant neoplasms. There were three deaths from malignant neoplasms of the bladder (0.63 expected) in males with over 5 years in high-exposure jobs. This excess was statistically significant (SMR 16.12; 95% CI 3.32-47.14). Members questioned the adequacy of exposure assessment by using job categories. It was noted that there had been potential exposure of the workers to benzidine, a known bladder carcinogen. Members advised that, overall, it was not possible to draw definite conclusions from this study. Further evaluation across all PFOS manufacturing sites would have provided more appropriate information. Members considered that the 'episode of care' analysis was unusual in design and uninformative.

58. In conclusion, the COC agreed that there was equivocal evidence for carcinogenicity limited to hepatocellular adenoma in the animal studies. The NOAEL for tumourigenicity was 0.15-0.57 and 0.19-0.56 mg/kg bw/day in males and females, respectively. COC were not convinced that adequate evidence had been provided for a mode of action incorporating peroxisome proliferation. Considering both the COM conclusions and the carcinogenicity data Members agreed that a threshold approach could be used for risk assessment.

Reproductive toxicity

59. Teratological studies have been conducted in rat, mouse, and rabbit with agreement of observation sacross the species examined. Observed developmental effects include reduction of fetal weight, cleft palate, anasarca, delayed ossification of bones (sternebrae and phalanges), and cardiac abnormalities (ventricular septal defects and enlargement of the right atrium). The majority of these findings were seen in the highest dose groups where significant reductions of weight gain and food consumption were also observed in the pregnant dams.

Rat

60. Time-mated female Sprague-Dawley rats were administered 0, 1, 5, and 10 mg/kg bw/day potassium PFOS by gavage from gestation day (GD) 6 to GD 15 [39]. Animals were sacrificed on GD 20. A NOAEL of 5 mg/kg bw/day and a LOAEL of 10 mg/kg bw/day for maternal toxicity were indicated based on significant reductions in mean body weights during GD 12-20. No other signs of maternal toxicity were reported. A LOAEL of 1 mg/kg bw/day for developmental toxicity was indicated on the basis of reductions in fetal weights. Developmental toxicity evident at doses of 10 mg/kg bw/day consisted of reductions in the mean number of implantation sites, corpora lutea, resorption sites and in the mean number of viable male, female and total fetuses, and fetal weights.

61. A repeat study in pregnant Sprague-Dawley rats [40], with the same dosing regime, reported NOAELs for maternal toxicity and developmental toxicity of 1 mg/kg bw/day. The LOAEL for maternal toxicity was 5 mg/kg bw/day, based on clinical signs of toxicity, decreases in body weight and food consumption, decreases in uterine weights, and an increased incidence in gastrointestinal lesions. The

LOAEL for developmental toxicity was 5 mg/kg bw/day, based on decreased fetal body weight and increases in external and visceral anomalies and variations. Signs of developmental toxicity included a dose-related trend toward an increased incidence of late resorptions, total resorptions, number of dead fetuses, and fetal loss, although these findings were not statistically significant. Significant decreases in mean fetal weights for both males and females were observed in the 5 and 10 mg/kg bw/day dose groups. Statistically significant increases in incomplete closure of the skull were observed in the low- and high-dose groups. Also observed in the high-dose group were delayed ossification and skeletal variations.

62. Thibodeaux *et al.*, [41] and Lau *et al.*, [42] reported maternal and developmental toxicity studies in rats. Pregnant Sprague-Dawley rats were given 1, 2, 3, 5 or 10 mg/kg bw/day by gavage from GD 2 to GD 21. Maternal weight gains were suppressed by PFOS in a dose-dependent manner (statistically significant in the 2 mg/kg bw/day and higher dose groups), attributed to reduced food and water intake (statistically significantly different from controls at 5 and 10 mg/kg bw/day). Serum PFOS levels increased with dosage and liver levels were approximately four-fold higher than serum levels. Serum T_4 and T_3 in the PFOS-treated dams were significantly reduced (1 week into treatment schedule). However, no feedback response of TSH was seen. Serum triglycerides (though not cholesterol) were significantly reduced, particularly in the high-dose group.

63. Fetuses had detectable levels of PFOS in liver tissue, at almost 50% that in the maternal livers, regardless of dose level. PFOS did not alter the numbers of implantations or live fetuses at term. Birth defects noted included, cleft palate, anasarca, ventricular septal defect and enlargement of the right atrium, primarily in the 10 mg/kg bw/day dose group. Maternal doses estimated, by the study authors, to correspond to the $BMDL_5$s for sternal defects and cleft palate were 0.12 and 3.3 mg/kg bw/day, respectively.

64. In the highest dose group (10 mg/kg bw/day) neonates became pale, inactive and moribund within 1 hour of birth, with death following quickly. Neonates in the 5 mg/kg bw/day dose group survived for between 8 and 12 hours and approximately 50% of offspring died at 3 mg/kg bw/day. Cross-fostering the 5 mg/kg bw/day dose group neonates to control nursing dams failed to improve survival. The maternal dose corresponding to the $BMDL_5$ for survival of rat pups at postnatal day 8 was estimated, by the study authors, at 0.58 mg/kg bw/day.

65. Small but significant and persistent growth lags were detected in surviving pups, and slight delays in eye opening were noted. Serum levels of PFOS in neonates were comparable to those of the dam at term, suggesting that PFOS equilibrated across the placenta. Unlike the situation in the adult there did not appear to be preferential accumulation of PFOS in the neonatal liver.

66. Grasty *et al.* [43] investigated critical windows of PFOS toxicity during gestation. Exposure of pregnant rats to 25 mg/kg bw/day PFOS for a 4 day period during pregnancy demonstrated an increased incidence of neonatal death when administration was later in gestation, reaching 100% mortality in the group treated on GD 17–20.

Mouse

67. Thibodeaux *et al.* [41] and Lau *et al.* [42] also reported maternal and developmental toxicity studies in mice. Pregnant CD-1 mice were treated with 1, 5, 10, 15, and 20 mg/kg bw/day from GD 1 to GD 17. Deficits in maternal weight gains were not as pronounced in the mouse as in the rat, and were only statistically significant in the 20 mg/kg bw/day dose group. Serum PFOS levels increased with dosage, and liver levels were approximately four-fold higher than serum levels. Serum T_4 levels were significantly reduced after 1 week of treatment. Serum triglycerides (though not cholesterol) were significantly reduced, particularly in the high-dose groups. Mouse dams in 10 mg/kg bw/day and higher dose groups had markedly enlarged livers.

68. PFOS did not alter the numbers of implantations or live fetuses at term. Birth defects noted were similar to those seen in the rat, namely cleft palate, anasarca, ventricular septal defect and enlargement of the right atrium, primarily in the 20 mg/kg bw/day dose group. The study authors estimated maternal doses corresponding to $BMDL_5$s for sternal and cleft palate defects in fetuses to be 0.016 and 3.5 mg/kg bw/day, respectively.

69. All animals were born alive and initially appeared to be active. In the highest dose group (20 mg/kg bw/day) neonates became pale, inactive and moribund within 1 hour with death following quickly. Neonate mice in the 15 mg/kg bw/day dose group also became moribund but survived for between 8 and 12 hours. Approximately 50% of offspring died at 10 mg/kg bw/day. The maternal dose corresponding to the $BMDL_5$ for survival of pups at postnatal day 6 was estimated at 3.9 mg/kg bw/day, approximately six times higher than that of the rat.

70. Serum levels of PFOS in neonates were comparable to those of the dam at term, suggesting that PFOS equilibrated across the placenta. There was no evidence of preferential accumulation of PFOS in the liver of the neonates.

Rabbit

71. Case *et al.*, [44] carried out oral developmental toxicology studies on mated female New Zealand white rabbits at dose levels of 0, 0.1, 1.0, 2.5, 5.0, 10, and 20 mg/kg bw/day by gavage. Treatment was from GD 6 to GD 20 and rabbits were sacrificed on GD 29. PFOS was not a selective fetal toxicant and did not cause fetal malformations in the rabbit.

72. A NOAEL and LOAEL of 0.1 and 1.0 mg/kg bw/day, respectively, were indicated for maternal toxicity, based on decreases in body weight gains and food consumption. The NOAEL and LOAEL indicated for developmental toxicity were 1.0 and 2.5 mg/kg bw/day, respectively, based on reductions in mean fetal body weight and increased incidences of fetal alterations such as delayed ossification. Abortions occurred in one 2.5 mg/kg bw/day dose group doe (GD 25) and ten of the 3.75 mg/kg bw/day dose group animals (between GD 22 and GD 28).

Two-generation reproductive study

73. A two-generation reproductive toxicity study was conducted in Sprague-Dawley rats [6]. Five groups of 35 rats/sex/dose were administered PFOS by oral gavage at 0, 0.1, 0.4, 1.6, and 3.2 mg/kg bw/day for six weeks prior to and during

mating. Treatment in males continued for approximately 22 days, and female rats were treated throughout gestation, parturition and lactation. F_1 generation rats were administered PFOS beginning on lactation day (LD) 22 and continuing through until one day prior to sacrifice. Only the 0, 0.1 and 0.4 mg/kg bw/day dose groups were continued into the F_2 generation because of excessive toxicity seen in the 1.6 and 3.2 mg/kg bw/day F_1 generation pups.

74. No mortality occurred in the F_0 generation females, and there did not appear to be any effects on oestrous cycling, mating and fertility parameters. There were no treatment-related signs of toxicity, effects on mating or on any of the fertility parameters evaluated in the F_0 generation male rats. The 1.6 and 3.2 mg/kg bw/day dose groups did exhibit reductions in body weight gains during the pre-mating period and terminal body weights were also significantly reduced. Absolute weights of seminal vesicles and the prostate in the 3.2 mg/kg bw/day dose group were significantly lower than controls.

75. The most significant finding in the F_1 generation offspring was reduced pup viability at the two highest dose levels. No pups survived beyond LD 1 in the 3.2 mg/kg bw/day dose group and in the 1.6 mg/kg bw/day dose group 10.6% (27/254) of pups were dead on LD1, and an additional 26% (59/227) died between LD 2 and 4. Clinical observations in the 0.1 and 0.4 mg/kg bw/day dose groups F_1 generation male and female rats were unremarkable.

76. Evidence of treatment-related effects in the F_2 generation pups consisted of reductions in mean pup body weights (on a per litter basis) observed at 0.1 and 0.4 mg/kg bw/day on LD 7. Body weights were comparable to control levels by LD 14 (0.1 mg/kg bw/day group) and by LD 21 (0.4 mg/kg bw/day group).

77. Based on reductions in body weight gain and food consumption, the NOAEL was 0.1 mg/kg bw/day for the F_0 generation and female F_1 generation. The NOAEL for the F_1 generation parental males was 0.4 mg/kg bw/day, the highest dose tested, as the 1.6 and 3.2 mg/kg bw/day groups were not continued. The NOAEL for the F_1 generation offspring was 0.1 mg/kg bw/day, based on statistically significant reductions in mean pup weight gain at higher doses. For the F_2 generation offspring the NOAEL was 0.1 mg/kg bw/day, based on statistically significant reductions in mean pup body weight, litter size, pup viability and survival at higher doses.

78. A cross-fostering study was conducted with female Sprague-Dawley rats administered 0 and 1.6 mg/kg bw/day PFOS beginning 42 days prior to mating with untreated males, and continued throughout gestation and into LD 21 [7]. Litters were placed with either a control or PFOS-treated dam for rearing, producing four groups of litters: *in utero* exposure only; un-exposed (controls); *in utero* and post-natal exposure; and post-natal exposure only.

79. Pups with post-natal exposure only had a similar mortality rate (1.1%) as pups in the control group (1.6%). Pups exposed to PFOS only *in utero* and those exposed both *in utero* and postnatally had mortality rates of 9.6% and 19.2%, respectively, indicating that *in utero* exposure is the main contributor to reduced pup survival.

Mechanistic studies

80. A small number of recently published studies have investigated more specific effects of PFOS.

81. An acute study demonstrated that PFOS, but not *N*-EtFOSE, administered via a single intraperitoneal injection at 100 mg/kg bw to male Sprague-Dawley rats, induced markers of peroxisome proliferation (induction of lauroyl CoA oxidation and lowering of serum cholesterol) in the absence of hepatomegaly [45]. PFOS did not cause a significant change in liver weight but there was a significant increase in liver-to-body weight ratio (a 12% increase) due to body weight loss.

82. With its highly hydrophobic and rigid perfluorinated carbon tail and strongly polar sulfonyl head group PFOS somewhat resembles a fatty acid. Luebker *et al.*, [46] demonstrated that PFOS and *N*-EtFOSE can interfere with the binding affinity and capacity of liver-fatty acid binding protein for fatty acids.

83. Hepatic gene expression studies in rats treated with PFOS (5 mg/kg bw/day for 3 days or 3 weeks) identified twenty three genes induced significantly and nineteen genes suppressed significantly [47]. Induced genes were primarily genes for fatty acid metabolising enzymes, cytochrome P450s, or genes involved in hormone regulation. One cytosolic enzyme, long-chain acyl-CoA hydrolase, showed a 90-fold induction on treatment. This enzyme cleaves acyl-CoA to free fatty acid and CoA, and leads to increased cytosolic free fatty acid concentrations. PPAR-α mRNA expression levels were unchanged on treatment, however, a number of genes that are indicative of peroxisome proliferation were affected. The activities of the phenobarbital inducible genes carboxyesterases and CYP2B1 were also increased by PFOS treatment, but no evidence for PFOS acting directly on the arylhydrocarbon receptor has been found.

84. One study in mice suggested that PFOS induced increases in peroxisomal fatty acid beta-oxidation, peroxisomal catalase activity, omega-hydroxylation of lauric acid, cytosolic epoxide hydrolase activity and cytosolic DT-diaphorase activity in liver [48], which are effects induced by peroxisome proliferators. The authors proposed that the study results challenge the hypothesis that the first step in peroxisome proliferation is formation of a thioester between the carboxylic group of a proliferator and coenzyme A.

COT evaluation

85. In accordance with the advice of COM and COC, the COT considered it appropriate to take a threshold approach to establishing a tolerable intake for PFOS. This is based upon the negative genotoxicity in standard assays and the equivocal evidence for carcinogenicity.

86. Given the bioaccumulative properties of PFOS it may be more appropriate to relate the toxic effects to a body burden rather than to a daily dose. However, there is incomplete understanding of the pharmacokinetics of PFOS in rodents and humans, and the Committee considered that equilibrium between plasma and target

organ concentration is unlikely to have been reached in the sub-acute studies in animals. The use of a body burden approach would therefore involve excessive uncertainty on the basis of the currently available data.

87. Conclusions on the rat and mouse teratology studies [41,42] were:

- The finding of delayed ossification (manifested as bipartite and bilobed sternebrae) would be more appropriately considered a “variation” rather than a “defect” as it regularly occurred in control animals;
- delayed ossification is often a sign of general developmental delay but this is not entirely clear in this study where fetal weight effects only occur in the highest dose group. There is a dose-response in both species (rats and mice) in terms of the number of sternebrae per fetus with the variation. However, in the absence of details about the extent of the effects it is not possible to draw firm conclusions about their significance;
- the authors description of “notable skeletal defects” is not explicitly explained but probably relates to the sternal and phalangeal findings in the rat and to the sternal findings in the mouse. In the mouse, roughly half of the litters show these “notable skeletal defects” in the control and at both highest doses, indicating that this is not a generalized phenomenon throughout all litters, and moreover, a dose-response is not apparent;
- taken together, the sternal findings should not be interpreted as malformations but as indications of delayed development. In view of the above and given the additional fetal observations in this study the sternal findings do not determine the developmental NOAEL in this study.

88. The $BMDL_5$ indicated for sternal defects in the mouse fetus was approximately two orders of magnitude below the lowest dose of PFOS tested [41,42], when modelled by the study authors. Insufficient information was provided on the modelling procedures to verify the validity of this value, which indicated considerable variability. In view of the uncertainties in the BMD modelling, it was considered more practical to define an overall developmental NOAEL, which was 2 mg/kg bw/day in the rat on the basis of anasarca [41], and 5 mg/kg bw/day in the mouse on the basis of heart defects [41].

89. Overall, the data from the mechanistic studies [45-48], the rat carcinogenicity study [26,37] and the 26-week capsule study in cynomolgus monkeys [12] provide evidence that PFOS is not a potent inducer of peroxisome proliferation. Electron microscopy of livers of PFOS-exposed rats did not reveal peroxisome proliferation. The 50-95% increases in liver palmitoyl CoA oxidase levels, although statistically significant, were not considered to be biologically significant. There is evidence for some liver growth inducing agents also increase the incidence of thyroid tumours, however, with respect to PFOS more information is required.

90. Re-analysis by COT of the data reported in a 14-week rodent study [26] derived a $BMDL_{10}$ of 0.20 mg/kg bw/day for increased relative liver weights in males and females, the most sensitive endpoint in this study. Non-neoplastic effects in the two-year rat carcinogenicity study [37] indicated NOAELs of 0.16 and 0.14 mg/kg bw/day for males and females, respectively, and the two-generation reproductive toxicity study in rats [6] indicated a NOAEL of 0.1 mg/kg bw/day for F_0, F_1 and F_2 generations.

91. The 26-week cynomolgus monkey study [11,12] provided the lowest NOAEL, of 0.03mg/kg bw/day for decreased serum T3 levels . This NOAEL was considered to be the most suitable basis for deriving a tolerable daily intake (TDI) for PFOS. The Committee noted that, on the basis of the pharmacokinetic data indicating an elimination half-life of between 110 and 180 days[10-12], the cynomolgus monkeys would be at approximately half steady state at the end of this study.

92. Taking into account that this was a primate study and the effects were mild, the Committee concluded that it was not necessary to apply an additional uncertainty factor to allow for the incomplete attainment of steady state. The Committee applied an uncertainty factor of 100 to allow for inter- and intra-species variation to the NOAEL of 0.03 mg/kg bw/day from the cynomolgus monkey study. Therefore, the TDI indicated for PFOS is 0.3 µg/kg bw/day. This value is provisional and should be reviewed as new information becomes available.

93. Because of the accumulative properties of PFOS, exposure should be averaged over prolonged periods for comparison with the TDI.

Exposure assessment

94. 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 PFOS [49]. The TDS models the typical UK diet and is fully described in Food Survey Information Sheet 38/03 [50].

95. PFOS was detected at concentrations above the limit of detection in the potatoes, canned vegetables, eggs and sugars and preserves food groups. Five of the other perfluorinated chemicals were not detected in any food groups and nine were detected only occasionally. Ten different fluorinated chemicals were found in the potatoes food group.

96. The estimated average and high level adult intakes of PFOS from the whole diet in 2004 were 0.01-0.1 µg/kg bw/day and 0.03-0.2 µg/kg bw/day (range of lower to upper bound figures)[†], respectively. The highest estimated high level dietary intake was 0.1-0.5 µg/kg bw/day (range of lower to upper bound figure) for 1.5-2.5 year olds. Only 10 to 20% of the estimated intakes is derived from the four food groups in which PFOS was detected. These estimated intakes of PFOS from the diet are below the TDI of 0.3 µg/kg bw/day recommended by the COT, with the exception of the high level intake for children aged 1.5-6 years (0.1-0.5 µg/kg bw/day; range of lower to upper bound figures). As PFOS can be formed by degradation from a large group of related perfluorinated substances, the

[†] Upper bound concentrations assume that PFOS is present at the reporting limit for those food groups in which PFOS 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 PFOS is absent for those food groups in which PFOS 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.

significance to the exposure assessment of detecting a number of other fluorinated chemicals in different food groups is currently uncertain.

Conclusions

97. We conclude that PFOS has the potential to cause a range of adverse health effects. Given the bioaccumulative properties of PFOS a body burden approach to setting health-based guidance values may be most appropriate, but the current knowledge of the pharmacokinetics of PFOS does not allow adequate estimation of the body burden. We recognise the need for further characterisation of human pharmacokinetics of PFOS but acknowledge that this may not be easily obtained or even feasible. In addition, we recommend that data be generated to support a body burden approach, including a better understanding of the magnitude of enterohepatic recirculation of PFOS in rodents.

98. We recommend a TDI of 0.3 µg/kg bw/day be provisionally proposed for PFOS. We consider that on the basis of available information this provisional TDI is adequate to protect against the range of identified effects.

99. We note the results of the Food Standards Agency analysis of composite food group samples from the 2004 Total Diet Study (TDS) that indicated that some groups of consumers may exceed the recommended TDI. There are considerable uncertainties in the dietary intake estimates, and therefore these potential exceedances do not indicate immediate toxicological concern.

100. We recommend that there is a need for generation of further information to reduce the uncertainties in the exposure assessment, including consideration of the impact of other perfluorinated chemicals in the diet on total PFOS exposure.

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