Per- and polyfluoroalkyl substances: evaluation of liver effects using in vivo data - PFAS/2023/06

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Introduction, Background and Literature Search

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Introduction

1. This paper is part of a series of papers supporting the Committee on Toxicity (COT) assessment of the toxicology of per- and polyfluoroalkyl substances (PFAS). It provides the evidence on *in vivo* liver toxicity, with individual studies tabulated in Annex A.

2. Future papers will include evidence on liver toxicity based on in vitro toxicity studies and human evidence for liver toxicity, and groups of papers covering other endpoints including developmental toxicity and immunotoxicity.

Background

3. The COT has previously considered PFAS on a number of occasions (see summary in TOX/2022/53), and has recently published a statement on the EFSA opinion. A paper summarising health-based guidance values (HBGV) was presented in December 2022 (TOX/2022/67) and following agreement in March 2023 the PFAS subgroup was established and an interim position published outlining future work.

Literature search

4. Search terms used previously by the European Food Safety Authority (EFSA) (2018 and 2020) were replicated. These search terms, the inclusion and exclusion criteria and the search results, are presented in Annex B to this paper.

5. A total of 53 published papers or reports were evaluated, some of which comprise more than one study and more than one PFAS. All papers and reports were evaluated for reliability using the ToxRTool (Klimisch et al., 1997) to determine data quality and reliability. Only those designated a score of 1 or 2 were further evaluated.

In vivo liver toxicity studies

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6. The current paper considers effects in adult animals following exposure to PFAS by gavage, intraperitoneal (i.p) injection, diet or drinking water.

7. From the 53 published sources, 50 studies were carried out on 10 perfluoroalkyl carboxylic acids (PFCAs) and 27 studies were carried out on three perfluoroalkyl sulphonic acids (PFSAs). Table 3 to Table 16 present no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) for PFCAs based on liver effects and Table 17 to Table 19 present data for PFSAs.

8. In vivo acute toxicity studies are available for perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) (Table 3 to Table 5) and perfluorooctane sulfonic acid (PFOS) (Table 6).

9. Repeated dose studies are available for perfluorobutanoic acid (PFBA), perfluorohexanoate (PFHxA), PFOA, PFNA, PFDA, perfluoroundecanoic acid (PFUnDA), perfluorododecanoic Acid (PFDoDA), perfluorotetradecanoic acid (PFTeDA) and perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA) (Table 7 to Table 16) and perfluorobutane sulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS) and PFOS (Table 17 to Table 19).

10. Developmental toxicity studies are available for PFBA and PFOA (Table 20 and Table 21).

11. Of the 12 acute studies identified, four were carried out with PFOA, one with PFNA, five with PFDA and two with PFOS.

12. Of the 62 repeated dose studies identified, two were carried out with PFDoDA, three with PFBA, PFHxA and PFDA, four with PFNA, six with PFHxS, seven were carried out with PFBS, 12 with PFOS and 18 with PFOA. Only one study was carried out with PFUnDA, PFTeDA, PFHxDA and PFPDA.

13. Of the three development toxicity studies identified, one was carried out with PFBA and two with PFOA. Only effects in the dam are discussed in the endpoint summaries below as developmental effects in offspring, as a result of exposure during gestation and/or lactation, will be evaluated in subsequent papers.

14. The majority of acute and repeated dose studies were conducted with rats and mice, with the exception of one repeated dose study with PFOS and PFOA, which was carried out in Cynomolgus monkeys.

15. An overview of the PFAS chemical structure and molecular weight is presented in Annex C to this paper. Depending on the PFAS, studies have investigated the acid form, or a sodium, ammonium or potassium salt.

Endpoints investigated and Summary of Results

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Endpoints investigated

16. Exposure to PFAS caused a number of liver effects in animals including effects on liver weight and DNA content, clinical chemistry, effects on histopathology and impacts on gene expression.

17. For PFCAs, 40 of the 50 assays (reported in the 34 published sources) measured liver weight, 37 carried out clinical chemistry, although not all parameters (i.e., AST, ALT, cholesterol, TG etc) were measured in each study, 37 included liver histopathology, and 24 included gene expression relating to liver effects.

18. For PFSAs, 17 of the 25 studies (reported in the 19 published sources) evaluated absolute and/or liver weight, 18 carried out clinical chemistry measurements, 17 assessed liver histopathology, and 11 measured gene expression changes relating to liver effects.

19. The data presented below relate to statistically significant changes in liver effects seen at the LOAEL. Effects seen at higher doses are not included.

Summary of results

20. Exposure to PFAS caused a number of liver effects in animals including effects on liver weight, clinical chemistry (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), cholesterol, triglycerides (TG), total protein (TP), bilirubin, albumin/globulin ratio), effects on histopathology, and impacts on gene expression.

Liver Weight

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21. For PFCAs, absolute and/or relative liver weight was measured in 40 of the 50 studies reviewed (four of the 10 acute studies, 33 of the 37 repeated dose studies and all three developmental studies).

22. In the acute studies, three of the four studies reported an increase in liver weight. Das et al. (2017) reported an increase in absolute and relative liver weight with PFOA and PFNA in male mice, whereas Kawashima et al. (1995) only reported an increase in relative liver weight with PFDA, but not PFOA, in male mice.

23. In the repeated dose assays, an increase in liver weight was seen in 19 of the 33 assays.

24. An increase in absolute liver weight was seen in male, but not female, rats following exposure to PFBA for 28 and 90 days (Butenhoff et al., 2012a). PFOA also increased absolute and/or relative liver weight in male mice (Botelho et al., 2015; Guo et al., 2019; Soltani et al., 2023; Son et al., 2008; Wu et al., 2018), rats (Butenhoff et al., 2012a; Elcombe et al., 2010; Li et al., 2019; NTP., 2022b; Qazi et al., 2010b), male and female mice (Kennedy Jr, 1987) and male Cynomolgus monkeys (Butenhoff et al., 2002). An increase in absolute liver weight was also seen in male and female mice following exposure to PFNA (Kennedy Jr, 1987; NTP., 2022b) and absolute and relative liver weight was increased in male and female rats with PFDA (NTP., 2022b). PFDA also increased relative liver weight in female rats (Frawley et al., 2018).

25. Increased relative liver weights in male rats were also reported with PFunDA (Takahashi et al., 2014) and in male and female rats with PFDoDA (Kato et al., 2014), whereas both absolute and relative liver weight was increased following exposure to PFHxDA (Hirata-Koizumi et al., 2015), PFTeDA (Hirata-Koizumi et al., 2015) and PFODA (Hirata-Koizumi et al., 2012).

26. No effects were seen on liver weight with PFHxA, (Chengelis et al., 2009; Loveless et al., 2009; NTP., 2022b), PFBA (Foreman et al., 2009), PFNA (Hadrup et al., 2016) or PFDoDA (Zhang et al., 2008).

27. In the developmental studies, increased liver weights were also increased in female rats with PFBA (Das et al., 2017) and PFOA (Xu et al., 2022; Zhang et al., 2021).

28. Where both male and female rats were included in repeated dose studies, increased liver weights were seen in males but not females with PFBA (Butenhoff et al., 2012a), PFOA (Butenhoff et al., 2012a; NTP., 2022b), PFUnDA (Takahashi et al., 2014), PFDoDA (Kato et al., 2014), PFHxDA and PFODA (Hirata-Koizumi et al., 2012).

29. Various authors measured the hepatic DNA content to help the mechanism by which the increased liver weights occurred following exposure to PFAS. Elcombe et al. (2010) reported a decrease in liver DNA content, when expressed as mg/g liver but not as mg/whole liver, in male rats following exposure to PFOA for 7 and 28 days. This was accompanied by an increase in absolute and relative liver weight at both time points.

30. Butenhoff et al. (2002) showed a dose-dependent decrease in liver DNA content in male Cynomologus monkeys, although it only reached statistical

significance at the top dose of 30 mg/kg bw/day (reduced to 20 mg/kg bw/day) whereas increased absolute liver weight was seen at 3 mg/kg bw/day.

31. For PFSAs, absolute and/or relative liver weight was measured in 17 of the 25 repeat dose toxicity studies reviewed, and an increase in absolute and/or relative liver weight was seen in 11 of the 17 studies.

32. An increase in absolute and relative liver weight was seen in male, but not female, rats following exposure to PFBS (Lieder et al., 2009b). PFBS also increased relative liver weight in male rats after 28 days exposure (NICNAS., 2005) and relative liver weight in male and female rats (NTP., 2022a).

33. PFHxS increased absolute liver weight in male mice (Bijland et al., 2011) and absolute and relative liver weights in male, but not female rats (NTP., 2022a).

34. PFOS also increased absolute liver weight in male mice (Bijland et al., 2011), relative liver weight in male rats (Elcombe et al., 2012), male and female rats (Kim et al., 2011) and male mice (Huck et al., 2018) and absolute and relative liver weights in male and female rats (NTP., 2022a) and female monkeys (Seacat et al., 2002).

35. No effects were seen on liver weight with PFBS (Bijland et al., 2011; Lieder et al., 2009a; NTP., 2022a), PFBS following 90 days exposure (NICNAS., 2005), PFHxS (Chang et al., 2018) and PFOS.

36. Where both male and female animals were included in repeated dose studies, increased liver weights were seen in male, but not female, rats with PFBS (Lieder et al., 2009b; NICNAS., 2005) and PFHxS (NTP., 2022a), and female, but not male, monkeys (Seacat et al., 2002).

Recovery

37. For both PFBA and PFOA, the increase in absolute liver weight seen in male rats after the 28- (PFBA and PFOA) and 90-day (PFBA only) exposure was comparable to controls following the 3-week recovery period (Butenhoff et al., 2012a). Butenhoff et al. (2002) also reported a transient increase in liver weight in Cynomolgus monkeys following a 90-day recovery period.

38. For PFBS, the increase in absolute and relative liver weights seen in male rats after a 28-day exposure was comparable to controls after a 14-day recovery period (NICNAS., 2005). For PFOS, liver weight was still greater than controls in male rats after 84 days recovery, although on days 28 and 56 liver

weight was comparable to controls (Elcombe et al., 2012).

Clinical chemistry

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Aspartate aminotransferase

39. For PFCAs, levels of AST were measured in 23 of the 50 studies reviewed (one of the ten acute studies, 21 of the 37 repeated dose studies and one of the three developmental studies).

40. No effect on AST was reported in the acute study in male rats following exposure to PFOA (Elcombe et al., 2010).

41. In the repeated dose studies, serum AST was only increased in three of the 21 studies, all with PFOA. (Elcombe et al. (2010) reported an increase in male rats following a 28-, but not 7-day, exposure. An increase in AST was also seen in male (Soltani et al., 2023; Zou et al., 2015) and female (Xu et al., 2022) mice.

42. In contrast, no effects were reported following exposure to PFBA for 28 or 90 days (Butenhoff et al., 2012a), PFHxA (Chengelis et al., 2009; Loveless et al., 2009; NTP., 2022b), PFOA (Butenhoff et al., 2002; Butenhoff et al., 2012a; Elcombe et al., 2010; Guo et al., 2019; Li et al., 2019; NTP., 2022b; Qazi et al., 2010a; Son et al., 2008; Zou et al., 2015), PFNA and PFDA (NTP., 2022b) and PFUnDA (Takahashi et al., 2014).

43. No studies that showed an increase in AST were carried out in both male and female animals so sex differences could not be evaluated.

44. For PFSAs, levels of serum AST were measured in 13 of the 25 studies reviewed (one acute study and 12 of the 25 repeated dose studies).

45. No effect on AST was reported in the acute study in male and female Cynomolgus monkeys following a single exposure to PFOS (Chang et al., 2017).

46. In the repeated dose studies, AST was only increased in two of the 12 studies, both of which reported an increase in male rats following exposure to PFOS (Han et al., 2018b; Kim et al., 2011).

47. In contrast, no effects were reported following exposure to PFBS (Lieder et al., 2009a; NICNAS., 2005; NTP., 2022a), PFHxS (Butenhoff et al., 2009; Chang et al., 2018; NTP., 2022a) and PFOS (Butenhoff et al., 2012b; Elcombe et al., 2012; Han et al., 2018a; NTP., 2022a).

48. Differences in response were seen between sexes only following exposure to PFOS, where decreases in AST were only seen in male rats (Kim et al., 2011).

Alanine aminotransferase

49. For PFCAs, serum levels of ALT were measured in 25 of the 50 studies reviewed (one of the ten acute studies, 23 of the 37 repeated dose studies and one of the three developmental studies).

50. No effect on ALT was reported in the acute study in male rats following exposure to PFOA (Elcombe et al., 2010).

51. In the repeated dose studies, ALT was only increased in four of the 23 studies. Loveless et al. (2009) reported an increase in male rats following exposure to PFHxA. NTP. (2022b) and Soltani et al. (2023) showed increases in male rats and mice, respectively, following exposure to PFOA and Xu et al. (2022) reported an increase in ALT in female mice in the developmental study.

52. In contrast, no effects were reported following exposure to PFBA for 28 and 90 days (Butenhoff et al., 2012a), PFHxA (Chengelis et al., 2009; NTP., 2022b), PFOA (Botelho et al., 2015; Butenhoff et al., 2002; Butenhoff et al., 2012a; Elcombe et al., 2010; Guo et al., 2019; Li et al., 2019; NTP., 2022b; Qazi et al., 2010a; Son et al., 2008; Zou et al., 2015), PFNA and PFDA (NTP., 2022b), PFUnDA (Takahashi et al., 2014) and PFDoDA (Kato et al., 2014).

53. Differences in response were seen between sexes following exposure to PFHxA (Loveless et al., 2009) and PFOA (NTP., 2022b) where decreases in ALT were only seen in males.

54. For PFSAs, serum levels of ALT were measured in 14 of the 25 studies reviewed (one acute study and 13 of the 25 repeated dose studies).

55. No effect on ALT was reported in the acute study in male or female Cynomolgus monkeys following exposure to PFOS (Chang et al., 2017).

56. In the repeated dose studies, ALT was increased in two of the 13 studies and was decreased in one study. An increase in ALT was seen male mice following exposure to PFHxS (He et al., 2022) and in male rats with PFOS (Han et al., 2018a), whereas a decrease was seen in female Cynomolgus monkeys after PFOS exposure (Seacat et al., 2002).

57. In contrast, no effects were reported following exposure to PFBS (Lieder et al., 2009a; NICNAS., 2005; NTP., 2022a), PFHxS (Butenhoff et al., 2009; Chang et al., 2018; NTP., 2022a) and PFOS (Butenhoff et al., 2012b; Han et al., 2018b; NTP., 2022a).

58. Differences in response were seen between sexes only following exposure to PFOS where decreases in ALT were only seen in female monkeys (Seacat et al., 2002).

Alkaline phosphatase

59. For PFCAs, serum levels of ALP were measured in 17 of the 50 studies reviewed (all of which were repeated dose studies).

60. In the repeated dose studies, ALP was increased in five of the 17 studies. Butenhoff et al. (2012a) reported increases in male rats following exposure to PFBA for 28 and 90 days.

61. PFOA increased ALP in male and female rats (NTP., 2022b) and in male mice (Qazi et al., 2010a; Zou et al., 2015) and PFDoDA increased ALP in male mice (Kato et al., 2014).

62. In contrast, no effects were reported following long term exposure to PFHxA (Chengelis et al., 2009; Loveless et al., 2009; NTP., 2022b), PFOA (Butenhoff et al., 2012a), PFNA and PFDA (NTP., 2022b), PFUnDA (Takahashi et al., 2014) and PFTeDA, PFHxDA and PFPDA (Hirata-Koizumi et al., 2015).

63. Differences in response were seen between sexes following exposure to PFBA (Butenhoff et al., 2012a) and PfDoDA (Kato et al., 2014) where a decrease in ALP was only seen in male rats.

64. For PFSAs, serum levels of ALP were measured in 10 of the 25 studies reviewed (one acute study and nine of the 25 repeated dose studies).

65. No effect on ALP was reported in the acute study in male or female Cynomolgus monkeys following exposure to PFOS (Chang et al., 2017).

66. In the repeated dose studies, ALP was only increased in female Cynomolgus monkeys following exposure to PFOS (Seacat et al., 2002).

67. In contrast, no effects were reported following long term exposure to PFBS (NICNAS., 2005; NTP., 2022a), PFHxS (Butenhoff et al., 2009; Chang et al., 2018; NTP., 2022a) and PFOS (Butenhoff et al., 2012b; Elcombe et al., 2012; NTP., 2022a)

68. Differences in response were seen between sexes following exposure to PFOS where increases in ALP were only seen in female monkeys (Seacat et al., 2002).

Triglycerides

69. For PFCAs, serum or plasma TG levels were measured in 15 of the 50 studies reviewed (five of the ten acute studies and 18 of the 37 repeated dose studies).

70. In the acute studies, four of the five studies reported an increase in serum TG. Das et al. (2017) reported an increase with PFOA and PFNA in male mice, and Kawashima et al. (1995) reported an increase with PFOA and PFDA in male rats. No effect was seen in male mice following exposure to PFOA (Elcombe et al., 2010).

71. In the repeated dose studies, TG was decreased in five of the 18 studies. Elcombe et al. (2010) reported a decrease in male rats following a 7- and 28-day exposure to PFOA in male rats. A decrease was also reported by NTP. (2022b) in male rats and by Qazi et al. (2010a) in male mice. In contrast, Wu et al. (2018) reported an increase in TG in male mice following PFOA exposure. PFNA also decreased TG in male rats (NTP., 2022b).

72. In contrast, no effects were reported following exposure to PFBA for 28 or 90 days (Butenhoff et al., 2012a), PFHxA (Chengelis et al., 2009; NTP., 2022b), PFOA (Botelho et al., 2015; Butenhoff et al., 2012a), PFDA (NTP., 2022b), PFDoDA (Kato et al., 2014; Zhang et al., 2008), PFTeDA and PFHxDA (Hirata-Koizumi et al., 2015) and PFODA (Hirata-Koizumi et al., 2012).

73. Differences in response were seen between sexes following exposure to PFOA and PFNA (NTP., 2022b) where a decrease in TG was only seen in male rats.

74. For PFSAs, serum or plasma TG levels were measured in 16 of the 25 studies reviewed (one acute study and 15 of the 25 repeated dose studies) and hepatic TG levels were measured in seven repeated dose studies.

75. No effect on TG was reported in the acute study in male or female Cynomolgus monkeys following exposure to PFOS (Chang et al., 2017).

76. In the repeated dose studies, serum or plasma TG was decreased in four of the 15 studies and increased in one study. A decrease in TG was seen in male mice after exposure to PFBS, PFHxS and PFOS (Bijland et al., 2011) and in male rats with PFOS (Kim et al., 2011), whereas an increase was seen in male Cynomolgus monkeys after PFOS exposure (Seacat et al., 2002).

77. Hepatic TG was increased in male mice following exposure to PFHxS (Bijland et al., 2011; Das et al., 2017; He et al., 2022) and PFOS (Bijland et al., 2011; Chen et al., 2022)

78. In contrast, no effects were reported following exposure to PFBS (Bijland et al., 2011; Chen et al., 2022; Lieder et al., 2009a; NICNAS., 2005; NTP., 2022a), PFHxS (Butenhoff et al., 2009; Chang et al., 2018; NTP., 2022a) and PFOS (Butenhoff et al., 2012b; Elcombe et al., 2012; Huck et al., 2018; NTP., 2022a).

79. Differences in response were seen between sexes following exposure to PFOS where a decrease in TG was only seen in male rats (Kim et al., 2011) and an increase was seen in male Cynomolgus monkeys (Seacat et al., 2002).

Cholesterol

80. For PFCAs, serum total cholesterol levels were measured in 22 of the 50 studies reviewed (three of the ten acute studies and 19 of the 37 repeated dose studies).

81. No effect on cholesterol was reported in the acute study in male rats following exposure to PFOA (Elcombe et al., 2010; Kawashima et al., 1995) or PFDA (Kawashima et al., 1995).

82. In the repeated dose studies, cholesterol was decreased in 11 of the 19 studies.

83. A decrease was reported in male rats following exposure to PFBA for 28 days (Butenhoff et al., 2012a), in male rats (Chengelis et al., 2009; NTP., 2022b) after exposure to PFHxA, in male rats following exposure to PFOA (Elcombe et al., 2010; NTP., 2022b; Qazi et al., 2010a), and in male rats PFNA and PFDA (NTP., 2022b). Takahashi et al. (2014) also reported decreases cholesterol in male and female rats following exposure to PFUnDA and Kato et al. (2014) noted decreases in male rats with PFDoDA.

84. In contrast, no effects were reported following exposure to PFBA for 90 days (Butenhoff et al., 2012a), PFHxA (Loveless et al., 2009), PFOA (Butenhoff et al., 2002; Butenhoff et al., 2012a), PFDoDA (Zhang et al., 2008), PFTeDA and PFHxDA (Hirata-Koizumi et al., 2015) and PFODA (Hirata-Koizumi et al., 2012).

85. Differences in response were seen between sexes following exposure to PFBA (Butenhoff et al., 2012a), PFHxA (Chengelis et al., 2009; NTP., 2022b), PFOA,

PFNA and PFDA (NTP., 2022b), and PFDoDA (Kato et al., 2014), where a decrease in cholesterol was only seen in male rats.

86. For PFSAs, serum total cholesterol levels were measured in 14 of the 25 studies reviewed (one acute study and 13 of the 25 repeated dose studies).

87. No effect on cholesterol was reported in the acute study in male or female Cynomolgus monkeys following exposure to PFOS (Chang et al., 2017).

88. In the repeated dose studies, cholesterol was decreased in seven of the 13 studies and increased in one study. NTP. (2022a) reported an increase in male rats following exposure to PFBS. In contrast, a decrease was observed in male mice (Bijland et al., 2011) and rats (Butenhoff et al., 2009; NTP., 2022a) following exposure to PFHxS, and in female rats (Butenhoff et al., 2012b), male rats (Elcombe et al., 2012; NTP., 2022a) and male and female Cynomolgus monkeys following exposure to PFOS (Seacat et al., 2002).

89. Plasma non-high-density lipoprotein (HDL) and HDL cholesterol were decreased in male mice following exposure to PFHxS and PFOS (Bijland et al., 2011).

90. In contrast, no effects were reported following exposure to PFBS (Bijland et al., 2011).

91. Sex differences were seen following exposure to PFBS, PFHxS and PFOS (NTP., 2022a) and PFOS (Butenhoff et al., 2012b) where an increase in cholesterol was seen in males with PFBS and a decrease was seen in males with PFHxS and PFOS.

Total protein

92. For PFCAs, serum TP levels were measured in 16 of the 50 studies reviewed (all of which were repeated dose studies.

93. In the repeated dose studies, TP was decreased in three of the 16 studies and increased in two studies.

94. A decrease was reported in male rats following exposure to PFBA for 90 days (Butenhoff et al., 2012a) and in male and female rats following exposure to PFNA and PFDA (NTP., 2022b), whereas PFOA increased TP in male mice (Guo et al., 2019) and male rats (NTP., 2022b).

95. In contrast, no effects were reported following exposure to PFBA for 28 days (Butenhoff et al., 2012a), PFHxA (Chengelis et al., 2009; Loveless et al., 2009; NTP., 2022b), PFOA (Butenhoff et al., 2002; Butenhoff et al., 2012a), PFUnDA (Takahashi et al., 2014), PFDoDA (Kato et al., 2014), PFTeDA and PFHxDA (Hirata-Koizumi et al., 2015) and PFODA (Hirata-Koizumi et al., 2012).

96. Differences in response were seen between sexes following exposure to PFBA (Butenhoff et al., 2012a), where a decrease in cholesterol was only seen in male rats.

97. For PFSAs, serum TP levels were measured in eight of the 25 studies reviewed (one acute study and seven of the 25 repeated dose studies).

98. No effect on TP was reported in the acute study in male or female Cynomolgus monkeys following exposure to PFOS (Chang et al., 2017).

99. In the repeated dose studies, TP was increased in two of the seven studies in male (NTP., 2022a) and female (NICNAS., 2005) rats following exposure to PFBS.

100. In contrast, no effects were reported following exposure to PFHxS (Butenhoff et al., 2009; Chang et al., 2018; NTP., 2022a) or PFOS (Butenhoff et al., 2012b; NTP., 2022a).

101. Differences in response were seen between sexes in both studies with PFBS as NTP. (2022a) reported an increase in TP in males but not females, but NICNAS. (2005) reported increases in females but not males.

Bilirubin

102. For PFCAs, serum bilirubin levels were measured in 12 of the 50 studies reviewed (all of which were repeated dose studies).

103. In the repeated dose studies, bilirubin was decreased in one of the 12 studies and increased in one study.

104. A decrease was reported in male and female rats following exposure to PFBA for 90 days (Butenhoff et al., 2012a) but increased in male and female Cynomolgus monkeys following exposure to PFOA (Butenhoff et al., 2002).

105. In contrast, no effects were reported following exposure to PFBA for 28 days (Butenhoff et al., 2012a), PFHxA (Chengelis et al., 2009; Loveless et al.,

2009; NTP., 2022b), PFOA (Butenhoff et al., 2012a; NTP., 2022b), PFNA and PFDA (NTP., 2022b), PFUnDA (Takahashi et al., 2014) and PFDoDA (Kato et al., 2014).

106. No differences in response were seen between sexes as effects were seen in both male and female rats with PFBA (Butenhoff et al., 2012a) and Cynomolgus monkeys with PFOA (Butenhoff et al., 2002).

107. For PFSAs, serum bilirubin levels were measured in 10 of the 25 studies reviewed (one acute study and nine of the 25 repeated dose studies).

108. No effect on bilirubin levels was reported in the acute study in male or female Cynomolgus monkeys following exposure to PFOS (Chang et al., 2017).

109. In the repeated dose studies, bilirubin was decreased in one of the 12 studies, in male Cynomolgus monkeys following exposure to PFOS (Seacat et al., 2002).

110. In contrast, no effects were reported following exposure to PFBS (Lieder et al., 2009a; NICNAS., 2005; NTP., 2022a), PFHxS (Butenhoff et al., 2009; Chang et al., 2018; NTP., 2022a) and PFOS (Butenhoff et al., 2012b; NTP., 2022a).

111. Differences in response were seen between sexes following exposure to PFOS where decreases in bilirubin were only seen in male Cynomolgus monkeys (Seacat et al., 2002).

Albumin

112. For PFCAs, serum albumin levels were measured in 17 of the 50 studies reviewed (all of which were repeated dose studies).

113. In the repeated dose studies, albumin was increased in one study in male mice following exposure to PFOA (Guo et al., 2019).

114. In contrast, no effects were reported following exposure to PFBA for 28 or 90 days (Butenhoff et al., 2012a), PFHxA (Chengelis et al., 2009; Loveless et al., 2009; NTP., 2022b), PFOA (Butenhoff et al., 2002; Butenhoff et al., 2012a; NTP., 2022b), PFNA and PFDA (Kennedy Jr, 1987; NTP., 2022b), PFDA (NTP., 2022b), PFUnDA (Takahashi et al., 2014), PFDoDA (Kato et al., 2014), PFTeDA and PFHxDA (Hirata-Koizumi et al., 2015) and PFODA (Hirata-Koizumi et al., 2012).

115. No studies that showed an increase in albumin were carried out in both male and female animals so sex differences could not be evaluated.

116. For PFSAs, serum albumin levels were measured in nine of the 25 studies reviewed (one acute study and eight of the 25 repeated dose studies).

117. No effect on albumin levels was reported in the acute study in male or female Cynomolgus monkeys following exposure to PFOS (Chang et al., 2017).

118. In the repeated dose studies, albumin was increased in one of the eight studies in which a decrease was reported in female rats following exposure to PFBS for 90 days (NICNAS., 2005).

119. In contrast, no effects were reported following exposure to PFBS (Lieder et al., 2009a; NTP., 2022a), PFHxS (Butenhoff et al., 2009; Chang et al., 2018; NTP., 2022a) and PFOS (Butenhoff et al., 2012b; NTP., 2022a).

120. Differences in response were seen between sexes following exposure to PFBS where increases in albumin were only seen in female mice (NICNAS., 2005).

Globulin

121. For PFCAs, serum globulin levels were measured in 9 of the 50 studies reviewed (all of which were repeated dose studies).

122. In the repeated dose studies, globulin was decreased in two of the nine studies and increased in two studies.

123. A decrease was reported in male and female rats following exposure to PFNA but only in males treated with PFDA (NTP., 2022b), but increased in male rats (NTP., 2022b) and male mice (Guo et al., 2019) following exposure to PFOA.

124. In contrast, no effects were reported following long term exposure to PFHxA (Chengelis et al., 2009; Loveless et al., 2009; NTP., 2022b), and PFOA (Butenhoff et al., 2002).

125. Differences in response were seen between sexes following exposure to PFOA and PFDA (NTP., 2022b), where the changes in globulin were only seen in male rats.

126. For PFSAs, serum globulin levels were measured in nine of the 25 studies reviewed (one acute study and eight of the 25 repeated dose studies).

127. No effect on globulin levels was reported in the acute study in male or female Cynomolgus monkeys following exposure to PFOS (Chang et al., 2017).

128. In the repeated dose studies, globulin was increased in one of the eight studies in which a decrease was reported in male rats following exposure to PFBS (NTP., 2022a).

129. In contrast, no effects were reported following exposure to PFBS (Lieder et al., 2009a; NICNAS., 2005), PFHxS (Butenhoff et al., 2009; Chang et al., 2018; NTP., 2022a) and PFOS (Butenhoff et al., 2012b; NTP., 2022a).

130. Differences in response were seen between sexes following exposure to PFBS where increases in albumin were only seen in male rats (NTP., 2022a).

Albumin/globulin ratio

131. For PFCAs, serum albumin/globulin ratio was measured in 11 of the 50 studies reviewed (all of which were repeated dose studies).

132. In the repeated dose studies, albumin/globulin ratio was decreased in three of the 11 studies and increased in two studies.

133. An increase was reported in male and female rats following exposure to PFDA (NTP., 2022b) and PFUnDA (Takahashi et al., 2014). In contrast, NTP. (2022b) reported an increase in male and female rats with PFNA and in male rats with PFOA, and Kato et al. (2014) noted an increase in female rats with PFDoDA.

134. In contrast, no effects were reported following long term exposure to PFHxA (Chengelis et al., 2009; Loveless et al., 2009; NTP., 2022b), PFTeDA and PFHxDA (Hirata-Koizumi et al., 2015) and PFODA (Hirata-Koizumi et al., 2012).

135. Differences in response were seen between sexes following exposure to PFOA (NTP., 2022), and PFDoDA (Kato et al., 2014), where increases in albumin/globulin ratio were only seen in male and female rats, respectively.

136. For PFSAs, serum albumin/globulin ratios were measured in eight of the 27 studies reviewed (one acute study and seven of the 25 repeated dose studies).

137. No effect on albumin/globulin ratio was reported in the acute study in male or female Cynomolgus monkeys following exposure to PFOS (Chang et al., 2017).

138. No effects were also reported in the repeat dose toxicity studies with PFBS (Lieder et al., 2009a; NICNAS., 2005; NTP., 2022a), PFHxS (Butenhoff et al., 2009; Chang et al., 2018; NTP., 2022a) and PFOS (NTP., 2022a).

Other endpoints

139. An increase in serum thiobarbituricacid (TBA), tumour necrosis factor (TNF)-α, interleukin (IL)-6, but not cholinesterase (CHE) was in male rats following exposure to PFOS (Han et al., 2018a; Han et al., 2018b).

140. PFOS decreased serum levels of SBA in male but not female Cynomolgus monkeys, decreased CK in males, increased CK in females and decreased sorbitol dehydrogenase (SDH) in male and females following exposure to PFOS (Seacat et al., 2002).

Recovery

141. Following the 28-day exposure to PFBA and a 3-week recovery period, cholesterol levels were comparable to controls in male rats although TP increased after the 3-week recovery period. No other parameters were measured after recovery. Similar results were seen following the 90-exposure where all parameters observed after treatment (TP, bilirubin) were comparable to controls after recovery (Butenhoff et al., 2012a).

142. Similar results were seen following the 7-day exposure to PFOS where all parameters observed after 1, 28 or 56 days after treatment were comparable to controls after 84 days (Elcombe et al., 2012). Seacat et al. (2002) also reported that changes to levels of cholesterol and histopathological changes seen at the end of exposure were comparable to controls after a one-year recovery period.

Liver histopathology

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143. For PFCAs, histopathology was carried out in 37 of the 50 studies reviewed (three of the ten acute studies, 33 of the 37 repeated dose studies and one of the three developmental studies).

144. In acute studies, no effects on liver histopathology were reported in male rats following exposure to PFOA (Elcombe et al., 2010) and in male mice with PFOA and PFNA (Das et al., 2017).

145. In the repeated dose studies, histopathological changes were seen in 14 of the 33 studies.

146. An increased incidence of hepatocellular hypertrophy was seen in male rats following exposure to PFBA for 90 days (Butenhoff et al., 2012a). PFOA caused hypertrophy of centrilobular hepatocytes in male mice (Botelho et al., 2015), hepatocellular hypertrophy and fatty vacuolation in male rats and hepatocellular hypertrophy and hepatocellular hyperplasia (Elcombe et al., 2010), hepatocellular hypertrophy in male mice (Guo et al., 2019), centrilobular hepatocellular hypertrophy, with elevated numbers of cytoplasmic acidophilic granules in male mice (Qazi et al., 2010a), mild lymphocytic infiltration around the central vein in male mice (Son et al., 2008), visible vacuoles around liver portal area in male mice (Wu et al., 2018) and deranged liver architecture, marked oedema, vacuolar degeneration, hepatocellular necrosis, and

inflammatory cell infiltration in male mice (Zou et al., 2015).

147. PFNA also caused hepatocytic cytoplasmic alterations in male and female rats and hepatocyte hypertrophy in males (NTP., 2022b). Takahashi et al. (2014) also reported hepatocyte centrilobular hypertrophy in male and female rats after exposure to PFUnDA, and Hirata-Koizumi et al. (2012) observed centrilobular liver hypertrophy and steatosis in males and females with PFTeDA, centrilobular liver hypertrophy in males and females with PFHxDA and centrilobular liver hypertrophy in males with PFODA.

148. Xu et al. (2022) also reported hepatocyte hypertrophy, disarrangement, cytoplasmic loss, nuclear migration, acidophil bodies and inflammatory cell infiltration in female mice in the developmental study with PFOA.

149. In contrast, no effects were seen on liver histopathology with PFBA following 28 days exposure (Butenhoff et al., 2012a), PFHxA (Chengelis et al., 2009; Loveless et al., 2009; NTP., 2022b), PFOA (Butenhoff et al., 2002; Butenhoff et al., 2012a; Li et al., 2019; NTP., 2022b; Soltani et al., 2023), PFNA (Fang et al., 2012; Hadrup et al., 2016), PFDA (Frawley et al., 2018; NTP., 2022b) and PFDoDA (Kato et al., 2014; Zhang et al., 2008).

150. Differences in response were seen between sexes following exposure to PFBA for 90 days (Butenhoff et al., 2012a), PPFA (NTP., 2022b) and PFODA (Hirata-Koizumi et al., 2012) where histopathological changes were only seen in males.

151. For PFSAs, histopathology was carried out in 17 of the 25 repeated dose studies reviewed and histopathological changes were seen in 11 of the 16 studies.

152. Following exposure to PFBS, Chen et al. (2022) reported increased apoptosis in male mice and Lieder et al. (2009b) observed hepatocellular hypertrophy in male rats.

153. Hepatocellular hypertrophy was also seen in male mice following exposure to PFHxS (Chang et al., 2018; Das et al., 2017) and with PFOS in male and female mice (Bagley et al., 2017), male rats (Butenhoff et al., 2012b; Elcombe et al., 2012; Han et al., 2018b), female rats (Kim et al., 2011; NTP., 2022a) and male and female Cynomolgus monkeys (Seacat et al., 2002).

154. Other histopathological effects observed following PFOS exposure included cytoplasmic alterations in males and necrosis in male and female mice

(Bagley et al., 2017), cystic hepatocellular degeneration in male rats and hepatocellular periportal vacuolation in females (Butenhoff et al., 2012b), lipid droplets, inflammation and apoptosis in male mice (Chen et al., 2022), apoptosis in male rats (Kim et al., 2011) and centrilobular vacuolation and lipid droplet accumulation in male and female Cynomolgus monkeys (Seacat et al., 2002).

155. In contrast, no effects were seen with PFBS (Lieder et al., 2009a; NICNAS., 2005; NTP., 2022a), PFHxS (He et al., 2022; NTP., 2022a) and PFOS (NTP., 2022a).

156. Differences in response were seen between sexes following exposure to PFBS (Lieder et al., 2009b) where hepatocellular hypertrophy was only seen in male rats.

Recovery

157. Following the 90-exposure to PFBA, all parameters observed after treatment (TP, bilirubin and histopathological changes) were comparable to controls after recovery (Butenhoff et al., 2012a).

158. Following the 90 day exposure to PFBS, the increased liver weight that was observed after treatment was comparable to controls after the recovery period (NICNAS., 2005). No data on liver weight were reported in Cynomolgus monkeys following exposure to PFOS for 182 days and a 90-day recovery period (Seacat et al., 2002).

Effects on gene expression

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159. For PFCAs, liver-related gene expression was assessed in 21 studies (five acute studies, 15 repeated dose studies, and one developmental study), 20 of which (ten in both mice and rats) reported changes in liver-related gene expression.

160. Following acute exposure, Cheng and Klaassen (2008) reported increased mRNA levels of cytochrome P450 (Cyp450) 2B10, 3A11 and 4A14 in liver of male mice treated with 40 mg/kg bw PFOA and 80 mg/kg bw PFDA. However, in the range-finding experiment, 10 mg/kg bw PFDA only increased Cyp4A14. Das et al. (2017) also reported changes in mRNA of genes relating to fatty acid, TG and cholesterol synthesis and omega oxidation in male mice.

161. For PFCAs, in the repeat dose studies, changes in mRNA expression were reported following exposure with PFBA, PFHxA, PFOA, PFNA, PFDA and PFDoDA.

162. Butenhoff et al. (2012a) reported an increase in mRNA of acyl-CoA oxidase 1 (Acox), uridine diphospho-glucuronosyl transferase (Ugt) 1A1 and CYP4A1 but a decrease in Cyp1A1, Ugt 1A6 and Ugt 2A in male rats following exposure to PFBA for 28 days. Similar results were reported after a 90-day exposure, with Acox, UGT1A1, CYP4A1, malic enzyme and cytochrome P450 oxidoreductase (Por) being increased and Cyp1A1 being decreased. No effects were seen in females. Foreman et al. (2009) reported an increase in Cyp4A10 and acyl-CoA oxidase (ACO) in male mice after PFBA exposure.

163. NTP. (2022b) reported increased expression of Acox1, Cyp4a1, Cyp2b1 and Cyp2b2 in male and Cyp2b1 and Cyp2b2 in female rats following PFHxA exposure.

164. Butenhoff et al. (2012a) reported an increase in mRNA of Acaca, Acox, Cyp4A1, Cyp2B2, Malic, Por, Fasn, Type 1 deiodinase, iodothyronine deiodinase type 1 (Dio1), Ugt 1A1, Ugt 1A6, Ugt 2B and Apolipoprotein (Apo) A1 in male rats following exposure to PFOA and an increase in Acox, Cyp3A1, Malic, Cyp7A1 in females.

165. Guruge et al. (2006) reported changes in the expression of genes involved in transport and metabolisms of fatty acids and lipids, cell communication, adhesion, growth, apoptosis, regulation of hormone, proteolysis and peptidolysis and signal transductionas well as apoptosis, regulation of hormone, metabolisms and G-protein coupled receptor protein signalling pathway in male rats.

166. Li et al. (2019) reported increased the expression of genes related to fatty acid metabolism (Cd36, Acox1, Sterol regulatory element-binding transcription factor 1 (Srebf1) and sterol regulatory element-binding transcription factor 2 (Srebf2), carnitine palmitoyltransferase 1A (Cpt-1A) and ApoB), Cyp2b10, Cyp3a11, Cyp4a10, constitutive androstane receptor (Car) and pregnane X receptor (Pxr), after 2 weeks, Cd36, Peroxisome proliferator-activated receptor (Ppar) -α, Ppar-γ, Cyp2b10, Cyp3a11, Car and Pxr after 8 weeks and Cd36, Fasn, Ppar-γ, Cyp2b10, Cyp3a11, Car and Pxr in male mice.

167. NTP. (2022) also demonstrated an increase in gene expression of Cyp4a1, Cyp2b1 and Cyp2b2 in male rats and Cyp2b1 and Cyp2b2 in females.

168. Son et al. (2008) showed that increased expression of mRNA for TNF-α, IL-1β and transforming growth factor (TGF) -β in the liver only occurred at 50-250 mg/kg bw/day PFOA in male mice. No changes were seen at the LOAEL.

169. PFNA was reported to decrease mRNA of Aldo-Keto Reductase 1C1 (AKR1C1), Ugt 2B15, Cyp2C11, Cyp1A2 and Cyp2B6 in male rats at the highest dose where toxicity was noted (Hadrup et al., 2016). In contrast, NTP. (2022b) reported increased gene expression of Acox1, Cyp4a1, Cyp2b1 and Cyp2b2 in males and female rats.

170. NTP. (2022b) also reported increased gene expression of Acox1, Cyp4a1, Cyp2b1 and Cyp2b2 in males following exposure to PFDA and Acox1, Cyp2b1 and Cyp2b2 in females.

171. Finally, Zhang et al. (2008) reported increased mRNA of PPAR-α/γ, Acox and CypA4 in male rats associated with PFDoDA exposure.

172. In the developmental study, Xu et al. (2022) reported an increase in mRNA of gene related to inflammation including toll-like receptor (Tlr)-4, Myeloid differentiation primary response 88 (Myd88), TNF receptor associated factor 6 (Traf6), IL1-β and Tnf-α following exposure to PFOA

173. For PFSAs, liver-related gene expression was assessed in 11 repeated dose studies, 10 of which (five in both mice and rats) reported changes in liver-related gene expression following exposure with PFBS, PFHxS and PFOS.

174. Bijland et al. (2011) observed changes in gene expression related to lipolysis, fatty acid uptake and transport, fatty acid binding and activation, fatty acid oxidation and very low density lipoprotein (VLDL) assembly in male mice following exposure to PFBS, PFHxS and PFOS.

175. NTP. (2022a) reported increased gene expression of Cyp4a1, Cyp2b1 and Cyp2b2 in males after PFBS, PFHxS and PFOS exposure whereas Cyp2b1, Cyp2b2 and Acox1expression was increased in female rats. Kim et al. (2011) also showed an increase in Cyp4A1 in male rats following exposure to PFOS.

176. Han et al. (2018b) saw increased expression of PCNA, c-Jun, c-MYC and CydD1 in male rats following PFOS exposure whereas a decrease in gene expression of APOA1, APOA2, PEPCK, G6PC was seen in male mice (Huck et al., 2018).

177. In contrast, no effects were seen in male and female rats following exposure to PFOS (Bagley et al., 2017).

Serum/plasma PFAS levels

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178. Levels of PFCAs in serum or plasma were measured in 11 repeated dose studies and in one developmental study and levels of PFSAs were measured in 13 repeated dose studies.

179. Levels of PFCAs and PFSAs in males were typically higher than their female counterparts at the same dose levels, suggesting a sex-specific difference in plasma concentrations for certain PFAS and that males and females respond differently to exposure. These results will be evaluated further in subsequent papers considering the toxicokinetics of PFAS.

Discussion

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180. Ten PFCAs (PFBA, PFHxA, PFOA, PFNA, PFDA, PFUnDA, PFDODA, PFTeDA, PFHxDA and PFPDA) and three PFSAs (PFBS, PFHxS and PFOS) are considered in this paper.

181. Table 1 and Table 2 below present the lowest point of departure (POD) based on liver effects. For PFHxA, PFOA, PFDA and PFHxS, only a LOAEL was determined, as effects were seen at the lowest dose tested.

182. Clinical chemistry parameters, liver weight and histopathological changes (hepatocellular hypertrophy) were the most sensitive endpoints on which the majority of the N/LOAELs have been determined. Changes in gene expression were also noted in some studies.

183. In repeated dose and developmental toxicity studies there were some changes in ALT, ALP, TP, bilirubin, albumin, globulin and albumin/globulin ratio and cholesterol, although for most parameters, there was a lack of consistency of the direction of effect across studies and with different PFAS. For example, PFOA and PFNA decreased serum TG male rats (NTP., 2022b) and in male mice (Qazi et al., 2010a) whereas Wu et al. (2018) reported an increase in male mice. However, the majority of studies did not show any effect on such parameters. Several studies show increases in AST, ALP or ALT at the LOAEL, which, in some cases, was accompanied by histopathological changes but overall, there were limited signs of overt hepatotoxicity observed.

184. The most prevalent effect observed as the decrease in serum cholesterol, which was observed in a number of studies, mainly in male rats after treatment with both PFCAs and PFSAs. Seacat et al. (2002) also noted that the decrease in serum cholesterol observed in Cynomolgus monkeys was the earliest reliable clinical response to PFOS.

185. An increase in liver weight was reported in multiple studies, which was generally accompanied by hepatocellular hypertrophy, due to the increased hepatic enzyme induction and hepatocellular function in response to the chemical exposure. In general, such effects appeared transient as they returned to control levels during a recovery period following cessation of treatment.

186. In rats and cynomolgus monkeys treated with PFBA and PFOA, respectively, Butenhoff et al. (2002 and 2012) noted that the increased liver weight did not appear to be a result of hepatocellular hyperplasia (no increase in nuclear DNA) and correlated it with increases in peroxisomes, endoplasmic reticulum, and mitochondria in both short term and chronic studies. Moreover, despite deriving a NOAEL of 6 mg/kg bw/day based on hepatocellular hypertrophy, increased liver weights, and slight clinical biochemistry changes at 30 mg/kg bw/day, Butenhoff et al. (2012a) noted that the NOAEL was conservative as such changes did not constitute clear functional or morphological deficits. NTP noted that increased absolute and relative liver weights were commonly seen with two or more PFCAs and PFSAs which correlated with histopathologic changes in the liver such as hepatocellular hypertrophy, and in some cases hepatocellular degeneration and necrosis, and that such changes often were seen at the lowest dose tested (NTP., 2022b). Other histological lesions in the liver varied across PFAS and between sexes.

187. Sex-specific differences were seen with effects on clinical chemistry parameters, with changes more frequently seen in male than in female animals at comparable doses. Similarly, histopathological changes or increased liver weight was more common in male animals.

188. Serum/plasma PFAS levels will be evaluated further in subsequent papers considering the toxicokinetics of PFAS.

189. It may be relevant to note the approach taken by two authoritative bodies, namely the ATSDR (ATSDR, 2021) and the United States Environmental Protection Agency (USEPA) (USEPA, 2023), in selecting liver effects as the basis for setting human health criteria values. These opinions will be explored in future papers.

190. Overall, the in vivo evidence indicates that doses of PFSAs and PFCAs can produce affect clinical chemistry parameters, can produce histopathological alterations in the liver and an increase in liver weight. However, some of these findings are inconsistent, and some endpoints appear to be sex-specific (with males being more sensitive than females).

191. The biological relevance and adverse nature of the most sensitive endpoints seen in the liver following PFSA exposure will be further explored in future papers.

Table 1 Lowest POD for PFAS based on liver effects - PFCAs

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*Derived by contractor; NA – not applicable.

Substance / reference / table	Sex, strain and species (Duration)	NOAEL/LOAEL mg/kg bw/day	Effect at LOAEL

			↑ absolute liver weight ↑ ALP ↓ TP ↓ bilirubin
PFBA Butenhoff et al. (2012a) Table 7	Male and female Sprague-Dawley rats, 90 days. Recovery group: 3 weeks.	Males: 6 / 30. Recovery 30 / NA*.	↑ hepatocellular hypertrophy ↑ mRNA of Acox, UGT1A1, CYP4A1, malic enzyme and Por ↓ mRNA for Cyp1A1 in liver. Recovery: Absolute liver weight, TP, bilirubin and hepatocellular hypertrophy comparable to controls.
PFHxA Loveless et al. (2009) Table 8	Male and female Crl:Cd Sprague-Dawley rats, 28 days. Recovery group: 30 and 90 days.	Males: NA / 20.	↑ ALT

PFOA Guo et al. (2019) Table 9	Male Balb/c mice, 28 days. Recovery not assessed.	Males: NA / 0.4.	↑ relative liver weight, ↑ TP ↑ albumin ↑ globulin ↑ hepatocellular hypertrophy.
PFNA Hadrup et al. (2016) Table 10	Male Wistar rats, 14 days. Recovery not assessed.	Males: 0.25 / 5.	↓ mRNA of AKR1C1, Ugt 2B15, Cyp2C11, Cyp1A2 and Cyp2B6.
PFDA Frawley et al. (2018) Table 11	Female Sprague-Dawley rats, 28 days. Recovery not assessed.	Females: NA / 0.125.	↑ relative liver weight.
PFUnDA Takahashi et al. (2014) Table 12	Crl:CD (SD) rats, 41-46 days, Recovery not assessed,	Males and females: 0.1 / 0.3,	Males ↑ relative liver weight ↓ total cholesterol ↑ albumin/globulin ratio ↑ hepatocyte centrilobular hypertrophy, Females: ↓ total cholesterol ↑ hepatocyte centrilobular hypertrophy,

PFDoDA Kato et al. (2014) Table 13	Male and female Crl:CD (SD) rats, 42 days (males), 41-46 days (females),	Males and females: 0.1 / 0.5,	Males ↑ relative liver weight ↑ ALP ↓ total cholesterol. Females: ↑ albumin/globulin ratio ↑ relative liver weight ↑ focal necrosis.
PFTeDA Hirata-Koizumi (2015) Table 14	Male and female Crl:CD (SD) rats, Males: 42 days beginning 14 days prior mating. Females: 14 days prior to mating, gestation and to PND5.	Males: 1 / 3.	↑ absolute liver weight ↑ relative liver weight ↑ centrilobular liver hypertrophy and steatosis.
PFHxDA Hirata-Koizumi (2015) Table 15	Male and female Crl:CD (SD) rats Males: 42 days beginning 14 days prior mating. Females: 14 days prior to mating, gestation and to PND5.	Males and females: 20 / 100.	↓ body weight ↑ absolute liver weight ↑ relative liver weight ↑ centrilobular liver hypertrophy. Females: ↑ centrilobular liver hypertrophy.

PFODA (Hirata-Koizumi et al., 2012) Table 16	Male and female Crl:CD (SD) rats Males: 42 days beginning 14 days prior mating. Females: 14 days prior to mating, gestation and to PND5.	Males and females: 40 / 200.	Males ↑ absolute liver weight ↑ relative liver weight ↑ centrilobular hypertrophy. Females ↑ relative liver weight.

Table 2 Lowest POD for PFAS based on liver effects - PFSAs

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*Derived by contractor; NA – not applicable.

Substance / reference / table	**Sex, strain and species** **(Duration)**	**NOAEL/LOAEL mg/kg bw/day**	**Effect at LOAEL**
PFBS Chen et al. (2022) Table 17	Male C57BL/6 mice, 28 days.	Males: 2 / 104.	↑ apoptosis ↓ CAT activity Changes in hepatic lipidome.
PFHxS Butenhoff et al. (2009) Table 18 Chang et al. (2018) Table 18	Male Crl:CD®(SD) IGS BR VAF/Plus® 42 days. Male Crl:CD1 mice 42 days.	Males: NA / 0.3.	↓ serum cholesterol, ↑ centrilobular hypertrophy.

			Male ↑ hepatocellular centrilobular hypertrophy
PFOS Butenhoff et al. (2012b) Table 19	Male and female Crl:CD®(SD) IGS BR rats, 104 weeks.	Males/females: 0.024 / 0.098.	↑ cystic hepatocellular Degeneration. Female ↑ hepatocellular periportal vacuolation ↓ serum total cholesterol.

Questions on which the views of the Committee are sought

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192. Members are invited to consider the following questions:

i). Are there any specific papers that the subgroup would like to review in more detail?

ii). Recovery is assessed in a minority of studies. Should the N/LOAEL be based on effects seen at the end of treatment or after the recovery period?

iii). Liver effects seen in developmental studies are presented for dams and offspring. The N/LOAELs are based on effects in the dam only. Does the subgroup agree with excluding effects seen in offspring, which will be reported in subsequent papers?

IEH Consulting under contract supporting the UKHSA COT Secretariat

December 2023

Per- and polyfluoroalkyl substances: evaluation of liver effects using in vivo data - PFAS/2023/06

List of Abbreviations

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ACO	Acyl-CoA oxidase
Acox1	Acyl-CoA oxidase 1
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AKR1C1	Aldo-Keto Reductase 1C1
Apo	Apolipoprotein
APOA	Apolipoprotein A
AST	Aspartate aminotransferase
CAR	Constitutive androstane receptor
CAS	Chemical abstracts service
CAT	Catalase

Cd36	Cluster of differentiation molecule 36
CHE	Cholinesterase
COT	Committee on Toxicity
Cpt-1A	Carnitine palmitoyltransferase 1A
Cyp	Cytochrome P450
DIO1	Type 1 deiodinase, iodothyronine deiodinase type 1
EFSA	European Food Safety Authority
FA	Fatty acid
FDA	Food and Drug Administration
Fasn	Fatty acid synthase
G6PC	Glucose 6-phosphatase enzyme
GD	Gestational day
GL	Guideline
GLP	Good laboratory practice
HBGV	Health-based guidance value
HDL	High density lipoprotein

IL-1β	Interleukin-1β
i.p.	Intraperitoneal
LOAEL	Lowest observed adverse effect level
mRNA	Messenger ribonucleic acid
Myd88	Myeloid differentiation primary response 88
NA	Not applicable
NAM	New approach methodology
ND	Not detected
NOAEL	No observed adverse effect level
NR	Not reported
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCNA	Proliferating cell nuclear antigen
PEPCK	Phosphoenolpyruvate carboxykinase
PFAS	Per- and polyfluoroalkyl substances
PFBA	Perfluorobutanoate / Perfluorobutanoic acid

PFCA	Perfluoroalkyl carboxylic acid
PFDA	Perfluorodecanoate / Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFHxA	Perfluorohexanoate / Perfluorohexanoic acid
PFHxDA	Perfluorohexadecanoic acid
PFNA	Perfluorononanoate / Perfluorononanoic acid
PFOA	Perfluorooctanoate / Perfluorooctanoic acid
PFODA	Perfluorooctadecanoic acid
PFTeDA	Perfluorotetradecanoate / Perfluorotetradecanoic acid
PFUnDA	Perfluoroundecanoic acid
PND	Postnatal day
POD	Point of departure
Por	Cytochrome P450 oxidoreductase
PPARα	Peroxisome proliferator-activated receptor-α
PXR	Pregnane X receptor
QA	Quality assurance

SD	Standard deviation
SDH	Sorbitol dehydrogenase
Srebf1/2	Sterol regulatory element-binding transcription factor 1/2
TG	Triglycerides
TGF-β	transforming growth factor-β
TBA	Thiobarbituricacid
TLR-4	Toll-like receptor-4
TP	Total protein
TNF-α	Tumour necrosis factor
Traf6	TNF receptor associated factor 6
UGT	Uridine diphospho-glucuronosyl transferase
VLDL	Very low-density lipoprotein

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