PFOS

In this guide

In vivo toxicity data

Butenhoff *et al*. 2012

62. Butenhoff *et al*. (2012) investigated the effects of perfluorooctane sulfonate (PFOS) exposure on thyroid toxicity and neoplastic potential in rats. In a combined toxicity and carcinogenicity study, SD rats (60-70/sex/group) were administered K^+PFOS at doses 0, 0.5, 2, 5 or 20 mg/kg diet (equivalent to 0, 0.024, 0.098, 0.242 or 0.984 mg/kg bw/day for males, and 0, 0.029, 0.120, 0.299 or 1.251 mg/kg bw/day for females) in the diet for 104 weeks (103 weeks for females in the 0.120 mg/kg bw/day group); a subset of 10/sex/group of the high dose and controls were terminated at 52 weeks. A recovery group (40/sex) was administered 20 mg/kg diet (equivalent to 1.144 mg/kg bw/day for males, and 1.385 mg/kg bw/day for females) for the first 52 weeks of the study, after which they were administered a control diet until study termination. Necropsies were scheduled on week 53 after 52 weeks of treatment (10/sex/group) for controls, for males treated with 0.984 mg/kg bw/day group and females treated with 1.251

mg/kg bw/day and at study termination after 103 or 104 weeks of treatment for remaining rats for all groups. Thyroids were removed from males administered 0.984 mg/kg bw/day and from females treated with 1.251 mg/kg bw/day at scheduled necropsy during week 53. Thyroid tissue samples were collected at terminal necropsy for histopathological evaluation.

63. Mortality: In males, mortality was significantly decreased at 0.299 and 1.251 mg/kg bw/day compared with controls. In females, mortality was significantly increased at 0.120 mg/kg bw/day. Mortality was unaffected by treatment in all other groups.

64. General toxicity and body weight: No clinical signs of general toxicity were observed. In males, body weight was significantly decreased at 0.984 mg/kg bw/day (weeks 9 – 37), and at 1.144 mg/kg bw/day in the recovery group (weeks 9 – 37), compared with controls. In females, body weight was significantly decreased at 1.251 mg/kg bw/day (weeks 3 – 101), and at 1.385 mg/kg bw/day in the recovery group (weeks 3 – 61). In female rats sacrificed at 53 weeks, body weight was significantly decreased at 1.251 mg/kg bw/day. Body weights in males sacrificed at 53 weeks were unaffected by treatment. At terminal sacrifice, body weights in both males and females were unaffected by treatment.

65. Gross pathology: In males, significantly decreased left thyroid/parathyroid weights were recorded at 0.984 mg/kg bw/day but were considered spurious by the authors due to there being no contralateral effect in the right thyroid/parathyroid and a lack of difference in organ-to-weight ratios between treated and control rats. In females, thyroid weights at 1.251 mg/kg bw/day were unaffected by treatment.

66. Non-neoplastic lesions: There were no non-neoplastic microscopic observations attributed to treatment in thyroid tissues.

67. Neoplastic lesions: In males, there was a significant increase in thyroid follicular cell adenoma and combined thyroid follicular cell tumours (adenoma and carcinoma) at 1.144 mg/kg bw/day in the recovery group, compared with controls. The authors noted that although the increased incidence of thyroid follicular cell tumours was outside the range of historical control values, there was no other microscopic evidence of thyroid abnormality. Further, the significantly increased incidence of thyroid follicular cell adenoma in the 1.144 mg/kg bw/day recovery group, without observation of similar increases at 0.984 mg/kg bw/day in males and/or at 1.251 mg/kg bw/day in females is contradictory and may represent a chance occurrence. In females, there was a significant

increase in combined thyroid follicular cell adenoma and carcinoma at 0.299 mg/kg bw/day, compared with controls. The authors noted these tumours are known to occur in historical controls. In addition, the absence of either a dose–response or non-neoplastic thyroid findings suggests that the thyroid follicular cell tumours at 0.299 mg/kg bw/day in females were a spurious finding.

68. Serum PFOS concentrations: In males, mean PFOS concentrations on week 105 were 0.012 µg/mL (control), 1.31 µg/mL (0.024 mg/kg bw/day), 7.60 µg/mL (0.098 mg/kg bw/day), 22.50 µg/mL (0.242 mg/kg bw/day) and 69.3 µg/mL (0.984 mg/kg bw/day). In females, mean PFOS concentrations on week 105 were 0.084 µg/mL (control), 4.35 µg/mL (0.029 mg/kg bw/day), 20.20 µg/mL on week 102 (0.120 mg/kg bw/day), 75.00 µg/mL (0.299 mg/kg bw/day) and 233.0 µg/mL (1.251 mg/kg bw/day).

69. The authors concluded there were no anatomical indications of a response of the thyroid to dietary treatment with K^+PFOS, including thyroid weight and microscopic histological changes. There were no treatment-related findings in thyroid tissue in rats fed K^+PFOS through study termination.

Chang *et al*. 2008

70. Chang *et al*. (2008) investigated the effect of PFOS on thyroid hormones and regulatory functions of the HPT axis in rats in a series of three experiments.

71. In experiment 1, serum thyroid levels were collected to assess whether PFOS competes for thyroxine (T4) protein binding sites, and so resulting in transiently elevated FT4. Female Sprague-Dawley (SD) rats (5 – 15/group) were given either a single oral dose (assumed by gavage) of potassium-PFOS (K^+PFOS) (15 mg/kg bw) or negative control. Rats were divided into three groups and sacrificed at either 2, 6 or 24 hours post-dosing and blood samples collected. Serum TSH, FT4, TT4, TT3, reverse triiodothyronine (rT3) levels and PFOS concentrations were measured. Liver tissues were collected, and hepatic biochemical markers, ME and UDP-glucuronosyltransferase 1A (UGT1A) mRNA transcript levels as well as ME activity were measured as these can also reflect impacts on activity in the thyroid.

72. Thyroid hormone levels: A transient significant increase of FT4 and decrease of TSH levels were seen after 6 hours. In contrast, these levels remained stable after 2 and 24 hours. A significant decrease in TT4 levels were seen after 2, 6 and 24 hours. TT3 and rT3 levels were relatively stable after 2 and 6 hours but

then significantly decreased after 24 hours.

73. Gene expression: ME mRNA transcripts were significantly increased after 2 hours returning to within control levels after 6 and 24 hours. ME activity was stable after 2 and 6 hours but significantly increased after 24 hours. Liver UGT1A mRNA transcripts were significantly elevated after 2 and 6 hours returning to control levels after 24 hours. The authors indicate that this may be representative of induction of increased glucuronidation and turnover of T4. Serum PFOS concentrations: Mean serum PFOS concentrations were significantly elevated at all time points compared with controls, peaking at 6 hours. Measured mean concentrations were 10 µg/mL (control), 37.28 µg/mL (2 hours), 66.90 µg/mL (6 hours) and 61.58 µg/mL (24 hours).

74. The second experiment investigated whether transiently elevated levels of FT4 in response to PFOS exposure leads to increased turnover and elimination of T4. Male and female SD rats were injected with either 11 µCi (4/group, males) or 9.3 µCi (5/group, females) ^{125}I-labelled T4 (specific activity 1250 µCi/µg). This was followed by a single oral dose (assumed by gavage) of either 15 mg/kg bw K^+PFOS or vehicle. Urine and faeces were collected over the 24-hour period following PFOS administration, whilst serum and liver were harvested after 24 hours. Serum TT4 levels were measured. Serum, liver, urine and faeces were all measured for ^{125}I radioactivity to determine elimination of T4.

75. Thyroid hormone levels and turnover: TT4 levels were significantly reduced in males and females compared with controls after 24 hours and this correlated with a decrease in ^{125}I radioactivity in both sexes. Liver ^{125}I radioactivity was reduced in both males and females, urine and faecal ^{125}I radioactivity was significantly increased in males, and in faeces in females, indicating increased turnover and loss of thyroid hormones.

76. In the third experiment, pituitary function was investigated by measuring release of TSH following inhibition of thyroid hormone synthesis in the thyroid. Propyl thiouracil (PTU) was used as a thyroid hormone synthesis inhibitor which leads to increased pituitary secretion of TSH. Adult male SD rats (6/group) were administered either negative control (gavage); K^+PFOS only (3 mg /kg bw/day, gavage); PTU only (10 µg/mL, drinking water); or combined PTU (10 µg/mL, drinking water) and K^+PFOS (3 mg /kg bw/day, gavage), all for 7 consecutive days. Interim serum samples were collected on days 1, 3 and 7, whilst terminal serum samples were collected post sacrifice on day 8, 24 hours after the last treatment. Samples were analysed for TT4, TT3 and TSH. Anterior

pituitary samples were removed on day 8 and placed in static culture for assessment of TRH-mediated release of TSH.

77. Thyroid hormone levels: In the combined PTU and PFOS treatment group, TT4, TT3 and TSH levels did not differ significantly from levels observed in the PTU-only treatment group. In the PFOS only treatment group, TSH levels were unchanged, whilst TT4 and TT3 levels were significantly decreased compared with controls. PFOS treatment had no effect on TRH- mediated release of TSH from the pituitary gland.

78. Overall, the authors concluded that a single oral dose of PFOS in rats results in a transient increase in tissue availability of thyroid hormones and turnover of T4, with a resulting reduction in serum TT4. Under the dosing conditions of the study, PFOS does not induce a classical hypothyroid state nor does it alter hypothalamic-pituitary-thyroid activities.

Chang *et al*. 2009

79. Chang *et al*. (2009) evaluated thyroid status and histomorphological factors associated with thyroid follicles following exposure to PFOS. As part of a developmental study in rats, groups of pregnant SD rats (25/group) were administered daily oral doses (assumed by gavage) of K^+PFOS at 0, 0.1, 0.3 and 1.0 mg/kg bw/day from GD0 through to PND20. Offspring were nursed until PND21 and then observed until PND72. Maternal and foetal serum and tissue samples were obtained from a further group (10/group) administered daily oral doses (assumed by gavage) of K^+PFOS at 0, 0.1, 0.3 and 1.0 mg/kg bw/day until GD19 and sacrificed on GD20.

80. Serum was collected on GD20 (dams and foetuses), PND4 (dams, and male and female offspring), and PND21 (dams, and male and female offspring) and TSH levels measured. Thyroids were collected for histopathological evaluation from the control and 1.0 mg/kg bw/day maternal dose groups from GD20 foetuses, PND4 and PND21 male and female offspring. Other evaluations included thyroid follicular epithelial cell height and dimensions (PND4 and PND21 male and female offspring) and immunohistochemical staining (GD20 foetuses). mRNA transcripts were obtained from liver samples and the gene expression of thyroid hormones was evaluated from GD20 dams and foetuses, and PND21 male offspring with 1.0 mg/kg bw/day maternal dose.

81. Mortality: There was no evidence of treatment-related effects on offspring survival (no data presented for dams).

82. General toxicity and body weight: No clinical signs of general toxicity were observed in dams or offspring. Maternal body weights were significantly decreased in the 1.0 mg/kg bw/day dose group from PND4 through to PND21, due to lower mean body weight gains during gestation and PND1 to PND4. Food consumption was also significantly decreased at this dose. Maternal weight gains during the remainder of lactation and offspring body weights and body weight gain were unaffected by treatment compared with controls.

83. Histopathology: No treatment-related histological changes, including the number or distribution of follicles, were observed in thyroid sections from GD20 foetuses or PND4 and PND21 offspring, compared with controls. There were no treatment related changes to follicular colloid area in PND4 and PND21 offspring. Follicular epithelial cell height was unaffected by treatment in PND4 male and female offspring, and PND21 female offspring. In PND21 male offspring, follicular epithelial cell height was significantly higher than controls, although this difference was considered spurious by the authors because of the low result in the male control group as compared to the female control group.

84. Histopathology: There was a significant increase in number of thyroid follicular epithelial cells in GD20 female foetal thyroids from dams treated with 1.0 mg/kg bw/day, compared with controls. Due to the range in corresponding control values, the authors were unable to determine the toxicological significance of this finding. No treatment-related effects were observed in males. Thyroid hormone levels: Mean serum TSH levels taken from treated dams and their offspring were unchanged relative to controls on GD20, PND4 or PND21.

85. Gene expression: mRNA transcripts with a potential relationship to thyroid status (ME, P450 oxidoreductase (Por), type 1 deiodinase (Dio1), UGT1A family, and apolipoprotein A1 (ApoA1)) were unaffected in dams and offspring following maternal treatment.

86. The authors concluded K^+PFOS administration up to 1.0 mg/kg bw/day to maternal rats during gestation and lactation has no clear adverse effect on thyroid status (morphology, hormone homeostasis, proliferation or liver gene expression, specifically hepatic genes mediated by thyroid hormones).

Chang *et al*. 2017

87. Chang *et al*. (2017) investigated the effects of PFOS exposure on TH levels in monkeys. Cynomolgus monkeys (6/sex/group) were assigned to three groups: group 1 was administered vehicle by gavage on days 1, 43, 106, 288 and

358; group 2 was administered a single dose of K^+PFOS at a dose of 9 mg/kg bw by gavage on day 106 and vehicle on days 1, 43, 288 and 358; group 3 was administered three doses of K^+PFOS at doses of 14 mg/kg bw on day 43, 14.8 mg/kg bw (males) or 17.2 mg/kg bw (females) on day 288, 11 mg/kg bw on day 358, and vehicle on days 1 and 106, all by gavage. Blood samples for PFOS and TT4, FT4, TT3 and TSH analysis were collected at numerous time points including prior to treatment, during treatment, and on the final day of the study on day 420.

88. Mortality: No mortalities occurred during the study.

89. General toxicity and body weight gain: No clinical signs of general toxicity were observed. Body weight and body weight gain were unaffected by treatment in either group 2 or group 3, compared with group 1.

90. Thyroid hormone levels: Non-significant decreases in TT4 levels were observed in males and females at 9 mg/kg bw (group 2), at 11 and 14.8 mg/kg bw in males, or 11 and 17.2 mg/kg bw in females (group 3) compared with controls. There were no significant changes to TSH, FT4 and TT3 levels in males or females from either group 2 or 3. The authors proposed that because unlike TSH and FT4, TT4 is not a clinically relevant endpoint for interpreting thyroid function, and these results do not indicate an adverse effect on thyroid function.

91. Serum PFOS concentrations: The highest mean PFOS serum concentrations for the duration of the study from each treatment group were: 0.013 µg/mL on day 365 and 0.007 µg/mL on day 365 from males and females, respectively (group 1); 67.7 µg/mL on day 113 and 68.8 µg/mL on day 113 from males and females, respectively (group 2); and 160.8 µg/mL on day 365 and 165.0 µg/mL on day 365 from males and females, respectively (group 3).

92. The authors concluded that, when compared with time-matched controls, the administration of a single dose or multiple single doses of PFOS to monkeys did not result in any toxicologically meaningful or clinically relevant changes in thyroid related hormones at serum PFOS concentrations up to 165 µg/mL.

Conley *et al*. 2022

93. Conley et al. (2022) investigated the effects of PFOS exposure on thyroid hormones in rats. In a developmental study, pregnant SD rats (5/group) were administered K+PFOS at doses 0, 0.1, 0.3, 1, 2 or 5 mg/kg bw/day by

gavage from GD8 – PND2. Dams and offspring were sacrificed on PND2 and blood was collected for various analyses: TT4, TT3, free triiodothyronine (FT3), FT4 and PFOS in dams, and TT4, TT3 and rT3 in offspring.

94. Mortality: In dams, mortality was unaffected, whereas offspring survival was significantly reduced at 5 mg/kg bw/day, compared with controls. General toxicity and body weight: No clinical signs of general toxicity were reported. In dams, there was a significant reduction in body weight at 5 mg/kg bw/day on GD22 and PND2, and body weight gain (GD8-GD22), compared with controls, while in offspring, body weights were significantly reduced at 2 mg/kg bw/day on PND2 and at birth at 5 mg/kg bw/day when adjusted for litter size and birthdate.

95. Thyroid hormone levels: In dams, TT4 and TT3 levels were significantly reduced at ≥ 0.1 and ≥ 0.3 mg/kg bw/day, respectively, compared with controls. FT4 levels were significantly reduced at 0.3, 2 and 5 mg/kg bw/day, whereas FT3 levels were unaffected. In offspring, TT4 levels were reduced at ≥ 0.3 mg/kg bw/day, and both TT3 and rT3 were significantly reduced at ≥1 mg/kg bw/day.

96. Serum PFOS concentrations: In dams, mean PFOS concentrations were ND (controls), 2.2 µg/mL (0.1 mg/kg bw/day), 5.8 µg/mL (0.3 mg/kg bw/day), 31.4 µg/mL (1 mg/kg bw/day), 68.8 µg/mL (2 mg/kg bw/day), and 203.3 µg/mL (5 mg/kg bw/day).

97. The authors concluded PFOS reduced serum TH concentrations, which occurred at nearly all doses depending on the specific hormone and life stage (maternal or neonatal). However, PFOS did not reduce maternal FT3.

Curran *et al*. 2008

98. Curran *et al*. (2008) investigated the effects of PFOS on thyroid weight and TH levels in rats. In a repeated dose study, SD rats (15/sex/group) were administered K^+PFOS at doses 0, 2, 20, 50 or 100 mg/kg diet (equivalent to 0, 0.14, 1.33, 3.21 or 6.34 mg/kg bw/day for males, and 0, 0.15, 1.43, 3.73 or 7.58 mg/kg bw/day for females) in the diet for 28 days. At necropsy, blood samples were collected for TT4, TT3 and PFOS analysis, and thyroids were removed.

99. General toxicity and body weight: No clinical signs of general toxicity were reported. In males, body weights were significantly reduced at 3.21 and 6.34 mg/kg bw/day on days 14, 21 and 28, compared with controls. In females, body weights were significantly reduced at 3.73 mg/kg bw/day on days 21 and

28, and at 7.58 mg/kg bw/day on days 14, 21 and 28, compared with controls. Feed consumption was significantly reduced in both males and females at these doses, leading the authors to propose that palatability may be a factor.

100. Gross pathology: Absolute thyroid weights in males and females were unaffected by treatment. Relative thyroid weight: body weight was significantly increased at 6.34 and 7.58 mg/kg bw/day in males and females, respectively, compared with controls.

101. Thyroid hormone levels: In males, TT4 levels were significantly decreased at 1.33, 3.21 and 6.34 mg/kg bw/day, and in females, at 1.43 mg/kg bw/day, 3.73 and 7.58 mg/kg bw/day. In males, TT3 levels were significantly decreased at 6.34 mg/kg bw/day, and in females, at 3.73 and 7.58 mg/kg bw/day.

102. Serum PFOS concentrations (converted from µg/g to µg/mL using serum density 1.018 g/mL): In males, mean PFOS concentrations at study termination were 0.48 µg/mL (control), 0.97 µg/mL (0.14 mg/kg bw/day), 13.69 µg/mL (1.33 mg/kg bw/day), 21.31 µg/mL (3.21 mg/kg bw/day) and 30.42 µg/mL (6.34 mg/kg bw/day). In females, mean PFOS concentrations on day 28 were 0.97 µg/mL (control), 1.53 µg/mL (0.15 mg/kg bw/day), 15.68 µg/mL (1.43 mg/kg bw/day), 32.50 µg/mL (3.73 mg/kg bw/day) and 43.98 µg/mL (7.58 mg/kg bw/day).

103. The authors concluded that serum thyroid hormone levels were decreased in PFOS-treated rats. Decreased TT4 and TT3 occurred concurrently with hepatic changes indicative of peroxisome proliferation. Significant treatment-related changes in thyroid weight, relative to body weight, were suggestive of altered endocrine functions.

104. The authors determined the following lowest observed effect levels (LOEL). Reduced TT4: 1.33 mg/kg bw/day (serum 13.69 µg/mL) in males and 1.43 mg/kg bw/day (serum 15.68 µg/mL) in females. Reduced TT3: 6.34 mg/kg bw/day (serum 30.42 µg/mL) in males and 3.73 mg/kg bw/day (serum 32.50 µg/mL) in females. Reduced relative thyroid weight:body weight: 6.34 mg/kg bw/day (serum 30.42 µg/mL) in males and 7.58 mg/kg bw/day (serum 43.98 µg/mL) in females.

Elcombe *et al*. 2012

105. Elcombe et al. (2012) examined thyroid parameters as part of a 7-day dietary study in rats. Male SD rats (40/group) were given either 20 ppm or 100 ppm K^+PFOS (equivalent to 1.93 and 9.65 mg/kg bw/day, respectively) in the

diet for 7 days, followed by a control diet for a recovery period of either 1, 28, 56 or 84 days. Subgroups of rats (10/group) were sacrificed on the first day of recovery (recovery day 1) and subsequently on recovery days 28, 56 and 84.

106. Upon necropsy, blood was collected, and thyroids removed from each rat. Thyroid tissue samples underwent histopathological examination and were used to determine cell proliferation rates and apoptotic index. Blood serum PFOS concentrations were determined.

107. Mortality: All rats survived to scheduled necropsy.

108. General toxicity and body weight: No clinical signs of general toxicity were observed. Body weights were significantly decreased at 1.93 mg/kg bw/day recovery days 21 and 28, and at 9.65 mg/kg bw/day on recovery days 16, 21, 28. Food consumption was unaffected due to treatment.

109. Histopathology: There were no treatment-related effects observed upon microscopic examination of thyroid gland sections. Similarly, K^+PFOS treatment had no effect on S-phase activity and hence cell proliferation. Levels of apoptosis in thyroid follicles were similar to controls for all time points.

110. Serum PFOS concentrations: mean serum PFOS concentrations following administration of 1.93 mg/kg bw/day were 39.49 µg/mL, 15.49 µg/mL, 8.03 µg/mL and 4.38 µg/mL, on day 1, 28, 56 and 84, respectively, and 140.4 µg/mL, 53.82 µg/mL, 43.68 µg/mL and 25.79 µg/mL on day 1, 28, 56 and 84, respectively following treatment with 9.65 mg/kg bw/day. PFOS concentrations in controls not reported.

111. The authors concluded that K^+PFOS at up to 9.65 mg/kg bw/day via the diet for 7 days did not appear to have any effect on the thyroid parameters evaluated (histology, follicular epithelial cell proliferation and follicular epithelial apoptosis).

Lau *et al*. 2003

112. Lau *et al*. (2003) investigated the effects of PFOS on thyroid hormones in rats and mice. As part of a developmental study in rats, pregnant SD rats (17 – 28/group) were treated with K^+PFOS at doses of 0, 1, 2, 3, 5 or 10 mg/kg bw/day by gavage from GD2 until GD21. Four offspring from each litter were sacrificed within 2-4 hours of birth and trunk blood collected. Remaining offspring were randomised and redistributed to nursing dams within their respective dosage groups, a process that was repeated every few days to

maintain litters sizes at 10 – 12 offspring each. Offspring were weaned on PND21. Male and female offspring were randomly chosen for sacrifice on PND2, 5, 9, 15, 21, 28 and 35, where blood was collected, and serum prepared.

113. As part of a developmental study in mice, pregnant CD-1 mice (21 – 22/group) were treated with K^+PFOS at doses of 0, 1, 5, 10, 15 or 20 mg/kg bw/day by gavage from GD1 until GD17. Offspring were weaned on PND21. Male and female offspring were randomly selected from litters and sacrificed within 2-4 hours of birth and on PND3, 7, 14, 21, 28 and 35, where blood was collected, and serum prepared. Litters were culled at intervals of several days to maintain sizes at 10–12 offspring each.

114. Serum TT4, FT4, triiodothyronine (T3) (assumed to be both TT3 and FT3 as not specified in the paper) and TSH levels and serum PFOS concentrations were measured in offspring at sacrifice. Mortality: In rats, neonatal exposure to PFOS reduced postnatal survival in offspring at 5 and 10 mg/kg bw/day, and in mice at 15 and 20 mg/kg bw/day. Greater than 95 % of offspring in these groups died within 24 hours of birth. The authors noted that approximately 50 % rat and mice offspring died at 3 and 10 mg/kg bw/day, by PND22 and PND24, respectively.

115. General toxicity and body weight: In surviving rat offspring, at 2 mg/kg bw/day body weight was significantly decreased between PND0 to PND3, and on PND9; at 3 mg/kg bw/day body weight was significantly decreased between PND0 to PND5; and at 5 mg/kg bw/day body weight was significantly decreased between PND0 to PND22. In surviving mice offspring, body weights were unaffected by treatment.

116. Thyroid hormone levels: In rat offspring, TT4 and FT4 levels were significantly reduced on PND2 at either 1 or 2 mg/kg bw/day maternal doses depending on the statistical method used, compared with controls. TT4 levels in all treated groups were similar to controls on PND21 and PND35, whereas FT4 levels remained depressed from PND2 to PND35 in all treated groups. There were no significant changes in serum TT3, FT3 or TSH levels in maternally exposed offspring compared with controls at any time point. In mice offspring, there were no significant changes in TT4, TT3, FT3 or TSH levels in maternally exposed mice compared with controls from PND3 until PND28, although a non-significant decrease in levels was observed at 5 and 10 mg/kg bw/day on PND14.

117. Serum PFOS concentrations: In rats, at birth serum PFOS concentrations increased with dose in a non-linear manner (numerical values not

provided). By PND5, serum PFOS concentrations were lower than at birth. Serum PFOS concentrations for mice were not reported.

118. The authors concluded that hypothyroxinemia was observed in PFOS-exposed rat offspring. Serum T4 levels (assumed TT4 and FT4) were suppressed in the PFOS-exposed rat offspring, although T3 (assumed to be TT3 and FT3) and TSH were not altered.

119. Luebker *et al*. (2005) investigated the effects of PFOS on TH levels and histopathology in rats and their offspring. In a two-generation reproduction study, female SD rats (20/group) were administered K^+PFOS at doses of 0, 0.4, 0.8, 1.0, 1.2, 1.6 and 2.0 mg/kg bw/day by gavage for 42 days pre- mating, through mating with untreated males, gestation (GD0 to GD21) until PND4. Dams and offspring were sacrificed on PND5 and blood was collected for TT4, FT4, TT3, FT3 and TSH analysis. Offspring were not dosed directly but were exposed in utero or via lactation. Liver ME activity was measured in livers collected from PND5 dams and offspring from the 0.0, 0.4, 1.6, and 2.0 mg/kg bw/day groups. Thyroids were collected from offspring from the 0 and 2.0 mg/kg bw/day groups for histopathological examination.

120. Mortality and morbidity: In dams, the authors indicate deaths occurred, but these were not attributed to treatment (no further details given). In offspring, the number of dams with stillborn pups was within the historical control range. The number of dams with all offspring dying PND1 to PND5 was significantly increased at 2.0 mg/kg bw/day, compared with controls.

121. General toxicity and body weight: No clinical signs of general toxicity in dams or surviving offspring were reported. In dams, body weights were significantly decreased at 1.6 and 2.0 mg/kg bw/day from GD0 to GD21, and at 2.0 mg/kg bw/day from PND1 to PND5, compared with controls. Body weight gain was significantly decreased at 2.0 mg/kg bw/day for day 1 to day 42 pre-mating, and at doses ≥ 0.8 mg/kg bw/day from PND1 to PND5 (with the exception of 1.2 mg/kg bw/day), compared with controls. The authors state a general trend for decreased feed consumption with increasing dose was seen during pre-mating through to PND4. In offspring, mean pup weights per litter were significantly reduced for all maternal dose groups at birth and on PND5, compared with controls. Histopathology: No microscopic changes were observed in thyroids collected from offspring from the 2.0 mg/kg bw/day group on PND5, compared with controls.

122. Thyroid hormone levels: In dams, TT4 levels were significantly reduced at doses ≥ 0.4 mg/kg bw/day on PND5, while TT3 levels were significantly reduced at doses ≥ 1.2 mg/kg bw/day on PND5, compared with controls. Both TSH and FT4 levels were unaffected by treatment (observations for FT3 levels were not reported). In offspring, TT4 and TT3 levels were significantly reduced at 1.0 mg/kg bw/day on PND5, compared with controls. TSH levels were also significantly elevated at 1.6 mg/kg bw/day on PND5, compared with controls. No significant changes to FT4 levels were observed. The authors considered that the use of the standard reference method for FT4 (analog radioimmunoassay (RIA) kit) can lead to artificially lower FT4 levels (especially when protein bound T4 and serum binding capacity is low, and because the protein bound T4 and FT4 equilibrium can be disturbed by dilution by assay components). A method using equilibrium dialysis followed by radioimmunoassay (ED/RIA) is not prone to these biases and was used here to measure FT4.

123. Liver ME activity measured in dam and offspring on PND5 was unaffected by treatment.

124. The authors concluded that although there were apparent reductions in serum TT3 and TT4 in offspring, on the whole, the data from this study did not suggest a hypothyroid state in offspring. The lack of a major increase in TSH and the fact that liver ME activity, a marker for hepatic response to thyroid hormones, was comparable to controls, suggest that offspring were in a normal (euthyroid) state. In addition, there were no histopathological changes in offspring thyroids based on evaluation of tissues from control and 2.0 mg/kg bw/day groups. In dams, FT4, TSH and liver ME activity were unaltered by PFOS treatment, and serum TT3 and TT4 were reduced.

125. NTP (2022b) investigated the effects of PFOS on thyroid weight, histopathology and TH levels in rats. In a repeated dose study, SD rats (10/sex/group) were administered PFOS at doses 0, 0.312, 0.625, 1.25, 2.5 or 5 mg/kg bw/day by gavage for 28 days. At necropsy on day 29, blood samples were collected for TT4, TT3, FT4, TSH and PFOS analysis, and thyroids were removed for histopathological evaluation.

126. Mortality: One female rat at 5 mg/kg bw/day died, all other rats survived to scheduled necropsy.

127. General toxicity and body weight: No clinical signs of general toxicity were observed. Terminal body weights were significantly reduced at 5 mg/kg bw/day in males and females, compared with controls.

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

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

130. Thyroid hormone levels: In males and females, TT4 and FT4 levels were significantly decreased at ≥ 0.312 mg/kg bw/day, compared with controls. TT3 levels were significantly decreased at ≥ 0.625 mg/kg bw/day, compared with controls. TSH levels were unaffected by treatment.

131. Plasma PFOS concentrations: In males, mean plasma PFOS concentrations on day 29 were ND (control), 23.730 µg/mL (0.312 mg/kg bw/day), 51.560 µg/mL (0.625 mg/kg bw/day), 94.260 µg/mL (1.25 mg/kg bw/day), 173.700 µg/mL (2.5 mg/kg bw/day) and 318.200 µg/mL (5 mg/kg bw/day). In females, mean plasma PFOS concentrations on day 29 were 0.054 µg/mL (control), 30.530 µg/mL (0.312 mg/kg bw/day), 66.970 µg/mL (0.625 mg/kg bw/day), 135.100 µg/mL (1.25 mg/kg bw/day), 237.500 µg/mL (2.5 mg/kg bw/day) and 413.556 µg/mL (5 mg/kg bw/day). 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).

Seacat *et al* 2002

132. Seacat et al. (2002) investigated the effect of PFOS exposure on thyroid organ weight and TH levels in monkeys. C*ynomolgus* monkeys (4 — 6/sex/group) were administered K^+PFOS at doses of 0, 0.03, 0.15 and 0.75 mg/kg bw/day by capsule for 182 days. Two monkeys from the 0, 0.15 and 0.75 mg/kg bw/day groups were monitored for a recovery period of one year following the end of treatment. Blood samples for serum PFOS and TT4, T3 (assumed TT3) and TSH analysis were collected at numerous time points prior to the commencement of treatment, during treatment and during the recovery period. FT4 and FT3 levels were measured on day 184 only. Monkeys were sacrificed on days 184 and 185 and thyroid glands removed for examination.

133. Mortality and morbidity: A male monkey from the 0.75 mg/kg bw/day group died on day 155. A second male monkey from the 0.75 mg/kg bw/day group was in a moribund condition on day 179 and was sacrificed.

134. General toxicity and body weight: No clinical signs of general toxicity were reported. Body weight gain from day 0 to day 184 was significantly reduced

in males and females at 0.75 mg/kg bw/day, compared with controls. Terminal body weights on day 184 were unaffected by treatment. No data are provided for the recovery groups.

135. Gross pathology: No adverse findings regarding thyroid weights were reported.

136. Thyroid hormone levels: A significant increase in TSH levels was observed in males and females at 0.75 mg/kg bw/day on days 182 and 184, compared with controls. A significant decrease in TT3 levels was observed in males at 0.75 mg/kg bw/day on days 62, 91, 182 and 184, and in females at 0.75 mg/kg bw/day on days 91, 182 and 184. FT3 levels were also significantly reduced in males and females at 0.75 mg/kg bw/day on day 184. On day 184, FT4 levels in males and females were unchanged from control values, but a statistically significant reduction in FT3 levels was observed in both sexes but only at a dose of 0.75 mg/kg bw/day. Other changes to TT4, TSH, FT4 and FT3 levels were observed over the various time points monitored throughout the study, however, the authors state these changes were not consistent over time or consistent with a dose-response. In the recovery groups, all TH levels returned to control levels between days 33 to 61.

137. Serum PFOS concentrations: In the 0.75 mg/kg bw/day group, mean PFOS serum concentrations were 173 µg/mL and 171 µg/mL on day 183, in males and females, respectively. In the 0.15 mg/kg bw/day group, mean PFOS serum concentrations were 82.6 µg/mL and 66.8 µg/mL on day 183, in males and females, respectively. No numerical data are presented for the recovery groups, although the authors commented that serum PFOS concentrations declined overall during the recovery period except for an initial increase in the week following cessation of treatment.

138. The authors concluded that significant adverse effects occurred only in the 0.75 mg/kg bw/day group and included compound-related mortality in 2 of 6 male monkeys, decreased body weights and lowered TT3 concentrations (without evidence of hypothyroidism).

Thibodeaux *et al* 2003

139. Thibodeaux *et al*. (2003) investigated the effects of PFOS on thyroid hormones in rats and mice.

140. As part of a developmental study in rats, pregnant SD rats (25 – 50/group) were administered K^+PFOS at doses of 0, 1, 2, 3, 5 or 10 mg/kg bw/day by gavage from GD2 until GD20. Blood samples were collected from dams on GD7, GD14 and at sacrifice on GD21 TT4, FT4, TT3 TSH levels and PFOS concentrations measured. In a separate study, adult female SD rats (6 – 8/group) were treated with K^+PFOS at doses of 0, 3 or 5 mg/kg bw/day by gavage for 20 days. Blood samples were collected from adults on days 3, 7, 14 and at sacrifice on day 20 TT4, FT4, TT3 and TSH levels measured.

141. As part of a developmental study in mice, pregnant CD-1 mice (60 – 80/group) were treated with K^+PFOS at doses of 0, 1, 5, 10, 15 or 20 mg/kg bw/day by gavage from GD1 until GD17. Dams were selected for sacrifice on either GD6, GD12 or GD18. Blood samples were collected at sacrifice for analysis of TT4, FT4, T3 (assumed TT3), TSH levels and PFOS concentrations.

142. General toxicity and body weight: In pregnant rats, PFOS treatment reduced body weight gain in a dose-dependent manner and was significantly reduced at ≥2 mg/kg bw/day compared with controls. The authors state weight gain deficits in pregnant rats corresponded to significant reductions in food consumption. No data are given for adult female rats. In pregnant mice, body weight gain was significantly reduced at 20 mg/kg bw/day during late gestation (inferred GD14 to GD18). Food consumption was unaffected.

143. Thyroid hormone levels: In pregnant rats, both TT4 and FT4 levels were significantly reduced at ≥1 mg/kg bw/day on GD7 to GD21, compared with controls. TT3 levels were significantly reduced at 10 mg/kg bw/day on GD7 to GD21; at 3, 5 and 10 mg/kg bw/day on GD14 and GD21; and at ≥ 1 mg/kg bw/day on GD21. TSH levels were unaffected by treatment. In adult female rats, both TT4 and FT4 levels were significantly reduced at 3 and 5 mg/kg bw/day on day 3 to day 20. TT3 levels were significantly reduced at 3 and 5 mg/kg bw/day on day 7 to day 20. TSH levels were dose dependent and significantly increased at 3 mg/kg bw/day on day 7, returning to within control levels from day 14 onwards. Conversely, TSH levels were decreased (not statistically significant) at 5 mg/kg bw/day from day 3 to day 20. In pregnant mice, TT4 levels were significantly reduced at 20 mg/kg bw/day on GD6, but unchanged compared with controls on GD12 and GD18. FT4, TT3 and TSH levels were unaffected by treatment.

144. Serum PFOS concentrations: In pregnant rats, PFOS concentrations increased with dose, reaching maximum concentrations at GD14 before reducing by PND21. Numerical values were not provided. In pregnant mice, the authors state that serum PFOS concentrations were similar to those in the rat (no further

data provided). The mean PFOS concentration at 10 mg/kg bw/day on GD18 was 190 µg/mL (no further data provided).

145. The authors concluded that serum PFOS levels increased with dosage. Serum T4 (assumed TT4 and FT4) and T3 (assumed TT3) in the PFOS-treated pregnant rats were significantly reduced as early as one week after chemical exposure, although no feedback response of TSH was observed. A similar pattern of reduction in T4 (assumed TT4) was also seen in the pregnant mice. T3 and T4 results from the study with adult female rats by and large substantiated the findings in pregnant rats, discounting potential confounding effects of pregnancy. The adverse effect of PFOS on TH was less pronounced in the mouse than in the rat.

Wang *et al*. 2011

146. Wang *et al*. (2011) investigated the effect of PFOS on TH levels in rats. As part of a developmental study evaluating the mixture effects of PFOS and 2,2’,4,4’-tetrabromodiphenyl ether (BDE-47), a known thyroid hormone disruptor, pregnant Wistar rats (3 – 9 dams/group, 9 – 12 offspring/group) were administered K^+PFOS in the diet at doses 0, 3.2, 32 mg/kg diet /day (converted to 0.38 and 3.8 mg/kg bw/day using conversion factor of 0.12 for rats for a subacute study (EFSA, 2012)) in the diet from GD1 to PND14. Only results from exposure to PFOS alone are reported.

147. Dams and offspring were sacrificed on PND1, 7 and 14 (numbers not specified) and blood samples collected for measurement of TT4 and TT3 levels and serum PFOS concentrations.

148. Mortality: No deaths were attributed to treatment in dams or offspring.

149. General toxicity and body weight: No clinical signs of general toxicity were observed in dams or offspring. At 3.8 mg/kg bw/day, male and female offspring body weights were significantly decreased on PND1, PND7 and PND14 compared with controls. Body weights at 0.38 mg/kg bw/day were unaffected.

150. Thyroid hormone levels: In dams, TT4 levels were significantly reduced at 0.38 and 3.8 mg/kg bw/day on PND1, PND7 and PND14 (no data available for 3.8 mg/kg bw/day on PND7), compared with controls. TT3 levels were significantly reduced at 3.8 mg/kg bw/day on PND1 and PND14 (no data for PND7 at 3.8 mg/kg bw/day). In offspring, TT4 levels were significantly reduced at

0.38 mg/kg bw/day and 3.8 mg/kg bw/day on PND1, PND7 and PND14, with the exception of PND1 (0.38 mg/kg bw/day). TT3 levels were significantly reduced at 0.38 mg/kg bw/day and 3.8 mg/kg bw/day on PND14.

151. Serum PFOS concentrations: In dams, at 0.38 mg/kg bw/day mean concentrations in serum were 2.29 µg/mL, 4.16 µg/mL and 3.15 µg/mL for PND1, PND7 and PND14, respectively; at 3.8 mg/kg bw/day mean concentrations were 16.9, 27.3 and 28.7 µg/mL for PND1, PND7 and PND14, respectively. In offspring, at 0.38 mg/kg bw/day mean concentrations were 5.85 µg/mL, 3.65 µg/mL and 4.89 µg/mL for PND1, PND7 and PND14, respectively; at 3.8 mg/kg bw/day mean concentrations were 32.9 µg/mL, 21.3 µg/mL and 25.2 µg/mL for PND1, PND7 and PND14, respectively. Controls were all ND.

152. The authors concluded that there were exposure- and time-dependent alterations in thyroid hormone concentrations.

Yu *et al*. 2009

153. Yu *et al*. (2009) investigated the effect on TH levels following prenatal and/or postnatal (lactational) exposure to PFOS. In a cross-foster study, pregnant Wistar rats (20/group) were administered K^+PFOS at 0 and 3.2 mg/kg diet/day (converted to 0.29 mg/kg bw/day using conversion factor of 0.09 for rats for a subchronic study (EFSA, 2012)) in the diet from GD0 until weaning on PND21. Two control litters and two PFOS-treated litters were used for sample collection on the day of birth (PND0). Remaining litters were cross-fostered to create the following three groups: litters from control dams fostered by other control dams (unexposed control, n = 8); litters from treated dams fostered by control dams (prenatal PFOS exposure, n = 8), litters from control dams fostered by treated dams (postnatal PFOS exposure, n = 8), and litters from treated dams fostered by treated dams (combined prenatal and postnatal PFOS exposure, n = 10). Litter sizes were adjusted to 10 offspring (5 male and 5 female where possible). Offspring were weaned on PND21.

154. Offspring were weighed and sacrificed on PND0, 7, 14, 21 or 35 and blood and liver samples collected. Serum TT4, TT3, and rT3 levels were measured at all time points from all cross-fostered groups. Total RNA was isolated from livers from offspring from all cross-fostered groups on PND0 and PND21 to estimate transcript levels and gene expression. Genes studied included hepatic genes associated with thyroid function (transthyretin (TTR)), Dio1, UGT1A1, and uridine diphosphoglucuronosyl transferase 1A6 (UGT1A6)), and thyroid receptors in the developing liver (thyroid hormone receptor α (TRα) and thyroid hormone

receptor β (TRβ)). Serum PFOS concentrations were determined for all time points from all cross-fostered groups.

155. General toxicity and body weight: No clinical signs of general toxicity were observed in offspring. Offspring body weights for all groups were unaffected by treatment.

156. Thyroid hormone levels: Combined prenatal and postnatal PFOS exposure resulted in significantly reduced TT4 levels in offspring on PND14, 21 and 35, compared with controls. Prenatal only exposure and postnatal only exposure significantly reduced TT4 levels on PND21 and 35. The authors noted that reduced TT4 levels in postnatally exposed offspring at PND21 and 35 did not occur at PND14, which was attributed to the cumulative effect of PFOS during the lactation and postweaning period. There were no treatment- related effects on TT3 or rT3 levels at any time point or exposure scenario.

157. Gene expression: No alteration in mRNA expression was observed. However, the transcript level of TTR was significantly increased compared with controls at PND21 following combined prenatal and postnatal exposure. The authors proposed that this may not be indicative of TH status due to the absence of TTR up-regulation in the corresponding prenatal or postnatal exposure groups.

158. Serum PFOS concentrations: Serum PFOS concentrations in postnatally exposed offspring increased in a dose-dependent manner. The mean concentrations on PND35 were 6.64 and 7.04 µg/mL in males and females, respectively. A similar trend was observed in offspring with combined prenatal and postnatal exposure. Mean concentrations on PND35 were10.61 and 11.53 µg/mL in males and females, respectively. Conversely, serum PFOS concentrations in prenatally exposed offspring decreased with age. On PND0 concentrations were 5.98 µg/mL from a pooled serum sample, on PND35 concentrations were 0.41 and 1.02 µg/mL, for males and females respectively. Controls were all ND. The authors proposed that these results indicate the transfer of PFOS from dams to offspring via placenta and milk.

159. The authors concluded that prenatal only PFOS exposure and postnatal only PFOS exposure at 0.29 mg/kg bw/day induced hypothyroxinemia in rat offspring to a similar extent, which suggested that prenatal PFOS exposure and postnatal PFOS accumulation, especially though maternal milk, are matters of great concern. Of particular note is that prenatal PFOS exposure significantly suppressed serum TT4 levels on PND21 and 35. The finding suggests that exposure to PFOS during gestation results in a long-lasting adverse effect on serum TH in rat offspring. Although no alteration of mRNA expression was

observed for selected genes that are important for TT4 deiodination, glucuronidation and TH receptors, this does not rule out possible changes in regulation at the translation or post translational levels.