Summary of results - PFAS/2023/04

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20. Exposure to PFAS caused a number of thyroid effects in animals, including effects on TH levels (principally triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH)), effects on thyroid histopathology and thyroid weight, and impacts on gene transcription and associated processes in the thyroid and other tissues.

Thyroid hormone levels

FT4

21. FT4 levels were measured in three of the eight acute studies, 14 of the 30 repeated dose studies, and eight of the 16 developmental studies.

22. In the acute studies, an increase in FT4 was seen in female rats following exposure to PFOS, although recovery was seen 24 hours after treatment (study 1 in Chang *et al*. (2008)). The authors concluded that the increase was transient and due to the ability of PFOS to compete with T4 for binding proteins. In contrast, no changes were seen in two acute studies in male and female

Cynomolgus monkeys following exposure to PFOS (Chang *et al*., 2017).

23. In the repeated dose studies, FT4 was decreased in 10 of the 14 studies following exposure to PFBS (NTP, 2022b), PFHxS (NTP, 2022b), PFOS (NTP, 2022b; Thibodeaux et al., 2003) and PFBA (in the 28 day study by Butenhoff *et al*. (2012a)), PFHxA (NTP, 2022a), PFOA (Butenhoff *et al*., 2012a; NTP, 2022a)), PFNA (NTP, 2022a) and PFDA (NTP (2022a). All of these studies were conducted in rats.

24. In contrast, no effects were reported following long term exposure to PFOS in male and female monkeys (Seacat *et al*., 2002) and male rats (Yu *et al*., 2009a), in male monkeys following exposure to PFOA (Butenhoff *et al*., 2002), and in male and female rats following exposure to PFBA (in the 90 day study by Butenhoff *et al*. (2012a)).

25. Eleven out of the 14 repeated dose studies included males and females. Sex differences were seen in five studies, all of which were in rats, where decreases in FT4 were only seen in males following exposure to PFHxS (NTP, 2022b), PFBA (in the 28 day study by Butenhoff *et al*. (2012a)), PFHxA (NTP, 2022a), PFOA (NTP, 2022a) and PFDA (NTP, 2022a). No studies reported effects only in female animals.

26. No changes in FT4 were seen in developmental studies in either mice or rats.

Total T4

27. Total T4 (TT4) levels were measured in seven of the eight acute studies, 18 of the 30 repeated dose studies, and 12 of the 16 developmental studies.

28. In the acute studies, an increase in TT4 was seen in female mice following exposure to PFDA (Harris *et al*., 1989). Decreases were seen with PFDA in male rats (Langley & Pilcher, 1985; Van Rafelghem *et al*., 1987). Decreases were seen in three studies with PFOS, two studies in male and female rats both reported in Chang et al. (2008), and in male and female Cynomolgus monkeys (group 3 in Chang *et al*. (2017)). In contrast, no effects were reported in male and female Cynomolgus monkeys following exposure to a single dose of 9 mg/kg bw/day PFOS (group 2 in Chang *et al*. (2017)), but a decrease was seen following treatment to variable doses on three different occasions (13.3 and 14 mg/kg bw/day, male and female respectively) (group 2 in Chang *et al*. (2017)).

29. Three acute studies (Chang *et al*., 2017; Chang *et al*., 2008; Langley & Pilcher, 1985) included recovery groups or a recovery period for the treated

animals, but no recovery of effects on TT4 was seen in any of the studies.

30. TT4 was decreased in 16 of the 18 repeated dose studies following exposure to PFBS (NTP, 2022b), PFHxS (NTP, 2022b), PFOS (Chang et al., 2008; Curran *et al*., 2008; NTP, 2022b; Thibodeaux *et al*., 2003; Yu *et al*., 2009a; Yu *et al*., 2011)), PFBA (in the 28- and 90-day studies by Butenhoff *et al*. (2012a)), PFHxA (NTP, 2022a), PFOA (Butenhoff *et al*., 2002; Butenhoff *et al*., 2012a; NTP, 2022a), PFNA (NTP, 2022a) and PFDA (NTP, 2022a). With the exception of a single study in Cynomolgus monkeys (PFOA, Butenhoff *et al*., 2002) all studies were carried out in rats.

31. In contrast, no effects on TT4 were reported in male and female *Cynomolgus* monkeys following exposure to PFOS (Seacat *et al*., 2002). In addition no effects on TT4 were seen in male and female rats following exposure to PFTeDA (Hirata-Koizumi, 2015).

32. Thirteen out of the 18 repeated dose studies included males and females. Sex differences were seen in five studies, all of which were in rats. Decreases in FT4 were seen only in males following exposure to PFBA in the 28- and 90-day studies by Butenhoff *et al.* (2012a), and to PFHxA (NTP, 2022a), PFOA (NTP, 2022a), and PFDA (NTP, 2022a). In no studies were effects seen only in female animals.

33. In developmental studies in mice, a decrease in TT4 was seen following exposure to PFBS (Feng *et al*., 2017). Conflicting results were reported for PFOS, as a decrease in TT4 was reported by Thibodeaux **et al**. (2003), but not Fuentes *et al*. (2006). No mouse developmental studies were conducted on PFCAs.

34. In developmental studies in rats, a decrease in TT4 was seen following exposure to PFHxS (Gilbert *et al*., 2021; Ramhøj *et al*., 2018) and the two studies by Ramhøj *et al*. (2018), to PFOS (Conley et al., 2022; Luebker *et al*., 2005; Thibodeaux *et al*., 2003; Wang *et al*., 2011)) and PFOA (Conley *et al*., 2022).

Free T3

35. Free T3 (FT3) was measured in one of the eight acute studies, two of the 30 repeated dose studies, and three of the 16 developmental studies.

36. In the acute study, no effect was reported following exposure to PFOS (Chang *et al*., 2008).

37. In the two repeated dose studies, FT3 was decreased in male and female *Cynomolgus* monkeys following exposure to PFOS (Seacat *et al*., 2002) but not in male *Cynomolgus* monkeys (females were not studied) following exposure to PFOA (Butenhoff *et al*., 2002).

38. In the developmental studies, PFOS exposure had no effect on FT3 levels in mice (Fuentes *et al*., 2006) or rats (Conley *et al*., 2022; Luebker *et al*., 2005), whereas PFOA decreased FT3 levels in rats (Conley *et al*., 2022) No mouse studies are available on PFOA, or other PFCAs.

Total T3

39. Total T3 (TT3) levels were measured in six of the eight acute studies, 15 of the 30 repeated dose studies, and nine of the 16 developmental studies.

40. In the acute studies, a decrease was seen in male rats following exposure to PFDA (Langley & Pilcher, 1985), with levels returning to be comparable to recovery group controls from study day four. No effect was seen following exposure to PFDA in female mice (Harris *et al*., 1989) and male and female rats (Van Rafelghem *et al*., 1987). No effect was also reported following exposure to PFOS in female rats (Chang *et al.*, 2008) and two studies with male and female Cynomolgus monkeys (Chang *et al*., 2017).

41. In repeated dose studies, TT3 was decreased in eight of the 15 studies following exposure to PFBS (NTP, 2022b), PFHxS (NTP, 2022b), PFOS (Chang *et al* ., 2008; Seacat *et al*., 2002; Thibodeaux et al., 2003), PFHxA (NTP, 2022a), PFOA (NTP, 2022a) and PFHxDA (Hirata-Koizumi, 2015). With the exception of the study in Cynomolgus monkeys by Seacat *et al*. (2002), all of the studies were in rats.

42. In contrast, no effects were reported following exposure to PFOS (Curran *et al*., 2008; NTP, 2022b; Yu *et al*., 2009a; Yu *et al*., 2011), PFOA (Butenhoff et al., 2002), PFNA (NTP, 2022a) and PFDA (NTP, 2022a). With the exception of the study in Cynomolgus monkeys by Butenhoff *et al*. (2002), all of the studies were in rats.

43. Ten out of the 15 repeated dose studies included males and females. Sex differences were seen in four studies, all of which were in rats. Decreases in TT3 were seen only in males following exposure to PFHxS (NTP, 2022b) and PFCAs (PFHxA (NTP, 2022a) and PFOA (NTP, 2022a)), and only in females following exposure to PFHxDA (Hirata-Koizumi, 2015).

44. In developmental studies in mice, no effect was reported on TT3 following exposure to PFOS (Fuentes *et al*., 2006; Thibodeaux *et al*., 2003), and a decrease was seen following exposure to PFBS (Feng *et al*., 2017). No mouse developmental studies were conducted on PFCAs.

45. In developmental studies in rats, PFOS exerted no effects (Conley *et al*., 2022; Luebker *et al*., 2005), but a decrease was seen with PFHxS (Gilbert *et al*., 2021; Ramhøj *et al*., 2020), PFOS (Thibodeaux *et al*., 2003) and PFOA (Conley *et al*., 2022).

TSH

46. TSH levels were measured in three of eight acute studies, 16 of the 30 repeated dose studies, and seven of the 16 developmental studies.

47. In the acute studies, a decrease was seen in female rats following exposure to PFOS (study 1 in Chang et al. (2008)), but no effect was seen in male and female Cynomolgus monkeys (Chang *et al*., 2017). TSH levels were comparable to controls in the study by Chang et al. (2008) at 24 hours post-treatment.

48. In repeated dose studies, TSH was decreased following exposure to PFOA (Butenhoff *et al*., 2012a), but increased in the study by NTP (2022a). Both studies were in rats, with the decrease seen in only in male rats and the increase seen only in female rats. Increases in TSH were also seen with PFOS in Cynomolgus monkeys and rats respectively (Seacat *et al*., 2002; Thibodeaux *et al*., 2003). No effects were seen with PFBS (NTP, 2022b), PFHxS (NTP, 2022b), PFOS (Chang *et al* ., 2008; NTP, 2022b; Yu *et al.*, 2009a), PFBA (in the 28 and 90 days studies by Butenhoff *et al*. (2012a)), PFHxA (NTP, 2022a), PFOA (Butenhoff *et al*., 2002), PFNA (NTP, 2022a), PFDA (NTP, 2022a) and PFTeDA (Hirata-Koizumi, 2015).

49. Twelve out of the 16 repeated dose studies included males and females. Sex differences were seen in two studies, both of which were in rats following exposure to PFOA. Decreases in TSH were seen only in male rats (Butenhoff *et al*., 2012a) and only in female rats (NTP, 2022a).

50. In developmental studies in mice, an increase in TSH was seen following treatment with PFBS (Feng *et al*., 2017), but no effect was reported with PFOS (Thibodeaux et al., 2003). No mouse developmental studies were conducted on PFCAs.

51. In developmental studies in rats, no effect on TSH was reported with PFHxS (Gilbert *et al*., 2021; Ramhøj *et al*., 2018) or PFOS (Chang *et al*., 2009; Luebker *et*

al., 2005; Thibodeaux *et al*., 2003).

Recovery

52. In repeated dose studies, recovery was assessed in five studies, one of which was with a PFSA and four were with PFCAs.

53. Following the 182-day exposure to PFOS, THs that showed differences to controls in male and female Cynomolgus monkeys at 0.75 mg/kg bw/day PFOS (decreased TT3 and increased TSH in both sexes, decreased TT4 in males, and decreased FT3 in females) at the end of treatment were comparable to recovery group controls between days 33 to 61 in both sexes (Seacat *et al*., 2002).

54. Following the 28-day exposure to PFBA, THs that were decreased in male rats (TT4 and FT4) at 6 mg/kg bw/day PFBA were comparable to recovery group controls after a three week recovery period (Butenhoff *et al*., 2012a). However, at the highest dose tested (150 mg/kg bw/day) the observed decrease in TT4 did not show recovery.

55. Following the 90-day exposure to PFBA, the decreased TT4 seen in males at the end of treatment was subsequently increased relative to recovery group controls after the three-week recovery period (Butenhoff *et al*., 2012a).

56. Following the 28-day exposure to PFOA, THs that were decreased in female rats (TT4, FT4) at 30 mg/kg bw/day were comparable to recovery group controls after the 3-week recovery period (Butenhoff *et al*., 2012a). However, in male rats, of the THs that were decreased (TSH, TT4, FT4) at 30 mg/kg bw/day PFOA, both TT4 and FT4 remained significantly lower than recovery group controls whereas TSH returned to levels comparable to recovery group controls.

57. Following the 42-day exposure to PFHxDA, TH that were decreased in female rats (TT3) at 4 mg/kg bw/day were comparable to recovery group controls after the 14-day recovery period (Hirata-Koizumi, 2015). It should be noted that in the repeated dose part of this OECD 422 study, recovery group females were unmated and are therefore not directly comparable to treated females that were exposed for the same duration but through mating, gestation and to PND5. No effect on THs was seen in males at the end of the 42-day exposure up to the highest dose tested of 100 mg/kg bw/day, but a decrease in TT4 was seen at the end of the 14-day recovery period at this dose.

Relationship between T4, T3 and TSH

58. Overall, in repeated dose studies, consistent decreases in TH levels were observed with PFSAs (PFBS, PFHxS and PFOS) and PFCAs (PFBA, PFDA, PFOA and PFNA), mainly FT4, TT4, TT3. These reductions were not associated with compensatory increases in TSH in 19 studies (two acute studies, 12 repeated dose studies and five developmental studies). The exceptions to this, where increases in TSH were seen, were following exposure to PFOS in male and female Cynomologus monkeys (Seacat *et al*., 2002) and in female rats (Thibodeaux *et al*., 2003), to PFOA in female rats (NTP, 2022a) and to PFBS in pregnant mice (Feng et al., 2017). The results are therefore not generally indicative of a classical induced hypothyroid state, where decreased T4 and T3 levels would be associated with increased TSH. This is highlighted by NTP where the authors state that the reason for a lack of TSH response when a decrease in TH concentrations is seen is not clear, and is not consistent with a disruption in the hypothalamic-pituitary-thyroid axis (NTP, 2022a, 2022b).

59. Rats are uniquely sensitive to TH perturbation in association with induction of liver enzymes (Capen, 1997 cited in Loveless *et al*. (2009)). Circulating T3 and T4 bind to albumins in all species, but bind to globulins (thyroid-binding globulin (TBG)) with high affinity in primates and humans, which leads to an approximately 10-fold shorter half-life of THs in plasma in rodents compared to primates and humans, leading to a more rapid turnover of THs and potentially different effects arising from changes in TH levels in rodents and primates or humans (Alison *et al*., 1994), Consequently, maintaining homeostasis will be different between rodents and primates, including humans (Alison *et al*., 1994).

Thyroid histopathology

60. Histopathology was carried out in 25 of the 50 studies reviewed (two acute studies, 21 repeated dose studies, and two developmental studies).

61. In acute studies, no effects on thyroid histopathology were reported in male rats following exposure to PFOS (Elcombe *et al*., 2012a) or PFDA (Van Rafelghem *et al*., 1987).

62. Notable changes in thyroid histopathology were only identified in four out of 21 repeated dose studies.

63. An increased incidence of hypertrophy and hyperplasia of follicular epithelial cells was seen in male rats following exposure to PFHxS (Butenhoff *et al*., 2009a), PFBA (in the 90-day study by Butenhoff *et al*. (2012a)) and PFOA (Butenhoff *et al*., 2012a). However, no effect was reported in female rats treated with either PFBA (in the 90-day study by Butenhoff *et al*. (2012a)) and PFOA (Butenhoff *et al*., 2012a). An increased incidence of thyroid follicular epithelial hypertrophy was seen in male and female rats with PFHxA (Loveless *et al*., 2009).

64. No effects on thyroid histopathology were reported in 17 out of 21 studies, namely in seven studies conducted with PFSAs (PFBS (NTP, 2022b), PFHxS (NTP, 2022b) and PFOS (Butenhoff *et al*., 2012b; Curran *et al*., 2008; Elcombe *et al*., 2012a; Elcombe *et al*., 2012b; NTP, 2022b), and in 10 studies with PFCAs (PFBA in the 28-day study by Butenhoff *et al*. (2012a), PFHxA (NTP, 2022a), PFOA (Butenhoff *et al*., 2002; Butenhoff *et al*., 2012b; Griffith & Long, 1980; NTP, 2022a), PFNA (NTP, 2022a), PFDA (NTP, 2022a), PFHxDA (Hirata-Koizumi, 2015) and PFTeDA (Hirata-Koizumi, 2015)). Moreover, no changes were seen in dams in the two developmental studies on PFHxS (Ramhøj *et al*., 2020) and PFOS (Chang et al., 2009). With the exception of the study on PFOA by Butenhoff *et al*. (2002) in Cynomolgus monkeys, all studies were in rats.

65. Seventeen out of the 21 repeated dose studies included males and females. In the three studies in rats where an effect on thyroid histopathology was seen, sex differences were seen in two studies. An effect was seen only in males following exposure to PFBA in the 90-day study by Butenhoff *et al*. (2012a) and following exposure to PFOA in the 28-day study by Butenhoff *et al*. (2012a). In contrast, effects were seen in both sexes following exposure to PFHxA in the 90-day study by Loveless *et al*. (2009).

Recovery

66. Recovery was assessed in three of the four repeated dose studies where effects on thyroid histopathology were seen, all of which were with PFCAs.

67. For PFBA, the increased incidence of follicular hypertrophy/hyperplasia seen in male rats was comparable to recovery group controls after a 3-week recovery period (Butenhoff *et al*., 2012a). For PFHxA, the increased incidence of thyroid follicular epithelial hypertrophy seen in male and female rats did not show evidence of recovery on day 30. However, at the end of the recovery period on day 90, in female rats the incidence was comparable to controls. No recovery was seen in male rats (Loveless *et al*., 2009). For PFOA, the increased thyroid follicular epithelial cell height in male rats was comparable to controls following a 3-week

recovery period, but the increased incidence of thyroid follicular hypertrophy/hyperplasia did not show evidence of recovery (Butenhoff *et al*., 2012a).

Thyroid weight

68. Thyroid weight was measured in 17 of the 50 studies reviewed (one acute study, 15 repeated dose studies, and one developmental study).

69. In the acute study, no effect was reported on thyroid weight in male rats treated with PFDA (Van Rafelghem et al., 1987).

70. In the repeated dose studies, an increase in thyroid weight was seen in only four of 15 repeated dose studies, all with PFCAs.

71. An increase in absolute thyroid weight was seen in male, but not female, rats treated with PFBA (Butenhoff *et al*., 2012a) and PFHxDA (Hirata-Koizumi, 2015), and conversely in female, but not male, rats treated with PFDA (NTP, 2022a).

72. No effects were seen on thyroid weight with PFBS (NTP, 2022b), PFHxS (NTP, 2022b), PFOS (Butenhoff *et al.*, 2012b; Curran *et al*., 2008; NTP, 2022b; Seacat *et al*., 2002), PFHxA (NTP, 2022a), PFOA (Griffith & Long, 1980; NTP, 2022a), PFNA (NTP, 2022a) and PFTeDA (Hirata-Koizumi, 2015). With the exception of the study on PFOS in Cynomolgus monkeys (Seacat *et al*., 2002), all studies were carried out in rats.

73. No changes were seen in the developmental study in rats with PFHxS (Ramhøj *et al*., 2020).

74. All of the 15 repeated dose studies included males and females. In the four studies in rats where an effect on thyroid weight was seen, there appears to be no clear sex-specific effect following exposure to PFBA (in the 28-day study by Butenhoff *et al*. (2012a)), PFHxA (Loveless *et al*., 2009), PFDA (NTP, 2022a) and PFHxDA (Hirata-Koizumi, 2015).

Recovery

75. Recovery was assessed in three of the four repeated dose studies where effects on thyroid weight were seen, all of which were with PFCAs.

76. For PFBA, the increase in absolute thyroid weight seen in male rats was comparable to controls following the 3-week recovery period (Butenhoff *et al*., 2012a). For PFHxA, a delayed increase in thyroid weight (relative or absolute not specified) was seen in female rats, as no effects were seen at the end of treatment but a transient increase was seen during the recovery period (at 30 days) which had returned to control levels after 90 days (Loveless *et al*., 2009). Although no effect on thyroid weight was seen following treatment, the authors concluded that the increased weight observed during the recovery period was adverse and treatment related. For PFHxDA, the increase in relative thyroid weight seen in male rats was comparable to controls following the 14-day recovery period (Hirata-Koizumi, 2015).

Effects on gene expression

77. Thyroid-related gene expression was assessed in six studies (three repeated dose studies, and three developmental studies). Four studies (one in mice and three in rats) reported changes in thyroid-related gene expression. All studies were on PFSAs.

78. Three studies with PFOS reported effects in the liver (hepatic malic enzyme (ME), which responds to changes in THs, mRNA levels relating to hepatic T4 glucuronidation, and proteins associated with the hepatic uptake of T4) (Chang *et al*., 2008; Yu *et al*., 2009a; Yu *et al.*, 2011), and one study with PFBS showed a transcriptional effect in rat hypothalamus (Feng *et al*., 2017).

79. Studies with PFHxS and PFOS (Chang *et al*., 2009; Ramhøj *et al*., 2020) reported no effect on gene expression at any dose tested.

Serum/plasma PFAS levels

80. Levels of PFAS in serum or plasma were measured in three acute studies with PFSAs, 10 repeated dose studies with PFSAs and seven with PFCAs, and in six developmental studies with PFSAs and one with PFCAs.

81. Levels of both PFSAs and PFCAs 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.

82. These results will be evaluated further in subsequent papers considering the toxicokinetics of PFAS.