In vitro thyroid toxicity studies and Endpoints investigated

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In vitro thyroid toxicity studies

6. For perfluorosulfonic acids (PFSAs), *in vitro* toxicity data are available for perfluorobutane sulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS), perfluorodecane sulfonate (L-PFDS) and perfluorooctane sulfonic acid (PFOS) (Table 2 to Table 5).

7. For perfluoroalkane sulfinic acids (PFSIAs), data are available for perfluorooctane sulfinate (L-PFOSi) (Table 6).

8. For perfluoroalkyl carboxylic acids (PFCAs), data are available for perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA),

perfluorohexanoate (PFHxA), 7H-perfluoroheptanoic acid (7H-PFHpA), 2H-perfluoro-2-octenoicacid (6:2) (6:2 FTUA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA) (Table 7 to Table 19).

9. For perfluoroalkyl phosphonic acids (PFPAs), data are available for pentafluoropropionic anhydride (C6 PFPA) (Table 20).

10. For fluorotelomer alcohols (FTOH), data are available for 6:2 fluorotelomer alcohol (6:2 FTOH), 8:2 fluorotelomer alcohol (8:2 FTOH) and 10:2 fluorotelomer alcohol (10:2 FTOH) (Table 21).

11. For N-alkylperfluorooctane sulfonamidoethanols (FOSE), data are available for N-methyl perfluorooctane sulfonamide (N-MeFOSA), 2-(N-ethylperfluoro-1-octane sulfonamido) ethanol (N-EtFOSA), 2-(N-methylperfluoro-1-octane sulfonamido) ethanol (N-MeFOSE) and 2-(N-ethylperfluoro-1-octane sulfonamido) ethanol (N-EtFOSE) (Table 22).

12. For perfluorinated sulfonamides (FOSA), data are available for perfluorinated sulfonamide (PFOSA), N,N-dimethyl perfluorooctane sulfonamide (N,N-Me2FOSA) (Table 23).

13. For new generation PFAS, data are available for perfluoro{acetic acid, 2-[(5-methoxy-1,3-dioxolan-4-yl)oxy]}, ammonium salt (C6O4) and 6:2 chlorinated polyfluorinated ether sulfonate (F-53B) (Table 24).

14. Abbreviations used in Table 2 to Table 24 are not spelled out in the tables but are included in the abbreviations list. 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 or anionic form.

Endpoints investigated

15. Multiple endpoints were investigated including competitive binding studies with thyroid hormone (TH) relevant proteins, aryl hydrocarbon receptor (AhR) function, effects on cell proliferation and viability, iodide accumulation, and gene expression.

16. Competitive binding assays (Ren et al., 2016; Weiss et al., 2009) investigated the ability of PFAS to bind with transport proteins (human

transthyretin (TTR) or human thyroxine-binding globulin (TBG)), thereby displacing thyroid hormones (THs) from the transport proteins and decreasing levels of THs in the blood. Ren et al. (2016) used a direct fluorescent ligand binding assay with a fluorescence probe (fluorescein-thyroxine (FT4)) and Weiss et al. (2009) used radiolabelled 125I-labeled thyroxine (T4). The results from these studies enable an assessment of relative potency, relative to T4, of different PFAS.

17. AhR function was investigated by AhR transcriptional activity (AhR-tract), using the AhR-luciferase reporter gene bioassay and transfected mouse Hepa1.1 2cR cells (Long et al., 2013). The assay can detect compounds that can activate or inhibit the AhR, and therefore AhR-dependent gene expression. In the bioassay PFAS were tested alone or in co-treatment with 2,3,7,8 -tetrachlorodibenzo-p-dioxin (TCDD), which is one of the most studied AhR ligands.

18. Cell proliferation and viability were investigated in various studies, using cell lines that are under the influence of THs. Cell lines used included rat pituitary tumour (GH3) cells used in a T-screen assay, Fisher rat thyroid line-5 (FRTL-5) cells and Normal Human Thyroid (NHT) cells.

19. Iodide uptake by thyroid follicular cells is an early step in the synthesis of THs, with sodium iodide symporter (NIS) playing a critical role in this process. Two studies investigated the effect of PFAS on iodide uptake using FRTL5-YFP cells (a clonal population of FRTL-5 cells with stable expression of YFPH148Q/I152L used to monitor intracellular iodide) (Conti et al., 2020) and FRTL-5 cells (De Toni et al., 2022).

20. Increased intracellular cyclic adenosine monophosphate (cAMP) results from the binding of TSH to its membrane receptor and the activation of adenylyl cyclase. cAMP is the main mediator of the stimulating effect of thyroid stimulating hormone (TSH) on TH production, thyroid cell differentiation and growth, and events mediated by cAMP drive the expression of genes such as NIS and thyroperoxidase (TPO). Two studies (Croce et al., 2019; De Toni et al., 2022) investigated the effects of PFAS on cAMP production using FRTL-5 cells.