PFSAs

In this guide

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PFBS

In vivo toxicity data

Feng *et al*. 2017

8. Feng *et al*. (2017) investigated the effects of perfluorobutanesulfonate (PFBS) on levels of thyroid hormones (TH) in pregnant mice and their female offspring. As part of a developmental study, pregnant ICR mice were administered daily doses of PFBS potassium salt (K^+PFBS) at doses 0, 50, 200, and 500 mg/kg bw/day by gavage from gestation day 1 (GD1) to GD20. Upon delivery offspring were housed with dams until weaning on postnatal day 21 (PND21).

9. Dams were split into two experimental groups examining thyroid effects. In the first experiment, TH levels and relative mRNA expression were analysed using orbital blood collected from dams on GD20 and female offspring on either PND1, PND30 or PND60. Hypothalami were removed from female offspring on PND1, PND30 or PND60.

10. Total thyroxine (TT4), total triiodothyronine (TT3) and thyroid stimulating hormone (TSH) in serum were measured in dams (10/group) and 50 offspring: PND1 (30/group), PND30 (10/group) and PND60 (10/group). Free thyroxine (FT4) was also measured in dams (10/group). Gene expression for Trh (thyrotropin-releasing hormone) mRNA was determined from hypothalamus samples.

11. In the second experiment, serum PFBS levels were measured in orbital blood collected from dams (10/group) on GD20.

12. Mortality: No deaths were observed in offspring. No data were provided on maternal survival.

13. General toxicity and body weight: No clinical signs of general toxicity in offspring were observed. No data regarding dams were reported. Female offspring body weight was significantly decreased at 200 and 500 mg/kg bw/day from PND1 to PND60, compared with controls. No data regarding male offspring were provided. Terminal body weight and body weight gain in dams was unaffected by treatment.

14. Thyroid hormone levels: In offspring, TT3 and TT4 levels were significantly decreased at 200 and 500 mg/kg bw/day on PND1, PND30 and PND60. TSH levels were significantly elevated at 200 and 500 mg/kg bw/day on PND30. In dams, TT4, TT3 and FT4 levels were significantly reduced, and TSH levels were significantly increased at 200 and 500 mg/kg bw/day groups. Hypothalamic gene expression: In offspring, Trh mRNA levels in were significantly elevated at 200 and 500 mg/kg bw/day on PND30.

15. Serum PFBS concentrations: PFBS treatment on GD1 – 20 led to a dose-dependent increase in serum PFBS concentration in dams. PFBS concentrations measured on GD20 were 1.73 ng/mL (control), 74.0 ng/mL (50 mg/kg bw/day), 332 ng/mL (200 mg/kg bw/day) and 721 ng/mL (500 mg/kg bw/day).

16. The authors concluded that prenatal exposure to PFBS ≥ 200 mg/kg bw/day causes permanent hypothyroxinemia in female mice.

NTP, 2022b

17. NTP (2022b) investigated the effects of PFBS on thyroid weight, histopathology and TH levels in rats. In a repeated dose study, SD rats (10/sex/group) were administered PFBS at doses 0, 62.6, 125, 250, 500 or 1000

mg/kg bw/day with half the dose being administered twice daily by gavage for 28 days. At necropsy on day 29, blood samples were collected for TT4, TT3, FT4, TSH and PFBS analysis, and thyroids were removed for histopathological evaluation.

18. Mortality: In males, all ten rats died at 1000 mg/kg bw/day before scheduled necropsy (one from a dosing accident). In females, eight rats died at 1000 mg/kg bw/day, one rat at 500 mg/kg bw/day and one rat at 250 mg/kg bw/day. With the exception of the dosing accident, the observed mortality was attributed to treatment.

19. General toxicity and body weight: Seizures were noted in one male rat at 1000 mg/kg bw/day. In females, seizures were noted in one rat at 1000 mg/kg bw/day, two rats at 500 mg/kg bw/day and one rat at 250 mg/kg bw/day. In females at 1000 mg/kg bw/day, lethargy was reported in one rat, ruffled hair was reported in two rats, and two were reported to be thin. In males, terminal body weights were unaffected by treatment, compared with controls. In females, terminal body weights were significantly reduced at 500 mg/kg bw/day.

20. Gross pathology: Thyroid weights in males and females were unaffected by treatment.

21. Histopathology: Histopathology in males and females was unaffected by treatment.

22. Thyroid hormone levels: In males and females, TT4, FT4 and TT3 levels were significantly decreased at ≥ 62.6 mg/kg bw/day in a dose-response manner, compared with controls. TSH levels were unaffected by treatment.

23. Plasma PFBS concentrations: In males, mean plasma PFBS concentrations on day 29 were 0.090 µg/mL (control), 2.222 µg/mL (62.6 mg/kg bw/day), 5.366 µg/mL (125 mg/kg bw/day), 12.430 µg/mL (250 mg/kg bw/day) and 43.160 µg/mL (500 mg/kg bw/day). There were no data for 1000 mg/kg bw/day due to mortality. In females, mean plasma PFBS concentrations on day 29 were ND (control), 0.154 µg/mL (62.6 mg/kg bw/day), 0.309 µg/mL (125 mg/kg bw/day), 0.931 µg/mL (250 mg/kg bw/day), 8.171 µg/mL (500 mg/kg bw/day) and 25.455 µg/mL (1000 mg/kg bw/day).

24. The authors concluded that TT4, FT4 and TT3 decreased in a dose-response manner. TSH was unaffected, nor were there any histopathologic changes in the thyroid gland (hyperplasia/hypertrophy).

PFHxS

In vivo toxicity data

Butenhoff *et al.* (2009)

25. Butenhoff *et al*. (2009) investigated the effect of potassium perfluorohexanesulfonate (K^+PFHxS) on thyroid histopathology. In a modified OECD Test Guideline 422 study (Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening), adult SD rats (18/sex/group) were administered K^+PFHxS daily by gavage at doses 0, 0.3, 1, 3 or 10 mg/kg bw/day. Adult males were dosed for 14 days prior to cohabitation until day 43. Adult females were dosed for 14 days prior to cohabitation until PND21, or presumed GD25 for rats that did not deliver a litter. Offspring were not dosed directly but were exposed in utero or potentially via milk during the lactation period. In each treatment group, adults were assigned to the main study or for collection of serum samples for PFHxS concentration determination.

26. Adults from the main study selected for histopathological evaluation were sacrificed on day 44 (males), or PND22 or GD25 (females). Thyroids were removed and submitted for histopathological analysis. Blood samples for serum PFHxS concentrations were collected from adults on day 14 (males and females). Adults selected for serum PFHxS concentrations were sacrificed on day 42 (males) and GD21 (females). Offspring selected for serum PFHxS concentrations were sacrificed on GD21 and PND22.

27. Mortality: All adults survived to scheduled necropsy (no data on offspring reported).

28. General toxicity and body weight: No clinical signs of general toxicity were observed in adults (no data on offspring reported). In males, significantly reduced body weight gain was observed at 10 mg/kg bw/day from day 1 to day 44, and at 0.3 and 3 mg/kg bw/day between day 29 to day 43, compared with controls. Regardless of these variations, body weights were unaffected by treatment. In females, significantly reduced body weights were observed at 10 mg/kg bw/day on PND8, compared with controls. Occasional significant reductions in body weight also occurred at 0.3, 3 and 10 mg/kg bw/day from PND4 to 14, compared with controls. However, body weight gain was unaffected by treatment. Food consumption was unaffected by treatment in adults.

29. Histopathology: An increase in the incidence of mild to moderate hypertrophy was observed in the thyroid glands of males at 3 mg/kg bw/day and 10 mg/kg bw/day on day 44. The observed changes included hypertrophy and hyperplasia of the follicular epithelium cells. No treatment-related changes were observed in females at any dose.

30. Serum PFHxS concentrations: Mean serum PFHxS concentrations in parental males on day 42 were 0.32 µg/mL (control), 44.2 µg/mL (0.3 mg/kg bw/day), 89.1 µg/mL (1 mg/kg bw/day), 129 µg/mL (3 mg/kg bw/day), and 202 µg/mL (10 mg/kg bw/day).

31. The authors concluded that K^+PFHxS-induced effects noted in parental males included increased hyperplasia of thyroid follicular cells at 3 mg/kg bw/day and 10 mg/kg bw/day.

Gilbert *et al.* 2021

32. Gilbert *et al*. (2021) studied the effect of PFHxS on TH levels and thyroid-responsive genes. As part of a neurological developmental study in rats, pregnant Long-Evans rats (9/group and 6/control) were administered PFHxS at doses of 0 and 50 mg/kg bw/day daily by gavage from GD6 to PND21. Offspring were exposed in utero and during lactation only. Offspring were sacrificed on days PND0, PND2, PND6 and PND14 and tissues collected, dams were sacrificed on PND22 when pups were weaned.

33. Blood was collected from dams on GD20 and PND22, and offspring on PND0, PND2, PND6 and PND14, and serum TT4, FT4, TT3 and TSH levels measured. Brain tissue T4 and T3 levels (assumed to be TT4 and TT3, respectively) were measured in offspring on PND0, PND2, PND6 and PND14. Samples collected for gene expression included thyroid glands from dams on PND22 and offspring on PND0, PND2 and PND14; liver from dams on PND22 and offspring on PND2, PND6 and PND14; and the anterior neocortex from offspring on PND14.

34. Body weight: Body weights in dams were slightly but not significantly reduced by treatment. Body weights in offspring were unaffected.

35. Gross pathology: Thyroid weight was not significantly different in offspring on PND14 compared with controls (no other data given). Thyroid hormone levels: In dams, TT4 and TT3 levels were significantly decreased on GD20 and PND22, and FT4 levels were significantly decreased on PND22,

compared with controls (no data provided for GD20). No change was detected in TSH levels in dams on PND22 (no data provided for GD20). In offspring, TT4 levels were significantly decreased on PND0, PND2, PND6 and PND14; TT3 levels were significantly decreased on PND6 and PND14 (no data provided for PND0 and PND6); FT4 levels were significantly decreased on PND14 (no data provided for PND0, PND2, PND6), all compared with controls. No change was detected in TSH levels on PND14 (no data provided for PND0, PND2, PND6). A significant reduction in brain TT4 levels occurred in offspring on PND0, compared with controls. No changes were observed at subsequent ages. There were no treatment related effects in offspring brain TT3 levels at any age.

36. Gene expression: The expression of thyroid gland genes involved in thyroid function (thyroid peroxidase, Tpo) or sodium-iodide symporter (Nis) was not significantly altered in samples collected from dams on PND22 or offspring on PND0, PND2 and PND14. No changes were seen in the relative expression of thyroid hormone responsive genes (Dio1, multidrug resistance 1(Mdra1), malic enzyme (ME), thyroid hormone-inducible hepatic protein (THRSP, or Spot14)) in livers taken from dams on PND22 and offspring on PND2, PND6 and PND14. Gene expression of eleven thyroid hormone- responsive genes remained unchanged in the neocortex of offspring on PND14.

37. The authors concluded that PFHxS reduced serum TT4 but did not increase TSH, whilst thyroid-responsive genes in the liver, thyroid gland and brain were largely unchanged. Brain tissue TT4 was reduced in offspring on PND0, but despite persistent TT4 reductions in serum, had recovered in the PND2 offspring brain.

NTP, 2022b

38. NTP (2022b) investigated the effects of PFHxS on thyroid weight, histopathology and TH levels in rats. In a repeated dose study, SD rats (10/sex/group) were administered K^+PFHxS at doses 0, 0.625, 1.25, 2.5, 5 or 10 mg/kg bw/day) for males, or 0, 3.12, 6.25, 12.5, 25 or 50 mg/kg bw/day for females by gavage for 28 days. At necropsy on day 29, blood samples were collected for TT4, TT3, FT4, TSH and PFHxS analysis, and thyroids were removed for histopathological evaluation.

39. Mortality: All rats survived to scheduled necropsy.

40. General toxicity and body weight: No clinical signs of general toxicity were observed. Terminal body weights were unaffected by treatment, compared

with controls.

41. Gross pathology: Thyroid weights in males and females were unaffected by treatment.

42. Histopathology: Histopathology in males and females was unaffected by treatment.

43. Thyroid hormone levels: In males, TT4, FT4 and TT3 levels were significantly decreased at ≥ 0.625 mg/kg bw/day, compared with controls. TSH levels were unaffected by treatment. In females, TT4 levels were significantly decreased at ≥ 6.25 mg/kg bw/day, and FT4 levels were significantly decreased at ≥ 12.5 mg/kg bw/day. TT3 and TSH levels were unaffected by treatment.

44. Plasma PFHxS concentrations: In males, mean plasma PFHxS concentrations on day 29 were 0.102 µg/mL (control), 66.760 µg/mL (0.625 mg/kg bw/day), 92.080 µg/mL (1.25 mg/kg bw/day), 129.000 µg/mL (2.5 mg/kg bw/day), 161.700 µg/mL (5 mg/kg bw/day) and 198.300 µg/mL (10 mg/kg bw/day). In females, concentrations on day 29 were 1.750 µg/mL (control), 37.030 µg/mL (3.12 mg/kg bw/day), 50.410 µg/mL (6.25 mg/kg bw/day), 63.820 µg/mL (12.5 mg/kg bw/day), 83.820 µg/mL (25 mg/kg bw/day) and 95.510 µg/mL (50 mg/kg bw/day).

45. The authors concluded that TT4 and FT4 decreased in a dose- response manner. TSH was unaffected, nor were there any histopathologic changes in the thyroid gland (hyperplasia/hypertrophy).

Ramhøj *et al*. 2018

46. Ramhøj *et al*. (2018) investigated the effect of low doses of PFHxS on thyroid hormone levels in rats as part of two developmental studies evaluating PFHxS alone or in combination with an endocrine disruptor mix. In Study 1, pregnant Wistar rats (8/group) were administered PFHxS at doses 0, 25 or 45 mg/kg bw/day by gavage on GD7 to PND22. In Study 2, pregnant Wistar rats (16 – 20/group) were administered lower doses of PFHxS at doses 0, 0.05, 5 and 25 mg/kg bw/ day by gavage from GD7 to PND22.

47. In Study 1, trunk blood was collected at sacrifice for TT4 measurement from offspring on PND16 and dams on PND22. Serum PFHxS concentrations were measured in dams (5 – 7/group) on PND22. In Study 2, tongue blood was collected from dams on GD15, and trunk blood was collected at sacrifice from male offspring on PND16, female offspring on PND17 and dams on PND22. TT4

levels were measured in these samples.

48. General toxicity and body weight: No clinical signs of general toxicity were observed in dams or offspring in either study. Maternal weight and weight gain was also unaffected by treatment. In Study 2, male offspring body weight was slightly decreased at 25 mg/kg bw/day on PND0. There was no significant effect on female offspring birth weights in either study, or in males in Study 1. No significant effect was observed on offspring body weight or weight gain on PND6, PND14 or PND22 in either study.

49. Thyroid hormone levels: Treatment with PFHxS reduced TT4 levels in both dams and offspring. In Study 1, TT4 levels in dams on PND22 were significantly reduced at both 25 and 45 mg/kg bw/day, compared with controls. TT4 levels in offspring on PND16 were significantly reduced at both 25 mg/kg bw/day and 45 mg/kg bw/day. In Study 2, TT4 levels in dams on GD15 and PND22 were significantly reduced at both 5 mg/kg bw/day and 25 mg/kg bw/day. TT4 levels in offspring on PND16/17 were significantly reduced at 5 and 25 mg/kg bw/day.

50. Serum PFHxS concentrations: PFHxS concentrations in dams at PND22 were ND (controls), 139 µg/mL (25 mg/kg bw/day) and 174 µg/mL (45 mg/kg bw/day).

51. Based on these results, the authors proposed that PFHxS is an effective thyroid hormone disruptor in rats as PFHxS administration significantly decreased serum TT4 levels in rat dams and their offspring. Significantly lower TT4 levels were seen at 5 mg/kg bw/day, after only 7 days of exposure.

52. The authors concluded that PFHxS can induce marked reductions in circulating serum TT4 in rats, which at critical developmental stages can lead to altered brain morphology and adverse behaviour.

Ramhøj *et al*. 2020

53. Ramhøj *et al*. (2020) investigated the effect of PFHxS on the thyroid system as part of a developmental study evaluating PFHxS alone or in combination with an endocrine disruptor mix. Pregnant Wistar rats (16 – 20/group) were administered K^+PFHxS at doses 0, 0.05, 5 and 25 mg/kg bw/day by gavage on GD7 to PND22.

54. Tongue blood was drawn from dams on PND15. Necropsy of one male and one female per litter took place on PND16 and PND17, respectively, and

dams on PND22. Trunk blood was collected for measurement of TT3 and TSH. Thyroid glands were excised, weighed and saved for histopathology. Sections of thyroid glands from dams on PND22 and male offspring on PND16 were extracted for histopathological examination. A sub-set of female offspring was weaned and sacrificed at PND22 for collection of thyroid glands. Both lobes of the thyroid gland from female offspring on PND17 were extracted for RNA analysis. Expression levels of six gene transcripts involved in thyroid hormone synthesis and regulation were analysed, namely solute carrier family 5 (Slc5a5(NIS)), NK2 homeobox 1 (thyroid transcription factor 1, TTF-1) (Nkx2.1), Tpo, Thyroid stimulating hormone receptor (Tshr), Paired box 8 (Pax8) and Dio1. Serum PFHxS concentrations were not determined in this study.

55. Note: TT4 levels reported in this study have been previously provided in the summary of Ramhøj *et al*. (2018) above, refer Paragraph 48. Ramhøj et al. (2018) reported exposure to PFHxS during gestation and lactation and showed dose-dependent reductions in serum TT4 levels in both offspring and dams.

56. Gross pathology: Dose-dependent decreases in thyroid gland weight were observed in female offspring on PND22. Reductions were significant at 5 and 25 mg/kg bw/day compared with controls. There were no treatment- related effects on thyroid weights in dams on PND22 or female offspring on PND17. No data regarding males were presented.

57. Histopathology: There were no treatment-related effects on maternal histopathology. Statistically significant mild histological changes were seen in male offspring on PND16 at 25 mg/kg bw/day compared with controls; however, these changes were no longer seen on PND22. No data regarding female offspring were presented.

58. Thyroid hormone levels: In dams, a significant decrease in TT3 levels was observed at 25 mg/kg bw/day on PND22, compared with controls. TSH levels in dams were not altered by exposure to PFHxS. In offspring, a significant decrease in TT3 levels was observed at 25 mg/kg bw/day on PND16/17. TSH levels in offspring were not altered by exposure to PFHxS.

59. Gene expression: There were no treatment related effects on expression levels of genes associated with thyroid hormone synthesis and regulation (Slc5a5(NIS), Nkx2.1, Tpo, Tshr, Pax8 and Dio1) in thyroid glands from dams on PND22. Similarly in female offspring on PND17, no treatment relate effects were detected in expression levels of four gene transcripts involved in thyroid hormone synthesis and regulation (Slc5a5 (NIS), Nkx2.1, Tpo, and Tshr).

60. The study revealed that exposure to PFHxS resulted in marked reductions in TT4 levels and moderate reductions in TT3 in both dams and offspring, but had no significant effects on TSH levels in dams (PND22) or in offspring (PND16 and PND17). The authors propose that the results do not correspond to the classic view of the hypothalamic–pituitary–thyroid (HPT) axis, whereby TRH activates the pituitary to release TSH, which binds to TSH receptors on the thyroid gland, upregulating synthesis and release of thyroid hormone into the blood. The absence of effects on maternal TSH levels, thyroid gland weight, histopathology or gene expression indicate a lack of thyroid gland perturbation in response to exposure or as a compensatory reaction to decreases in serum T4.

61. The authors concluded that PFHxS at doses up to 25 mg/kg bw/day lowered TH levels in both dams and offspring in a dose-dependent manner, but did not change TSH levels, thyroid weight, thyroid histology, or expression of marker genes of the thyroid gland. PFHxS reduced TT3 and TT4 in pregnant dams and their progeny, but did not appear to activate the HPT axis at doses up to 25 mg/kg bw/day.