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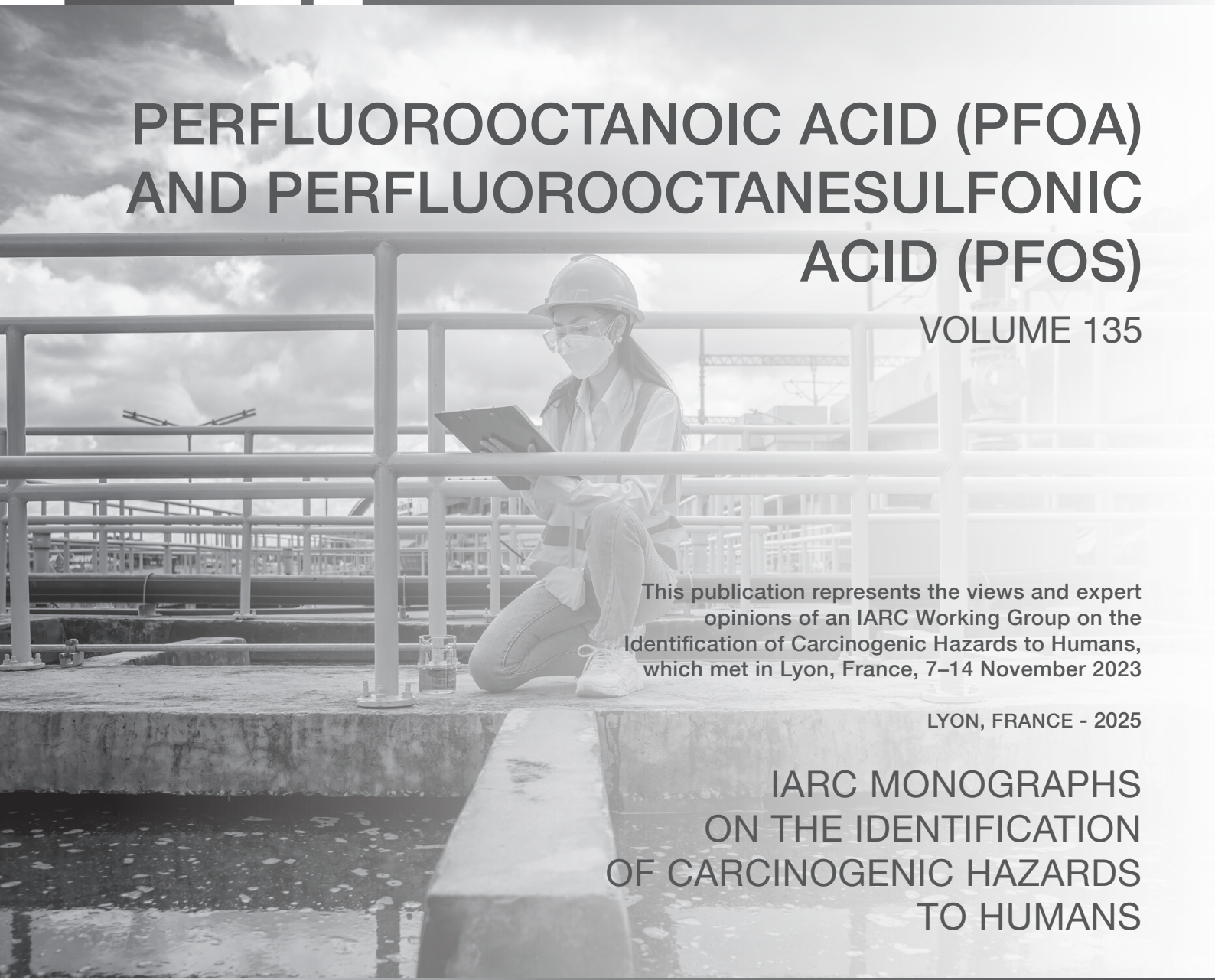

PERFLUOROOCTANOIC ACID (PFOA) AND PERFLUOROOCTANESULFONIC ACID (PFOS)

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4. MECHANISTIC EVIDENCE

4.1 Absorption, distribution, metabolism, and excretion

Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are characterized by long half-lives in humans (years) and much shorter half-lives in experimental animals (days or weeks). Numerous toxicokinetic (TK) studies in laboratory animals were available, and several physiologically based pharmacokinetic (PBPK) models of PFOA and PFOS have been developed. However, human TK data are still scarce in comparison with those from experimental animals, and the mechanistic bases for the observed TK differences among species remain to be fully elucidated.

4.1.1 Humans

(a) Exposed humans

(i) Absorption

Exposure to PFOA and PFOS in non-occupational settings occurs mainly via the oral route. After a single oral dose of ammonium perfluorooctanoate (APFO, 50–1200 mg, as a gelatin capsule) was administered during a clinical trial of patients with cancer, the serum concentrations reached a plateau after approximately 600 hours for the highest dose, but they were still increasing after 864 hours, the latest time point at which they were assessed, for lower doses (Convertino et al., 2018). [The Working Group noted that these doses were much higher than estimated human environmental exposures and that there were no healthy controls in the study. The Working Group also noted that the salt and protonated forms used in this study were comparable to those used in other studies described in this section.] No direct estimates of the oral absorption efficiency of PFOA or PFOS were available for humans. The oral absorption efficiency is assumed to be close to 100%, according to TK models that simulate the human serum concentrations of PFOA, on the basis of comparisons with data obtained from experimental animals. PFOA and PFOS are excreted in the bile (Fujii et al., 2015) and are reabsorbed from the gastrointestinal tract into the enterohepatic circulation; a high absorption efficiency would be required to be consistent with the observed long half-lives of these compounds (Harada et al., 2007).

The inhalation and dermal routes can significantly contribute to PFOA and PFOS exposure in occupational settings, such as in firefighters (Christensen and Calkins, 2023; Mazumder et al., 2023). In a transdermal absorption study of a single volunteer, whole-skin application of [^{13}C]PFOA mixed into sunscreen resulted in the maximum serum concentration 22 days post-application, and the absorption efficiency was estimated to be 1.6% of the administered dose (Abraham and Monien, 2022). No data regarding

the absorption of PFOA or PFOS by inhalation were available to the Working Group.

(ii) Distribution

Few data were available regarding the distribution of PFOA and PFOS, and most of the existing data were obtained from postmortem samples. The liver, blood, and lungs appear to be important sites of accumulation for both compounds, and there are generally lower levels of accumulation in the kidneys, bone, muscle, brain, and fat (Olsen et al., 2003a; Maestri et al., 2006; Pérez et al., 2013; Yeung et al., 2013a; Fàbrega et al., 2014; Mamsen et al., 2019; Di Nisio et al., 2022). In one study, PFOA concentrations in the liver were found to be similar to those in the blood, whereas PFOS concentrations in the liver were higher by approximately 2.7-fold (Maestri et al., 2006). In contrast, accumulation in the liver of laboratory animals appeared to be more marked, as discussed below.

In two other studies, PFOA and PFOS concentrations in human follicular fluid were found to be comparable to those in the serum (Petro et al., 2014; McCoy et al., 2017). PFOA and PFOS have been detected in the thyroid at concentrations just below those in the serum (Pirali et al., 2009). PFOA and PFOS have also been found in semen and cerebrospinal fluid, but at much lower levels than in serum (Raymer et al., 2012; Wang et al., 2018a; Di Nisio et al., 2019). In addition, PFOA and PFOS have been detected in the amniotic fluid, placenta, cord blood, and embryonic tissues at lower concentrations than those in the maternal serum (Zhang et al., 2013a; Mamsen et al., 2019).

The elimination of PFOA and PFOS from human serum has been demonstrated to follow first-order kinetics, and therefore the overall distribution can be characterized using the volume of distribution (V_d) (Harada et al., 2005; Olsen et al., 2007; Russell et al., 2015). Based on a Bayesian TK analysis of data from 13 studies in which PFOA and PFOS serum levels, PFOA and PFOS concentrations in drinking-water, and background PFOA and PFOS exposures were considered, the V_d for a one-compartment TK model was estimated to be 0.43 L/kg and 0.32 L/kg for PFOA and PFOS, respectively (Chiu et al., 2022). [The Working Group noted that these values are higher than some of the previously proposed PFOA and PFOS V_d estimates, but they appeared to be based on the largest dataset, analysed using the best available methods.] The previously widely used PFOA V_d estimate of 0.17 L/kg was derived from a single dataset using a similar approach (Thompson et al., 2010). This value was used as the initial estimate for the PFOA V_d in the Bayesian analysis performed by Chiu et al. (2022).

On the basis of their structure and physicochemical properties, PFOA and PFOS are unlikely to cross cellular membranes directly. Various membrane transporters are thought to mediate their transmembrane transport, as described in more detail in the section regarding in vitro studies below.

Blood is an important compartment for the accumulation of PFOA and PFOS. PFOA and PFOS in the blood primarily distribute to the serum, but a significant fraction also partitions to blood cells, indicating that the use of a default factor of two to account for the volume of plasma in whole blood (a common practice when no prior knowledge about chemical distribution between blood fractions is available) may not be an appropriate method for the blood-to-serum concentration conversion (Jin et al., 2016; Poothong et al., 2017; Liu et al., 2023a). Indeed, in two studies in which the mass fraction in plasma (F_p) was measured, it was 0.7–0.8 for PFOA and 0.8–0.85 for PFOS (Jin et al., 2016; Liu et al., 2023a). [The Working Group noted that F_p would equal 0.5 if the compound distributed to plasma.] In fractionated plasma collected from healthy volunteers (n = 4), PFOA and PFOS were bound to albumin, with little affinity for lipoproteins (Forsthuber et al., 2020).

An analysis of biomonitoring data using PBPK modelling showed that serum concentrations of PFOA and PFOS were lower in women of reproductive age than in age-matched men. This was thought to be the result of menstrual blood loss and placental and lactational transfer associated with pregnancy and childbirth (Wong et al., 2014; Gomis et al., 2017).

PFOA and PFOS cross the placenta and can also be transferred to infants via breast milk. In a review of the available studies, it was calculated that the placental transfer (median and range), defined as the ratio of PFAS fetal (cord blood) concentration to the maternal serum concentration, was 0.79 (0.60–1.5) for PFOA and 0.37 (0.29–0.56) for PFOS (Pizzurro et al., 2019). PFOS concentrations in maternal serum during the first trimester were significantly higher than those during the second and third trimesters, but there were no significant differences in PFOA concentration between trimesters (Mamsen et al., 2019). In another study, PFOA and PFOS concentrations both significantly decreased by approximately 40% between 16 weeks of gestation and time of delivery (Kato et al., 2014). [The Working Group noted that decreases in PFOA and PFOS levels in pregnancy were probably because of blood volume expansion and increase in the glomerular filtration rate (GFR) and, to a minor extent, transfer to the fetus. Differences among studies could be caused by differences in PFOA levels and experimental variation.]

Lactational transfer (median and range of the values obtained from the available studies), defined as the ratio of the breast milk to the maternal serum concentration, was calculated to be 0.04 (0.03–0.12) for PFOA and 0.01 (0.01–0.03) for PFOS (Pizzurro et al., 2019). Lactational PFOA transfer probably accounts for the higher (2.7–4.6-fold) PFOA serum concentrations in infants aged 2–6 months than in mothers. However, PFOS concentrations were similar in infants and mothers (Fromme et al., 2010; Gyllenhammar et al., 2018). By age 18 months, the mean PFOA concentration in the infants' serum was similar to that in the mothers' serum (Højsager et al., 2022), probably because of the cessation of breastfeeding and the growth-dependent dilution of PFOA according to body burden. There was very high inter-individual variability in the time-course profiles for PFOA and PFOS concentrations in infants (Mogensen et al., 2015a). Serum PFOA and PFOS concentrations in children were similar at age 2, 4, and 6 years (Kim et al., 2020). On the basis of the available data, Goeden et al. (2019) developed a TK model that predicts serum PFOA concentrations in people of all ages, including the very young.

The available evidence suggested that PFOA and PFOS undergo enterohepatic circulation. Although these compounds were not detected (< 0.5 ng/g) in the faeces of a single participant (serum concentrations 6.8 and 26.0 ng/g for PFOA and PFOS, respectively), the addition of cholestyramine, which inhibits the reabsorption of bile acids and thereby increases their excretion, to the diet for 1 week (4 g, three times per day) resulted in detectable faecal PFOA (0.96 ng/g) and PFOS (9.06 ng/g) (Genuis et al., 2010). Harada et al. (2007) measured the PFOA and PFOS concentrations in bile (4 participants) and, using the total and urinary clearances of these compounds, calculated their biliary reabsorption rates (the proportions reabsorbed) to be 0.89 and 0.97, respectively. The biliary reabsorption rate of PFOA was found to be 0.98 in another study (n = 5) in which similar methodology was used (Fujii et al., 2015).

PFOA and PFOS are present in the environment as a mixture of linear and branched isomers. PFOA is primarily present as a linear isomer in various exposure matrices, whereas the PFOS isomer composition is more variable (Shan et al., 2016). In human blood and serum, the fraction of the linear PFOA isomer (n-PFOA) is 96–100% (Zhang et al., 2014a, 2017a), which is consistent with the high proportion of n-PFOA in food and

drinking-water and its expected slower elimination compared with branched isomers (Zhang et al., 2013b; Zhou et al., 2014). In contrast, the proportion of *n*-PFOS in the blood or serum (35–80% of total blood PFOS) is lower than would be expected in most exposure scenarios (Zhang et al., 2014a, 2017a; Gebbink et al., 2015; Salihović et al., 2015; Nilsson et al., 2022). This apparent relative enrichment of branched isomers could be the result of the metabolism of unknown PFOS precursor(s) to form branched isomers (Gebbink et al., 2015; Shan et al., 2016).

The proportion of *n*-PFOA in cord serum has been reported to be 98–99% (Beesoon et al., 2011; Zhang et al., 2017a), and that of *n*-PFOS to be 52–75% of the total, which was lower than that in the maternal serum in studies in which such data were available (Hamm et al., 2010; Beesoon et al., 2011; Zhang and Qin, 2014; Zhang et al., 2017a).

(iii) Metabolism

No in vivo data on the metabolism of PFOA and PFOS were available to the Working Group. On the basis of their chemical structure and data demonstrating a lack of metabolism in experimental animals, PFOA and PFOS are not expected to be metabolized in humans.

The metabolism of precursor compounds, including fluorotelomer alcohols, perfluoroalkyl sulfonamides, and amidoalcohols, to form PFOA and PFOS was estimated to contribute to 2–8% and 2–5% of the internal doses of PFOA and PFOS, respectively, in an intermediate-level exposure scenario, and to 28–55% and 60–80%, respectively, in a high-level exposure scenario (Vestergren et al., 2008). By using exposure and biomonitoring data in a TK model, Gomis et al. (2016) estimated that the metabolism of 8:2 fluorotelomer alcohol contributed to 45% of the serum concentration of PFOA in ski-waxers who have a high level of occupational exposure.

(iv) Excretion

PFOA and PFOS are characterized by very long half-lives in humans, with estimates of 2–5 years reported in most studies (Harada et al., 2005; Olsen et al., 2007; Spliethoff et al., 2008; Costa et al., 2009; Bartell et al., 2010; Brede et al., 2010; D'eon and Mabury, 2011; Seals et al., 2011; Glynn et al., 2012; Olsen et al., 2012; Yeung et al., 2013b; Zhang et al., 2013b; Russell et al., 2015; Fu et al., 2016; Gomis et al., 2016, 2017; Worley et al., 2017a; Li et al., 2018a; Xu et al., 2020a). [The Working Group noted that these were observational population kinetic studies, in which participants may have been subject to unspecified background exposures, in addition to known contamination sources.] Recently, Chiu et al. (2022) applied a Bayesian TK analysis to data from 13 studies of the association between exposure and serum concentration; they reported serum half-life ($T_{1/2}$) estimates for the population geometric mean of 3.14 years for PFOA and 3.36 years for PFOS.

[The Working Group noted that several studies have investigated the serum half-lives of PFOS isomers (Xu et al., 2020a; Li et al., 2022a; Nilsson et al., 2022), but the results were inconsistent among studies.]

Xu et al. (2020a) reported a longer $T_{1/2}$ for *n*-PFOS than for the sum of branched-chain forms of PFOS, but the other two studies conducted by the same research group reported shorter $T_{1/2}$ estimates for *n*-PFOS than for the branched isomers (Li et al., 2022a; Nilsson et al., 2022).

In humans, PFOA and PFOS are primarily eliminated in the urine and faeces. In women of reproductive age, blood loss during menstruation, fetal transfer during pregnancy, and lactational transfer during breastfeeding are also thought to reduce the body burden of PFOA and PFOS (Mondal et al., 2014; Wong et al., 2014; Gomis et al., 2017; Pizzurro et al., 2019).

Multiple studies reported renal clearance of PFOA and PFOS, with estimates of 0.03–0.8 mL/(kg-day) and 0.01–0.03 mL/(kg-day), respectively, calculated in a 24-hour period and assuming an average human body weight of 50–70 kg (Harada et al., 2005; Zhang et al., 2013b; Zhou et al., 2014; Fujii et al., 2015; Gao et al., 2015a; Zhang et al., 2015a; Fu et al., 2016).

Linear PFOA and PFOS isomers appeared to be eliminated more slowly than branched isomers in the urine (Zhang et al., 2013b; Gao et al., 2015a). In only one study, PFOA and PFOS levels were reported to be above the limit of detection in the faeces, and only after addition of the bile acid sequestrant cholestyramine to the diet (Genuis et al., 2010).

A comparison of the urinary and total elimination rates by Fujii et al. (2015) showed that the faecal and urinary PFOA clearances contribute approximately 54% (0.052 mL/(kg-day) and 46% (0.044 mL/(kg-day), respectively, to the overall (serum) clearance, calculated in a 24-hour period and assuming a human body weight of 50 kg.

Using the same approach, Harada et al. (2007) found that urinary PFOA clearance constitutes approximately 20% of the total serum clearance – 0.03 mL/(kg-day) to 0.15 mL/(kg-day) – and urinary PFOS clearance constitutes approximately 14% – 0.015 mL/(kg-day) to 0.106 mL/(kg-day).

[The Working Group noted that the urinary clearance appears to be of less importance as an excretion route for PFOA and PFOS in humans than the estimated faecal clearance. The limits of detection for PFOA and PFOS in faeces appear to be higher than for other physiological matrices, which may explain the scarcity of published data.]

The body burden of PFOA and PFOS, indicated by serum concentrations, increases in response to exposure to these chemicals. Their excretion increases with increasing serum concentration until, if the intake is constant, an equilibrium is reached. The relation between intake and the serum concentration at steady state can be estimated, assuming first-order kinetics. One-compartment TK models are widely used in the literature to back-calculate PFOA and PFOS exposure (ng/kg per day) from serum levels (ng/mL). The outcome in these models depends on selected V_d and $T_{1/2}$ values. Several two-compartment human TK models have also been developed for PFOA and PFOS, to investigate gestational and lactational transfer or to fit TK data from a clinical trial with PFOA (Verner et al., 2016; Convertino et al., 2018; Goeden et al., 2019). Several multicompartment PBPK human models have been developed for PFOA and PFOS that included renal reabsorption, to account for the long retention times in humans (Loccisano et al., 2011, 2013; Fàbrega et al., 2014; Worley et al., 2017b; Chou and Lin, 2019). Of note, these models do not include either the faecal elimination route (Loccisano et al., 2011, 2013; Fàbrega et al., 2014) or the complete enterohepatic loop (Worley et al., 2017b; Chou and Lin, 2019).

[The Working Group noted that not fully accounting for faecal elimination would probably cause an overestimation of the effect of renal elimination relative to total elimination, with possible implications for PBPK studies of confounders dependent on renal elimination. The Working Group noted that a recently published PBPK model for PFOA (Husøy et al., 2023) appeared to include the faecal elimination route; however, this model was not evaluated in the present monograph.]

(b) Human cells in vitro

Approximately 24% of the applied dose of PFOA was found to penetrate human skin in vitro, and the skin permeability coefficient for ionized PFOA (a physiologically relevant form) was 6.0×10^{-5} cm/hour (Franko et al., 2012).

Cell-free and cell culture in vitro experimental systems have been used to characterize the interactions of PFOA and PFOS with membrane transporters, serum albumin, liver fatty acid-binding protein (L-FABP), thyroid receptor,

and transthyretin. Interactions with membrane transporters probably mediate the absorption and reabsorption of PFOA and PFOS from the gastrointestinal tract, their reabsorption in the kidney, and their ability to cross the placenta. High-affinity binding to serum albumin and L-FABP may underly the accumulation of PFOA and PFOS in serum and the liver.

Most available data regarding the interactions of PFOA and PFOS with transporters concern organic anion transporting polypeptides (OATPs), a family of proteins that are expressed in the gut, kidney, and placenta.

In uptake studies of cells that were stably transfected with human membrane transporters, PFOA was found to be transported by (human) OAT1 (K_m = 48 μM), OAT3 (K_m = 49.1 μM), OAT4 (K_m = 310 μM), URAT1 (K_m = 64.1 μM) and Na^+/taurocholate cotransporting polypeptide (NTCP) (K_m = 1.8 mM) (Nakagawa et al., 2008, 2009; Yang et al., 2010; Kummu et al., 2015), but not by OAT2, OATP1A2, or ABCG2 (Nakagawa et al., 2008; Yang et al., 2010; Kummu et al., 2015). In uptake studies of cells that were transiently transfected with human membrane transporters, PFOS was transported by (human) OSTα/β (no reported K_m) and NTCP (K_m = 130 μM), but not by ABCG2 (Zhao et al., 2015).

Kummu et al. (2015) proposed that OAT4 is involved in the transport of PFOA across the placenta, on the basis of a positive correlation between protein expression and the transport of PFOA by isolated placenta cells. Kimura et al. (2017) showed a decrease in the uptake of PFOA across the apical membrane of human intestinal Caco-2 cells when they were co-incubated with PFOA and inhibitors of OATPs or with the OATP substrate sulfobromophthalein. Furthermore, PFOA inhibited the uptake of sulfobromophthalein at this location (Kimura et al., 2020).

HEK293 cells stably transfected with NTCP exhibited PFOA uptake (K_m = 1.8 mM) and the inhibition of taurocholate uptake by PFOA (K_i = 7.5–28 μM) (Ruggiero et al., 2021).

PFOA and PFOS bind to several human proteins in cell-free systems, including to serum albumin, with dissociation constants $K_d = 3.7 \times 10^{-6}$–4×10^{-4} M for PFOA, and $4.5–20 \times 10^{-5}$ M for PFOS, and to L-FABP with $K_d = 2.4–50.4 \times 10^{-6}$ M for PFOA and 18.5×10^{-6} M for PFOS (Han et al., 2003; Messina et al., 2005a; Chen and Guo, 2009; Li et al., 2009; Wu et al., 2009; Luo et al., 2012; Zhang et al., 2013c; Sheng et al., 2016; Maso et al., 2021). [Where appropriate, the Working Group converted association or absorption constants to dissociation constants for the purposes of comparison; see Supplementary Table S4.1, in Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636.] PFOA was able to displace the endogenous ligand oleic acid from human serum albumin indicating the potential to interfere in fatty acid transport in the blood (D'Eon et al., 2010).

The linear isomers *n*-PFOA and *n*-PFOS bind much more tightly to human serum albumin than do the branched isomers, with dissociation constants that are several orders of magnitude lower (Beesoon and Martin, 2015). As described in more detail in Section 4.2.8, PFOA and PFOS were able to displace thyroid hormones (T3, T4) from the human thyroid receptor and the thyroid hormone transporter protein transthyretin (Weiss et al., 2009; Ren et al., 2015, 2016).

(c) Considerations regarding exposure metrics

Cross-sectional measurements of serum concentrations of PFOA and PFOS are often used to study associations in epidemiological investigations, because of cost constraints, and with the assumption that such metrics would represent quantitative indicators of exposure over an appropriate time frame for the chronic

effects studied (see Section 1.6.2, and Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans, available from: https://publications.iarc.who.int/636). In support of this approach, consecutive measurements of the serum concentrations of PFOA and PFOS, measured in the same non-occupationally exposed individuals in 1979, 1986, 1994, 2001, and 2007, were closely correlated (Spearman ρ coefficient, > 0.6), even though the mean levels increased 3.66-fold, peaking in 2001, and then decreased in 2007 (Nøst et al., 2014). In another investigation of the applicability of the use of a single serum PFOA measurement in human studies, retrospective PFOA exposure was reconstructed for participants in the C8 cohort, using geocoding, documented air and water concentrations, and a pharmacokinetic model with an approximate Bayesian computation (Zhu et al., 2022). These data were combined with the measured PFOA serum concentrations to produce a better longitudinal estimate of PFOA exposure. Associations between PFOA and pre-eclampsia based on reconstructed historical PFOA exposure estimates in this population were found to be similar to those reported, based on one-time PFOA measurements (Savitz et al., 2012), suggesting that the rank order of exposure estimates of study participants was important for statistically significant associations, and not the exact exposure amount (Zhu et al., 2022).

On the basis of current understanding of PFOA and PFOS kinetics, various physiological factors can affect their serum concentrations and lead to possible reverse causation effects in epidemiological analyses of associations. This type of bias has been termed pharmacokinetic bias, and several examples regarding PFOA and PFOS have been described in a recent review (Andersen et al., 2021). These examples are detailed below.

Dhingra et al. (2017) examined associations of menopause and estimated glomerular filtration rate (eGFR) with the measured or modelled serum concentrations of PFOA in a highly exposed cohort. Serum PFOA concentrations were from single sample collections, and the modelled PFOA concentrations were based on the retrospective exposure to PFOA and a one-compartment TK model (Shin et al., 2011). The modelled PFOA concentrations were generated independently of the measured concentrations and had a Spearman correlation of $\rho = 0.71$ (Winquist et al., 2013; Dhingra et al., 2017). Menopause and eGFR were found to be significantly associated with the measured, but not modelled, serum PFOA concentration (Winquist et al., 2013; Dhingra et al., 2017). Specifically, there was an inverse relation (negative trend) of eGFR with the quintile of measured serum PFOA concentration, but neither the modelled serum PFOA concentration nor the modelled cumulative exposure showed an association with eGFR (Dhingra et al., 2017). In the same study, a significant increasing trend for reported menopause was identified alongside an increase in the measured serum PFOA categories, but not in the modelled metrics (Dhingra et al., 2017). [The Working Group noted that these findings can be interpreted as an instance of reverse causation, in which changes in eGFR, which also occur as a consequence of menopause, would directly affect PFOA excretion and serum PFOA concentration.]

The same reverse causation mechanism, in which changes in GFR would affect concentrations of PFOA and/or PFOS and the outcome of interest, has been investigated for potential associations with respect to low birth weight (Verner et al., 2015). A human pregnancy PBPK model of PFOA/PFOS was updated and parametrized by Loccisano et al. (2013) to incorporate the relation between GFR and birth weight, based on the results of previous studies of substances other than perfluoroalkyl and polyfluoroalkyl substances (PFAS). A PBPK model-driven meta-analysis was performed, investigating associations of serum PFOA and PFOS concentrations with birth weight, using data obtained from seven

studies. The use of the modified PBPK model was intended to "remove" the confounding effect of low birth weight on GFR, and to simulate direct effects of increases in serum PFOA and/or PFOS concentrations on birth weight. The resulting levels were approximately 50% of those reported on the basis of measured PFOA and PFOS concentrations. The authors concluded that a substantial proportion of the reported associations of prenatal PFOA and PFOS concentrations with birth weight was attributable to confounding by GFR (Verner et al., 2015). [The Working Group noted that the TK adjustment for GFR confounding in the described examples relied on models that assumed urinary excretion to be the only excretion route. However, faecal elimination in humans also appeared to be equally, if not more, important. Thus, such adjustments may overestimate the effects of GFR and urinary elimination on serum PFOA/PFOS levels.]

Hypotheses regarding reverse causation have been also investigated with respect to associations of serum PFOA and PFOS concentrations with serum cholesterol (EFSA, 2018; Steenland et al., 2020). Two possible mechanisms were proposed. Firstly, PFOA and PFOS might preferentially distribute to cholesterol-containing lipoprotein particles in the blood, resulting in greater accumulation in the presence of higher cholesterol concentrations, therefore explaining the reverse causation. The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain provided a detailed analysis of this hypothetical mechanism and concluded that it was not supported by the available mechanistic data (EFSA, 2018). Secondly, the concentrations of serum PFOA and PFOS might be influenced by inter-individual variability in bile acid transporters, for which they are substrates, in enterohepatic resorption and would correlate with bile acid concentrations by virtue of sharing the same retention mechanism. However, an increase in bile acid concentrations would inhibit the metabolism of cholesterol to form bile acids, resulting in increases in cholesterol concentrations and an indirect relationship of the PFOA and PFOS concentrations with those of cholesterol. This mechanism was also briefly discussed in the updated EFSA report of 2020, in light of the available findings (EFSA, 2020).

One of the proposed reasons against this mechanism was a study that found significant associations for serum cholesterol based on estimated PFOA/PFOS external exposures, and not just serum levels (Li et al., 2020a). Additionally, in a study that reported associations between 20 measured PFAS (including PFOA and PFOS) and 19 bile acids in samples from healthy individuals (n = 20), the overall trend was that the concentrations of the majority of bile acids were negatively associated with those of PFAS (Salihović et al., 2020a), also indicating a lack of support for the shared retention mechanism. [The Working Group noted that the existing evidence does not support the proposed mechanisms of reverse causality between the measured PFAS and serum cholesterol.]

The effects of advanced cancers on the TKs of PFOA and PFOS remain unclear. PFOA has been previously tested in a phase 1 therapeutic trial of 43 patients with cancers at various stages, and the TK data obtained were made publicly available in a patent application (Elcombe et al., 2013). [The Working Group noted that these data have not been curated or peer-reviewed.] In this trial, up to 1200 mg of PFOA was administered to the patients once per week for 6 weeks, and their serum PFOA concentrations were monitored. The experimental set-up and the results have been described and discussed in separate publications (Convertino et al., 2018; Dourson et al., 2019), in which the authors noted that secondary to this very high level of exposure, which exceeded the typical level of environmental exposure by several orders of magnitude, the serum PFOA concentration appeared to reach steady state within weeks, which would correspond to a much

shorter half-life estimate than the 2–5 years that has generally been recorded in the literature (see above). [The Working Group noted that it was not clear whether the apparently faster elimination was because of higher PFOA doses or altered physiological parameters in the study participants, all of whom were patients with cancer. The study did not appear to include healthy controls.] [The Working Group noted that there was no information available on the kinetic effects of cancer or cancer treatment on PFOA or PFOS levels in patients with cancer.]

4.1.2 Experimental systems

(a) Experimental systems in vivo

The TK mechanisms associated with PFOA and PFOS have been extensively investigated in the mouse and rat, and a brief review of the findings obtained using these two species follows. In addition, information obtained from primates is included, where available.

The oral absorption efficiencies of PFOA and PFOS have been estimated in animal studies by comparing the area under the curve (AUC) of the serum/plasma concentration, with the dose-adjusted AUC obtained using data collected after an intravenous (i.v.) dose (an oral/i.v. ratio of 1 indicates 100% absorption efficiency), or alternatively by estimating the amount of the administered compound that remained in the faeces during the 24 hours after administration. [The Working Group noted that the second method could underestimate absorption efficiency, because some excretion via the faecal route would be expected within the observation period.] Using the first method, a comparison of AUCs yielded an oral absorption efficiency for PFOA of close to 100% in rats (Kim et al., 2016a; Dzierlenga et al., 2020) and mice (Fujii et al., 2015), and also for PFOS in rats (Kim et al., 2016a; Huang et al., 2019a, 2021). There were no studies available measuring PFOS in mice. With the second method (subtraction of the excretion via faecal route from 100%), the PFOA oral absorption efficiency was ≥ 93% in rats (Cui et al., 2010), and the PFOS oral absorption efficiency in mice was ≥ 98% (Jandacek et al., 2010).

PFOA exposure via inhalation increased the plasma PFOA concentrations of male and female rats in a dose-dependent manner (Hinderliter et al., 2006), and these authors concluded that a route-to-route extrapolation would be possible using the plasma PFOA concentration as an index of the internal dose metric. [No other TK studies of inhalation were available to the Working Group.]

In rats, the dermal application of APFO increased blood organofluorine concentration (a surrogate for PFOA concentration) in a dose-dependent manner, indicating that PFOA is absorbed through the skin (Kennedy, 1985). Similarly, the topical application of PFOA increased serum PFOA concentration of mice in a dose-dependent manner (Franko et al., 2012).

The Working Group identified more than 50 studies on PFOA and PFOS distribution in rats and mice, including studies of single and multiple dosing; studies of oral administration by gavage, in the drinking-water or in the diet; i.v. and intraperitoneal (i.p.) administration; and studies in pregnant animals (e.g. Ylinen et al., 1990; Vanden Heuvel et al., 1991; Austin et al., 2003; Luebker et al., 2005; Hundley et al., 2006; Kudo et al., 2007; Benskin et al., 2009; Cui et al., 2009; Bogdanska et al., 2011; Fujii et al., 2015; Iwabuchi et al., 2017; Dzierlenga et al., 2020). The data on organ distributions identified for PFOA and PFOS were generally consistent across the studies, with liver being the primary site of accumulation of both compounds, followed by the kidney and the blood/serum. Lower levels of accumulation were detected in the brain, fat, muscle, thyroid, testes, thymus, and skin than in the serum. PFOS was found to preferentially accumulate in the lungs in both mice and rats, but PFOA was not. The data available for monkeys were limited to the liver and serum, and showed preferential

accumulation of PFOS in the liver (Seacat et al., 2002), but much lower levels of PFOA in the liver than in the serum (Griffith and Long, 1980; Butenhoff et al., 2004). Most animal studies were conducted using low to intermediate milligram per kilogram doses (either single or repeated administration). Several of the studies included doses in the microgram per kilogram range (e.g. Seacat et al., 2002; Bogdanska et al., 2011; and Li et al., 2017a), and these generated organ distribution data that were comparable to the results obtained using higher doses.

PFOA and PFOS are efficiently transferred to fetuses during pregnancy and to pups via lactation (Lau et al., 2003; Chang et al., 2009; Fenton et al., 2009; Macon et al., 2011; Lai et al., 2017a). Similar to humans, PFOA and PFOS are involved in enterohepatic resorption, because cholestyramine treatment in the diet for 14 days in rats substantially increased the levels of [^{14}C]PFOA and [^{14}C]PFOS in the faeces after a single i.v. dose of PFOA at 13.3 or PFOS at 3.4 mg/kg (Johnson et al., 1984). PFOA and PFOS isomers showed minor differences in their organ distributions in male and female rats (Benskin et al., 2009; De Silva et al., 2009).

In the literature, there were several estimates of V_d in rats, mice, and monkeys. For comparison purposes, the following examples are from studies in which a one-compartment TK model was assumed or in which a non-parametric analysis was applied.

In rats treated with a single oral dose of PFOA (1 mg/kg) or PFOS (2 mg/kg), the PFOA V_d estimates were approximately 106 and 154 mL/kg for males and females, respectively, and the PFOS V_d estimates were approximately 280 and 289 mL/kg for males and females, respectively (Kim et al., 2016a).

In mice treated with a single i.v. dose of PFOA (0.13 mg/kg), V_d estimates were approximately 180 mL/kg in males and 150 mL/kg in females (Fujii et al., 2015). In mice treated with a single oral dose of PFOS (1 or 20 mg/kg), the V_d estimates were approximately 263–290 mL/kg in males and 258–261 mL/kg in females (Chang et al., 2012).

In monkeys treated with a single i.v. dose of PFOA (10 mg/kg), the V_d estimates were 181 and 198 mL/kg for males and females, respectively (Butenhoff et al., 2004). In monkeys treated with a single oral dose of PFOS (9 or 14 mg/kg), the V_d estimates were 127–135 mL/kg and 127–141 mL/kg in males and females, respectively (Chang et al., 2017).

[The Working Group noted that the magnitude of the V_d estimates for rats, mice, and monkeys (150–250 mL/kg) is consistent with the current understanding of the PFOA and PFOS distribution mechanism, with strong binding to proteins in the blood and accumulation in several organs.]

Two detailed studies of PFOA metabolism in rats were available. Vanden Heuvel et al. (1992) treated male and female rats with a single i.p. dose of [1-^{14}C]PFOA (9.4 µmol/kg or 3.9 mg/kg) and investigated the tissue distribution and tissue-specific concentrations at various time points up to 28 days. They did not find PFOA metabolites or conjugates in many tissues (liver, kidney, heart, muscle, fat, or testes) or in the plasma, urine, bile, or faeces, based on the similarity of the high-performance liquid chromatography (HPLC) chromatograms of the sampled extracts to the chromatogram of the parent compound. In addition, they found no increases in the serum or urine concentrations of fluoride, which would have indicated PFOA defluorination (Vanden Heuvel et al., 1991). In a study by Goecke et al. (1992), a single i.p. dose of PFOA (50 mg/kg) was administered to male rats, and ^{19}F-nuclear magnetic resonance spectra were acquired from blood, urine, bile, and liver homogenates 3 days after dosing. Only resonances referable to the parent compound were identified in all the analyses performed, which implies a lack of covalent modification of the PFOA molecule, i.e. absence of metabolites or

conjugates (Goecke et al., 1992). Other studies in rats confirmed the lack of phase I or phase II metabolism of PFOA (Ophaug and Singer, 1980; Ylinen et al., 1989; Kuslikis et al., 1992).

[The Working Group noted that, on the basis of the structural similarities between PFOA and PFOS and similarities in their TK mechanisms among species, PFOA and PFOS are not expected to be metabolized in mammals.]

There are species- and sex-specific differences with respect to the elimination of PFOA and PFOS. Estimates of the serum $T_{1/2}$ for PFOA in male rats after oral administration ranged from 6.4 days to 13.4 days on the basis of five studies that included a total of 12 dose groups (Kudo et al., 2002; US EPA, 2003; Benskin et al., 2009; Iwabuchi et al., 2017; Dzierlenga et al., 2020). Only one study with a single dose group produced a lower estimate of oral PFOA serum $T_{1/2}$ of 1.64 days (Kim et al., 2016a). However, the serum $T_{1/2}$ estimates for PFOA for female rats ranged from 2.75 to 13.9 hours, based on the results of three studies comprising a total of 10 dose groups (US EPA, 2003; Kim et al., 2016a; Dzierlenga et al., 2020). It was hypothesized that the sex-specific differences in rats with respect to the elimination of PFOA are the consequence of differential expression of specific transporter proteins in the kidneys, resulting in more effective reabsorption in males (Weaver et al., 2010; Pizzurro et al., 2019).

After a single oral dose by gavage, the serum $T_{1/2}$ for PFOA was 21.7 days in male mice and 15.6 days in female mice (Lou et al., 2009). The only available estimates for primates were obtained in monkeys (*Simia cynomolgus* or *Macaca fascicularis*). Butenhoff et al. treated monkeys with PFOA as an i.v. dose of 10 mg/kg, yielding a serum $T_{1/2}$ estimate for PFOA of 13.6–35.3 days in males and 26.8–41.7 days in females (Butenhoff et al., 2004).

In rats, the serum $T_{1/2}$ estimates for PFOS after gavage were 8.23–41.19 days for males and 23.5–71.13 days for females in three studies of a total of 5 dosage groups (Chang et al., 2012; Kim et al., 2016a; Huang et al., 2019a, 2021).

Longer $T_{1/2}$ estimates were obtained for female rats than for males in one of these studies (Chang et al., 2012), but no sex-specific differences were identified in the other two.

In mice, the serum $T_{1/2}$ estimates for PFOS after oral administration were 36–43 days for males and 30–38 days for females, with no significant sex-specific differences (Chang et al., 2012). In monkeys, the serum $T_{1/2}$ estimates for PFOS after oral administration were 117–200 days for males and 102–200 days for females, also with no significant sex-specific differences (Seacat et al., 2002; Chang et al., 2017).

Linear *n*-PFOA and *n*-PFOS isomers appear to be eliminated more slowly than the branched isomers. When mixtures of PFOA or PFOS isomers were administered to male rats by oral gavage, isomer-specific blood elimination $T_{1/2}$ was determined (Benskin et al., 2009). In this study, *n*-PFOA was found to have the longest blood elimination $T_{1/2}$, 13.4 days, compared with 1.28–9.10 days for the branched isomers; the $T_{1/2}$ for *n*-PFOS (33.7 days) was at the top end of the range obtained (the geometric mean for all the PFOS isomers was 23.8 days) (Benskin et al., 2009).

In rats and mice, PFOA and PFOS are eliminated in the urine and faeces. Vanden Heuvel et al. (1991) measured [^{14}C]PFOA elimination in male and female Sprague-Dawley rats. They found that, in females, 91% of the i.p. dose (9.4 µmol/kg or 3.9 mg/kg) was eliminated in the urine during the first 24 hours, with negligible amounts being present in the faeces, whereas in males, over the 28-day collection period, 36.4% and 35.1% of the administered dose was recovered in the urine and faeces, respectively (Vanden Heuvel et al., 1991). However, Cui et al. (2010) found greater excretion (approximately two-fold) of PFOA in the urine than in the faeces during the daily administration of PFOA by gavage at 5 or 20 mg/kg to male Sprague-Dawley rats. In this

study in male Sprague-Dawley rats, the rate of PFOS excretion in urine exceeded the excretion rate in faeces, at doses of either 5 or 20 mg/kg (Cui et al., 2010). Another study in male and female Sprague-Dawley rats also showed that urinary excretion was the primary means of eliminating PFOS after a single oral dose of 2 or 15 mg/kg of potassium perfluorooctanesulfonate (Chang et al., 2012). Furthermore, the urinary PFOA clearance, in male or female FVB/NJc1 mice, was approximately twice as high as the faecal clearance after either i.v. or oral gavage administration (0.313 µmol/kg and 3.13 µmol/kg, respectively, equal to 0.13 and 1.3 mg/kg) (Fujii et al., 2015).

Similarly, the clearance rate of PFOS in CD-1 mice after a single oral dose (1 or 20 mg/kg of the potassium salt, potassium perfluorooctanesulfonate) exceeded the faecal clearance rate (Chang et al., 2012). [The Working Group noted that urinary excretion appears to be the primary route of elimination for PFOA and PFOS in rats and mice.]

In monkeys, larger amounts of PFOA were also excreted in the urine than in the faeces at steady state (Butenhoff et al., 2004). [The Working Group noted that urinary excretion is more relevant than faecal excretion in laboratory animals, while in humans the opposite is true, at least for PFOS.]

Several TK and PBPK models were available regarding PFOA and PFOS in rats, mice, and monkeys (reviewed in Bernstein et al., 2021). [The Working Group noted that most of these models included the assumption that urinary elimination is the primary route, on the basis of the experimental evidence. Accordingly, the kidney reabsorption loop has been extensively studied using PBPK models. However, the enterohepatic reabsorption component in rodents, despite the evidence for its presence (Johnson et al., 1984), has not received much attention, and it has not been fully incorporated into the existing rodent PBPK models.]

Organic anion transporters are thought to be involved in the renal elimination and reabsorption of PFOA also in non-human mammals. The expression levels of OATP1a1, OAT2, and OAT3 were found to be sex-dependent and responsive to changes in steroid hormone concentrations in rats (Kudo et al., 2002; Cheng et al., 2006). In rats, multiple regression analysis suggested that the clearance of PFOA was at least in part dependent on the renal expression of OAT2 and OAT3 (Kudo et al., 2002).

The renal clearance of PFOA was increased by mannitol infusion (which increases urine flow) but was reduced by a low-phosphate diet in both male and female rats (Katakura et al., 2007).

The renal clearance of PFOA did not change in the rat knockout model for multidrug resistance protein (MRP2) compared with wildtype rats (Katakura et al., 2007), indicating that MRP2 is not involved in PFOA transport.

(b) Experimental systems in vitro

Approximately 39% of the applied concentration of PFOA was found to penetrate mouse skin in vitro (Franko et al., 2012). [The Working Group noted that the PFOA penetration of mouse skin samples in vitro was higher than that for human skin samples in this study, which was 24% of the dose administered.]

The transport of PFOA by rat transporters has been reported for OAT1 (K_m = 43.2–51 µM), OAT3 (K_m = 65.7–80.2 µM), and OATP1a1 (K_m = 126.5–162 µM), using transiently transfected oocytes of *Xenopus laevis* and human embryonic kidney HEK293 cells in vitro (Katakura et al., 2007; Nakagawa et al., 2009; Weaver et al., 2010). No transport was observed in vitro for rat OAT2 or URAT1 (Nakagawa et al., 2009; Weaver et al., 2010).

Woodcroft et al. (2010) investigated the binding of PFOA to bacterially expressed rat L-FABP and, depending on the method used (direct displacement or isothermal titration calorimetry), predicted the existence of two

Table 4.2 Serum half-life ($T_{1/2}$) estimates for key species

Substance	Human[a] Population GM (95% CI)	Monkey Range	Mouse Range or mean	Rat Range
PFOA	3.14 yr (2.69–3.73 yr)	M, 13.6–35.3 d F, 26.8–41.7 d	M, 21.7 d F, 15.6 d	M, 6.4–13.4 d F, 2.75–13.9 h
PFOS	3.36 yr (2.52–4.42 yr)	M, 117–200 d F, 102–200 d	M, 36–43 d F, 30–38 d	M, 8.23–41.19 d F, 23.5–71.13 d

CI, confidence interval; d, day(s); F, female; GM, geometric mean; h, hour(s); M, male; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; yr, year(s).

[a] Estimates for humans are for both men and women.

Data were obtained from Kudo et al. (2002); Seacat et al. (2002); US EPA (2003); Butenhoff et al. (2004); Benskin et al. (2009); Lou et al. (2009); Kim et al. (2016a); Chang et al. (2017); Iwabuchi et al. (2017); Huang et al. (2019a, 2021); Dzierlenga et al. (2020); Chiu et al. (2022).

or three binding sites, with K_m estimates of $3.1–52.6 \times 10^{-6}$ M. Furthermore, PFOA and PFOS displaced a fluorescent fatty acid analogue from its binding site on rat L-FABP in vitro, indicating their potential to interfere with fatty-acid binding in vivo (Luebker et al., 2002). PFOA bound to rat and human serum albumin with similar affinities in vitro ($K_m = 4 \times 10^{-4}$ M) (Han et al., 2003).

(c) Interspecies differences

There are marked interspecies differences in the $T_{1/2}$ of PFOA and PFOS (summarized in Table 4.2). The rat demonstrates sex-specific differences with respect to PFOA but not PFOS, and the female rat has the shortest PFOA $T_{1/2}$ overall. However, no sex-specific differences have been identified in other species with respect to either PFOA or PFOS. The mechanisms responsible for the long $T_{1/2}$ in humans have not been elucidated. It has been hypothesized that tighter binding to endogenous proteins, such as albumin in the serum or L-FABP in the liver, or more efficient reabsorption by OATs in the kidney may be responsible. However, in vitro studies of these molecular targets did not report differences in the affinities of the rat and human proteins for PFOA (Nakagawa et al., 2008). Of note, owing to a shorter $T_{1/2}$ for PFOA, implying more rapid excretion, female rats appear to represent a more complex combination of laboratory species and sex, such that extrapolation of data on PFOA to humans is more difficult than from male rats or mice. [The Working Group noted that longer human PFOA and PFOS $T_{1/2}$ appear to correlate with relatively greater faecal excretion, whereas the shorter half-lives in monkeys, mice, and rats appear to favour the urinary excretion of PFOA and PFOS.]

Most previous research has focused on OATs and the involvement of bile acid transporters in the intestinal reabsorption of PFOA and PFOS; therefore data regarding possible interspecies differences in this mechanism are scarce. The human kidney reabsorbs bile acids during filtration (Stiehl, 1974), suggesting that that bile acid transporters may be present, and these may also play a role in the renal reabsorption of PFOA and PFOS.

Although PFOA and PFOS accumulate in the liver in humans, the liver/serum and liver/blood concentration ratios for each agent are much lower than those reported in animal studies. For example, in postmortem samples collected from adults, Maestri et al. (2006) found liver/blood ratios of 1 for PFOA and 2.7 for PFOS (Maestri et al., 2006). However, a study of human fetal postmortem tissues showed that liver levels of PFOA and PFOS were lower than those in maternal serum (Mamsen et al., 2019). Furthermore, in repeated oral dosing studies, PFOA and PFOS were found to accumulate in the rat and mouse liver and yield much higher concentration ratios versus blood (e.g. liver/blood ratios for PFOA of

3.3–5.6 and for PFOS of 4.8; Cui et al., 2009). Moreover, PFOA and PFOS have been shown to accumulate in the livers of fetal mice and rats (Luebker et al., 2005; Chang et al., 2009; Macon et al., 2011; Ishida et al., 2017; Lai et al., 2017a).

The kidney is another organ that shows interspecies differences with respect to PFOA accumulation. In postmortem kidney samples obtained from adult humans, PFOA and PFOS concentrations were slightly higher than those in the blood, with kidney/blood ratios of 1.2 for both compounds (Maestri et al., 2006). In rats, the kidney/blood ratio was approximately 0.7 for PFOA and approximately 1.0 for PFOS in 3-month oral multiple dose studies (Gao et al., 2015b; Iwabuchi et al., 2017), and similar values were obtained using other study designs. In studies conducted in mice exposed to PFOS, the kidney/blood ratio was estimated to be 0.5–0.9 (Bogdanska et al., 2011; Chang et al., 2012), whereas studies in mice exposed to PFOA yielded an estimated kidney/blood ratio of 0.15–0.2 (Lou et al., 2009; Fujii et al., 2015). Thus, the kidney/blood ratio appears to be lower by 6–8-fold in mice than in humans, indicating that these agents accumulate to a lesser extent in this organ in mice. [The Working Group acknowledged that limited data were available and noted that differences in the site-specific preferential accumulation of PFOA and PFOS may contribute to species differences in adverse effects.]

Synopsis

[In summary, the Working Group noted that there were few available data regarding the absorption and distribution of PFOA and PFOS in humans. PFOA and PFOS are absorbed after oral, inhalation, or dermal exposure. On the basis of their structures, PFOA and PFOS are not likely to cross cellular membranes directly, and various membrane transporters are thought to mediate transmembrane transport. The liver, blood, and lungs are important sites of accumulation for both agents. The distribution of PFOA and PFOS appears to be driven by binding to specific proteins, such as albumin in the blood and L-FABP in the liver. The available evidence suggests that enterohepatic circulation of PFOA and PFOS and bile acid transporters in the gut could be responsible for this effect. PFOA and PFOS cross the placenta and are transferred to infants via breast milk. Neither agent is metabolized. PFOA and PFOS are excreted in the faeces, which is an important route in humans, and the urine. In women of reproductive age, blood loss during menstruation, fetal transfer during pregnancy, and transfer during breastfeeding also contribute to the loss of PFOA and PFOS. The half-lives of PFOA and PFOS in humans are approximately 3.14 and 3.36 years, respectively, whereas those in monkeys, mice, and rats are in the order of hours to months. Rats and mice also have PFOA and PFOS distribution patterns that are distinct to those in humans. The mechanisms underlying the interspecies TK differences in PFOA and PFOS are not well understood.]

4.2 Evidence relevant to key characteristics of carcinogens

This section reviews the mechanistic data for the 10 key characteristics (KCs) of carcinogens (Smith et al., 2016) encompassed by the agents PFOA and PFOS. The studies in exposed humans used to support the mechanistic evidence were evaluated for the quality of the study design, exposure assessment, and assay accuracy and precision, and were found to reflect suitable methods for human environmental epidemiological studies. The evaluated human studies also accounted for important confounding and modifying variables. The determination of the Working Group may also have been buttressed by mechanistic evidence from human primary cells and tissues or from experimental systems.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

(a) Humans

No studies in exposed humans or human cells in vitro were available to the Working Group.

(b) Experimental systems

The fluoro-carbon chain of PFOA and PFOS forms hydrophobic bonds with DNA, which may cause strand unwinding and steric hindrance for covalent binding (Lu et al., 2012, 2016a; Qin et al., 2022a). Several studies have demonstrated that PFOA binds to proteins in plasma (i.e. albumin), liver (especially L-FABP), kidney (i.e. alpha2u-globulin) and testes of rats (Vanden Heuvel et al., 1992; Luebker et al., 2002; Han et al., 2003, 2004, 2005). In addition, it has been shown that PFOA binds to cysteine residues in murine acetyl-coenzyme A (CoA) carboxylase A and B (Shao et al., 2018). PFOA has been shown to bind to rat and human albumin with similar strengths (Han et al., 2003; Messina et al., 2005b). Studies on bovine serum albumin have indicated that PFOA mainly binds by Van der Waals forces and hydrogen bonds at the Sudlow site 1 on albumin (Yang et al., 2023). PFOA binds to haemoglobin (Perera et al., 2023). [The Working Group noted that while the binding affinity to haemoglobin in this study was comparable to the binding affinity to albumin, as found in multiple studies, this finding probably had little biological relevance, because PFOA preferentially distributed to plasma proteins in vivo (see Section 4.1).] It has been shown that PFOS binds more strongly than PFOA to rat liver L-FABP (Luebker et al., 2002).

Synopsis

[The Working Group noted that PFOA and PFOS do not appear to have electrophilic properties or to be metabolized to electrophilic compounds (see also Section 4.1.1). PFOA and PFOS form non-covalent bonds with DNA. In addition, studies have shown that PFOA can interact with proteins, possibly through non-covalent hydrogen bonds and Van der Waals forces.]

4.2.2 Is genotoxic

See Tables 4.3 to 4.10.

(a) Humans

(i) Exposed humans

Genotoxic effects in humans were reported in the Flemish Environment and Health Study, a cross-sectional environmental study of adolescents from the Flanders region, Belgium, exposed to various hazardous compounds, including heavy metals, polycyclic aromatic hydrocarbons (PAHs), benzene, phthalates, and organophosphate pesticides (n = 606, in the entire cohort) (Franken et al., 2017). PFOA concentrations in serum were measured in a subpopulation from Menen (Flanders, Belgium), that was the location of a shredding factory (geometric mean, 2.55 ng/mL; 95% CI, 2.44–2.65 ng/mL; n = 197). The study participants were recruited between May 2010 and February 2011. [The Working Group noted that the PFOA concentrations in

serum were consistent with background levels of PFOA, indicating that the participants from Menen were not highly exposed.] The study showed a positive association between serum levels of PFOA and DNA strand breaks in leukocytes, measured using the alkaline comet assay (9% interquartile range, IQR; 95% CI, 1.5–17%, adjusted for sex, age, smoking status and maximum temperature, 7 days before sample collection). However, the positive association was not statistically significant after controlling for multiple comparisons using the method of Benjamini and Hochberg, which controlled the false discovery rate (FDR) at $P = 0.05$ (Franken et al., 2017). [The Working Group noted that PFOA serum concentrations were not correlated (Pearson correlation test) with marker levels of exposure to heavy metals, PAHs, benzene, phthalate, and organophosphate pesticides.]

[The Working Group noted that the study by Franken et al. (2017) had reliable measurements of exposure biomarkers and DNA damage end-points, and the positive association between PFOA and DNA strand breaks did not appear to be confounded by heavy metals, PAHs, benzene, phthalate, or organophosphate pesticides, and it was not statistically significant after controlling for multiple comparisons. The DNA damage was not mediated by oxidative stress, because no association between PFOA exposure and damaged DNA was observed using the formamidopyrimidine-DNA glycosylase (Fpg)-modified comet assay in leukocytes (see also Section 4.2.5).]

DNA fragmentation in spermatozoa has been assessed in two studies that aimed to evaluate semen quality and male fertility. One study recruited male partners of pregnant women at their first antenatal care visit to hospitals in Greenland ($n = 199$), Poland ($n = 197$), and Ukraine ($n = 208$) (Specht et al., 2012). The exposure levels differed between the populations, as reflected in the serum concentrations of PFOA (mean concentrations, 4.8, 5.1, and 1.8 ng/mL) and PFOS (51.9, 18.6, and 8.1 ng/mL in the populations from Greenland, Poland, and Ukraine, respectively). There was no association of the PFOA and PFOS serum concentrations with DNA fragmentation index in spermatozoa of this population of fertile men (Specht et al., 2012).

Another study recruited male partners of couples at their first visit to a reproductive medical clinic in Nanjing, China (Pan et al., 2019). The population was described as heterogeneous because it included both men with fertility issues and fertile men who were partners to infertile women ($n = 664$). The study included PFOA and PFOS measurements in serum and semen. There were similar serum concentrations of PFOA (median, 8.6 ng/mL; lower and upper cut-off values, 6.8 ng/mL and 11.0 ng/mL) and PFOS (median, 8.4 ng/mL; lower and upper cut-off values, 5.6 ng/mL and 13.1 ng/mL). Likewise, semen concentrations were similar for PFOA (median, 0.23 ng/mL; lower and upper cut-off values, 0.15 ng/mL and 0.36 ng/mL) and PFOS (median, 0.10 ng/mL; lower and upper cut-off values, 0.06 ng/mL and 0.18 ng/mL). There were strong correlations between serum and semen concentrations of PFOA ($r = 0.70$; $P < 0.001$) and PFOS ($r = 0.8$; $P < 0.001$). Serum concentrations of PFOA and PFOS or the increase per 1-unit increase in the log-transformed concentration were not associated with the DNA fragmentation index (PFOA: $\beta = 0.046$; 95% CI, −0.052 to 0.144; PFOS: $\beta = 0.040$; 95% CI, −0.037 to 0.116), adjusted for age, body mass index (BMI), BMI^2, smoking, alcohol intake, and the duration of abstinence from both, whereas there were positive associations between the DNA fragmentation index and concentrations of PFOA ($\beta = 0.136$; 95% CI, 0.064–0.209) and PFOS ($\beta = 0.087$; 95% CI, 0.033–0.142) in seminal fluid (Pan et al., 2019). [The Working Group considered that the two studies were less relevant than the findings reported in the study by Franken et al. (2017) described above, because DNA fragmentation was measured using the sperm

Table 4.3 End-points relevant to genotoxicity in human cells in vitro exposed to PFOA

End-point (assay)	Cells	Result[a]	Concentration (LEC or HIC)	Comments	Reference
DNA strand breaks (comet assay)	HepG2	+	1.9 µM	Positive control included (H_2O_2)	Ojo et al. (2022a)
DNA strand breaks (comet assay)	HepG2	+	10 µM	Positive control included (H_2O_2)	Wielsøe et al. (2015)
DNA strand breaks (comet assay)	TK6	+	125 µg/mL [0.3 µM]	No positive control included	Yahia et al. (2016)
DNA strand breaks (comet assay)	HepG2	+	50 µM	No positive control included	Yao and Zhong (2005)
DNA strand breaks (comet assay)	Sperm (primary cells)	–	1000 µM	Positive control included (H_2O_2)	Emerce and Çetin (2018)
DNA strand breaks (comet assay)	HepG2	–	400 µM	Positive control included (Ro19-8022 + light)	Eriksen et al. (2010)
DNA strand breaks (comet assay)	HepG2	–	400 µM	Positive control included (H_2O_2)	Florentin et al. (2011)
DNA double-strand breaks (γH2AX assay)	HaCaT	(+)	50 µM	Single concentration; no positive control included	Peropadre et al. (2018)
Chromosomal aberrations	Primary lymphocytes (from males)	–	2010 µg/mL	Increased frequency of endoreduplication in the cells observed at an exposure level that also caused a significant decrease in mitotic index; positive controls included (mitomycin C and cyclophosphamide for assays without or with metabolic activation, respectively)	US EPA (1996a)
Chromosomal aberrations	Primary lymphocytes (from males)	–	100 µg/mL	Positive controls included (mitomycin C and cyclophosphamide for assays without or with metabolic activation, respectively)	Butenhoff et al. (2014)
Micronuclei (CBMN assay)	HepG2	–	400 µM	Positive control included (mitomycin C)	Florentin et al. (2011)
Micronuclei (CBMN assay)	HepG2	+	100 µM	No positive control included	Yao and Zhong (2005)

CBMN, cytokinesis-block micronucleus assay; H_2O_2, hydrogen peroxide; HIC, highest ineffective concentration; LEC, lowest effective concentration; PFOA, perfluorooctanoic acid; γH2AX, γ-H2A histone family member X.

[a] –, negative; +, positive; (+), positive in a study of limited quality.

chromatin structure assay, which can be considered an indirect indicator of DNA damage.]

(ii) Human cells in vitro

See Table 4.3 and Table 4.4.

No studies conducted in human tissues were available. Four studies aiming to assess genotoxic effects induced by PFOA or PFOS treatment were conducted in human primary cells. The amount of DNA strand breaks (represented by DNA % tail as measured by comet assay) was not altered in human sperm cells by exposure to PFOA or PFOS (Emerce and Çetin, 2018). Exposure to PFOA or PFOS did not affect the frequency of chromosomal aberrations in human lymphocytes (US EPA, 1996a, 1999b; Butenhoff et al., 2014).

Six studies have assessed the formation of DNA strand breaks using the comet assay in human hepatoma (HepG2) and in human lymphoblastoid permanent TK6 cells; four of these studies have shown high levels of

Table 4.4 End-points relevant to genotoxicity in human cells in vitro exposed to PFOS

End-point (assay)	Cells	Result[a]	Concentration (LEC or HIC)	Comments	Reference
DNA strand breaks (comet assay)	HepG2	+	0.8 µM	Positive control included (H_2O_2)	Ojo et al. (2022a)
DNA strand breaks (comet assay)	HepG2	+	0.2 µM	Positive control included (H_2O_2)	Wielsøe et al. (2015)
DNA strand breaks (comet assay)	Sperm	–	1000 µM	Positive control included (H_2O_2)	Emerce and Çetin (2018)
DNA strand breaks (comet assay)	HepG2	–	400 µM	Positive assay control included (THP-1 cells treated with Ro19-8022 + light)	Eriksen et al. (2010)
DNA strand breaks (comet assay)	HepG2	–	300 µM	Positive controls included (H_2O_2 and benzo[*a*]pyrene)	Florentin et al. (2011)
Chromosomal aberrations	Primary lymphocytes (from males)	–	349 µg/mL	Negative with and without metabolic activation; positive controls included (mitomycin C and cyclophosphamide); one experiment with technical replicates	US EPA (1999b)
Micronuclei (CBMN assay)	HepG2	–	300 µM	Positive control included (mitomycin C)	Florentin et al. (2011)

CBMN, cytokinesis-block micronucleus assay; H_2O_2, hydrogen peroxide; HIC, highest ineffective concentration; LEC, lowest effective concentration; PFOS, perfluorooctanesulfonic acid.

[a] –, negative; +, positive.

genotoxicity (Yao and Zhong, 2005; Wielsøe et al., 2015; Yahia et al., 2016; Ojo et al., 2022a). The other two studies showed unaltered levels of DNA strand breaks in human HepG2 cells after exposure to both PFOA and PFOS when compared with a positive control group (Eriksen et al., 2010; Florentin et al., 2011). No change in the frequencies of chromosome aberrations and micronuclei in the HepG2 cells was observed after exposure to PFOA and PFOS in one of these studies (Florentin et al., 2011). Yao and Zhong instead reported an increase in the frequency of micronuclei in HepG2 cells after PFOA exposure (Yao and Zhong, 2005).

Exposure to PFOA induced an increase in the frequency of phosphorylated H2A histone family member X (γH2AX) foci in human HaCaT keratinocytes, which is an indicator of either DNA double-strand breaks or stalled replication forks (Peropadre et al., 2018).

(b) Experimental systems

(i) Non-human mammalian systems

See Table 4.5 (PFOA in vivo), Table 4.6 (PFOS in vivo), Table 4.7 (PFOA in vitro), Table 4.8 (PFOS in vitro).

DNA strand breaks (measured using the comet assay) were not induced in the liver and testes of mice exposed to PFOA in drinking-water (Crebelli et al., 2019). However, in the oocytes of mice that were exposed orally to PFOA there was an increased frequency of γH2AX foci, which is an indicator of either DNA double-strand breaks or stalled replication forks (Zhang et al., 2022a). No increases in the frequency of micronuclei in blood reticulocytes or spleen lymphocytes were observed in mice (Crebelli et al., 2019). In addition, oral exposure to PFOA did not alter the frequency of micronucleated bone marrow cells in mice (US EPA, 1995b, 1996d; Butenhoff et al., 2014) (see Table 4.5). [The Working Group considered that the negative results from the micronucleus assays were reliable, because the

Table 4.5 End-points relevant to genotoxicity in non-human mammals in vivo exposed to PFOA

End-point (assay)	Species, strain (sex)	Tissue(s)	Result[a]	Dose (LED, HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks (comet assay)	Mouse, C57BL/6 (M)	Liver, testis	–	5 mg/kg	Oral, 5 wk, drinking-water	Positive control included (MMS)	Crebelli et al. (2019)
DNA double strand breaks (γH2AX assay)	Mouse, ICR (F)	Oocytes	(+)	5 mg/kg per day	Oral, 28 days, drinking-water	Method for the detection of γH2AX foci was not described; no positive control group	Zhang et al. (2022a)
Micronuclei	Mouse, C57BL/6 (M)	Blood (reticulocytes) and spleen (lymphocytes)	–	5 mg/kg	Oral, 5 wk, drinking-water	Positive control included (MMS)	Crebelli et al. (2019)
Micronuclei	Mouse, ICR (F and M)	Bone marrow	–	1000 mg/kg	Oral, 24–72 h, single dose	Positive control included (cyclophosphamide)	Butenhoff et al. (2014)
Micronuclei	Mouse, Crl:CD-1 (ICR) BR (F and M)	Bone marrow	–	1990 mg/kg	Oral, single dose, killed 24, 48 or 72 h post-exposure	Positive control included (cyclophosphamide)	US EPA (1996d)
Micronuclei	Mouse, Crl:CD-1 (ICR) BR (F and M)	Bone marrow	–	5000 mg/kg	Oral, single dose, killed 24, 48 or 72 h post-exposure	Positive control included (cyclophosphamide)	US EPA (1995b)

F, female; h, hour(s); γH2AX, γ-H2A histone family member X; HIC, highest ineffective concentration; HPLC-ECD, high-performance liquid chromatography-electrochemical detection; LEC, lowest effective concentration; M, male; MMS, methyl methanesulfonate; PFOA, perfluorooctanoic acid; wk, week(s).

[a] –, negative; +, positive; (+), positive in a study of limited quality.

Table 4.6 End-points relevant to genotoxicity in non-human mammals in vivo exposed to PFOS

End-point (assay)	Species, strain (sex)	Tissue	Result[a]	Dose (LED, HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks (comet assay)	Rat, Wistar (M)	Bone marrow	+	0.6 mg/kg	Oral, 30 d, gavage at 48-h intervals	Positive control group included (mitomycin C)	Çelik et al. (2013)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Leukocytes (whole blood)	+	0.6 mg/kg	Oral, 30 d, gavage at 48-h intervals	Positive control group included (mitomycin C)	Eke and Çelik (2016)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Liver	+	0.6 mg/kg	Oral, 30 d, gavage at 48-h intervals	Positive control group included (mitomycin C)	Eke et al. (2017)
Mutations (*Spi*$^-$ assay)	Mice, *gpt* delta transgenic (M)	Liver	–	10 mg/kg per day	Oral, 28 d, gavage at 24-h intervals	No positive control group	Wang et al. (2015a)
Micronuclei	Mice, *gpt* delta transgenic (M)	Bone marrow	–	10 mg/kg per day	Oral, 28 d, gavage at 24-h intervals	No positive control group	Wang et al. (2015a)
Micronuclei	Rat, Sprague-Dawley (F and M)	Erythrocytes (blood)	–	5 mg/kg per day	Oral, 28 d, gavage once daily	A slightly increased percentage of micronucleated cells in female rats was related to PFOS-induced bone-marrow toxicity	NTP (2019)
Micronuclei	Rat, Wistar (M)	Bone marrow	+	1.25 mg/kg	Oral, 30 d, gavage at 48-h intervals	Positive control group included (mitomycin C)	Çelik et al. (2013)
Micronuclei	Rat, Wistar (M)	Leukocytes (whole blood)	+	0.6 mg/kg	Oral, 30 d, gavage at 48-h intervals	Positive control group included (mitomycin C)	Eke and Çelik (2016)
Micronuclei	Rat, Wistar (M)	Liver	+	0.6 mg/kg	Oral, 30 d, gavage at 48-h intervals	Positive control group included (mitomycin C)	Eke et al. (2017)
Micronuclei	Mouse, Crl:CD-1 (ICR) BR (F and M)	Bone marrow	–	950 mg/kg	Oral, single dose, killed 24, 48 or 72 h post-exposure	Positive control group included (cyclophosphamide)	US EPA (1996e)

d, day(s); F, female; h, hour(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; M, male; PFOS, perfluorooctanesulfonic acid.

[a] –, negative; +, positive.

Table 4.7 End-points relevant to genotoxicity in non-human mammalian cells in vitro exposed to PFOA

End-point (assay)	Cells	Result[a]	Concentration (LEC or HIC)	Comments	Reference
DNA strand breaks (comet assay)	Oocytes (porcine)	(+)	40 µM	Increased comet tail length, although changes in tail intensity and tail moment were not statistically significant; it was uncertain whether the statistical analysis was based on all comets (n = 250) or independent experiments; no positive control included	Mario et al. (2022)
DNA strand breaks (comet assay)	SHE cells	–	300 µM	Positive control included (H_2O_2)	Jacquet et al. (2012a)
DNA strand breaks (comet assay)	Rat testicular cells	–	300 µM	Positive control included (1,2-dibromo-3-chloropropane)	Lindeman et al. (2012)
DNA double-strand breaks (γH2AX assay)	Mouse oocytes	(+)	300 µM	No positive control included in the experiments; toxicity at the highest concentration (approximately 30% cell death and decreased oocyte maturation at lowest concentration)	Guo et al. (2021a)
DNA double-strand breaks (γH2AX assay)	Mouse oocytes	(+)	200 µM	Single concentration; statistical analysis was based on all oocytes (n = 50–58), rather than independent experiments; no positive control group included	Zhou et al. (2022)
HGPRT locus mutations	CHO-K1	–	39 µg/mL [90 µM]	Positive control included (ethylmethanesulfoxide and dimethylbenzanthracene for the test conditions without and with metabolic activation, respectively)	US EPA (2002)
CD59 locus mutations	Human–hamster hybrid (A_L) cells	+	200 µM	Mutagenic effect observed after extended period of exposure (16 days, but not after 1, 4 or 8 days of exposure); no positive control group included	Zhao et al. (2011a)
Micronuclei (assay not specified)	CHL V79 fibroblasts	–	10 µM	Single concentration; positive controls included (ethylmethylsulfonate and cyclophosphamide)	Buhrke et al. (2013)
Chromosomal aberrations	CHO cells	–	996 µg/mL	Positive control included (mitomycin C and cyclophosphamide for the test conditions without and with metabolic activation, respectively)	Butenhoff et al. (2014)
Chromosomal aberrations	CHO cells	–	2250 µg/mL	Negative without metabolic activation; increased frequency of endoreduplication in cells and polyploidy in cells with metabolic activation; positive controls included (mitomycin C and cyclophosphamide)	US EPA (1996b)
Chromosomal aberrations	CHO cells	+ (with metabolic activation)	3740 µg/mL	Induction of chromosomal aberrations in cells with metabolic activation; negative without metabolic activation; positive controls included (mitomycin C and cyclophosphamide)	US EPA (1996c)

CHL, Chinese hamster lung; CHO, Chinese hamster ovary; γH2AX, γ-H2A histone family member X; H_2O_2, hydrogen peroxide; HIC, highest ineffective concentration; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; LEC, lowest effective concentration; PFOA, perfluorooctanoic acid; SHE, Syrian hamster embryo.

[a] –, negative; +, positive; (+), positive in a study of limited quality.

Table 4.8 End-points relevant to genotoxicity in non-human mammalian cells in vitro exposed to PFOS

End-point (assay)	Cells	Result[a]	Concentration (LEC or HIC)	Comments	Reference
DNA double-strand breaks (γH2AX assay)	Porcine ovary	(+)	100 µM	Single concentration; no positive control included; the statistical analysis was based on all the oocytes (n = 27–30), rather than independent experiments	Chen et al. (2021)
DNA strand breaks (comet assay)	Sperm (boar)	(+)	461 µM	Single concentration; no positive control included	Oseguera-López et al. (2020)
DNA double-strand breaks (γH2AX assay; western blot)	MEF	(+)	20 µM	Genotoxic response occurred at concentrations with < 50% viability; no positive control included	Wang et al. (2015a)
DNA strand breaks (comet assay)	SHE cells	–	93 µM	Positive control included (H_2O_2)	Jacquet et al. (2012b)
Unscheduled DNA synthesis	Rat hepatocytes	–	25 µg/mL	Positive control included (2-acetylaminofluorene)	US EPA (1999c)
Mutations (*Spi*$^-$ mutation assay; *redBA/gam* locus)	MEF	+	10 µM	No positive control included	Wang et al. (2015a)
Mutations (CD59$^-$ mutants)	Human–hamster (A_L) hybrid cells	–	200 µM	No positive control included	Wang et al. (2013)
Micronuclei	CHL fibroblasts (V79)	–	12.5 µg/mL	Single concentration with metabolic activation (S9 mix); positive control included (cyclophosphamide)	Jernbro et al. (2007)
Aneuploidy	Mouse oocytes	(+)	600 µM	Single concentration; aneuploidy caused by dysfunction of spindle assembly and chromosome alignment in mitosis; no positive control included	Wei et al. (2021)

CHL, Chinese hamster lung; H_2O_2, hydrogen peroxide; γH2AX, γ-H2A histone family member X; HIC, highest ineffective concentration; LEC, lowest effective concentration; MEF, mouse embryonic fibroblasts; PFOS, perfluorooctanesulfonic acid; S9, 9000 × g supernatant; SHE, Syrian hamster embryo.

[a] –, negative; +, positive; (+), positive, in a study of limited quality.

studies included positive controls for genotoxic effects (Butenhoff et al., 2014; Crebelli et al., 2019).]

In three consecutive studies performed in rats by the same research group, oral exposure to PFOS was shown to induce an increase in DNA strand breaks, measured using the comet assay, and in the frequency of micronuclei in bone marrow cells (Çelik et al., 2013), leukocytes (whole blood) (Eke and Çelik, 2016), and liver (Eke et al., 2017).

One study assessed genotoxic effects in *gpt* transgenic mice, which were exposed by oral gavage to PFOS at 1.5, 4, or 10 mg/kg once daily for 28 days (Wang et al., 2015a). Compared with the control group, there were higher frequencies of mutations in the liver (2.2 and 6.8 λ mutants/10^6 plaques) and of micronucleated polychromatic erythrocytes in the bone marrow (3.0% and 2.9%, equal to 0.52- and 0.43-fold) at the two highest doses, although the increase was not statistically significant (Wang et al., 2015a). [The Working Group noted that the authors considered the fold increases as evidence that PFOS is mutagenic in vivo. However, the greater mutagenic effect in the highest dose group may have been because of an outlier, and genotoxic effects occurred only at doses that caused increases in the serum levels of alkaline phosphatase and alanine aminotransferase, suggesting genotoxicity may have been because of tissue toxicity.]

The study presented in a report by the National Toxicology Program (NTP) showed no effect on micronucleated cells in the peripheral blood of rats exposed orally (NTP, 2019). Lastly, one study showed unaltered frequency of micronuclei in bone marrow cells after a single oral administration of PFOS in mice (US EPA, 1996e) (see Table 4.6).

Two in vitro studies showed that PFOA did not induce the formation of DNA strand breaks, assessed using the comet assay, in rodent cells (Jacquet et al., 2012a; Lindeman et al., 2012).

In contrast, more recently, Mario et al. (2022) reported that PFOA exposure generated DNA strand breaks, assessed using the comet assay in porcine oocytes. [The Working Group noted that the study was of limited relevance because the statistical analysis seemed to be based on individual comets, rather than the mean results from independent experiments, and the study did not include a positive control group.]

In two different studies in mouse oocytes, PFOA treatment induced an increase in γH2AX foci in comparison with the negative control (Guo et al., 2021a; Zhou et al., 2022). [The Working Group noted that the statistical analysis in the study of Zhou et al. (2022) also seemed to be based on individual comets, rather than the mean results from independent experiments, and the two studies did not include a positive control group.]

US EPA (2002) reported no increase in mutation frequency in Chinese hamster ovary CHO-K1 cells, using the hypoxanthine-guanine phosphoribosyl transferase assay. An increase in mutation frequency was observed at the CD59 locus in human–hamster hybrid (A_L) cells exposed to PFOA for 16 days, whereas a shorter exposure to PFOA for 1, 4, or 8 days was not associated with increases in mutation (Zhao et al., 2011a). No increases of chromosome aberrations or in the frequency of micronuclei were observed in PFOA-exposed rodent cells (US EPA, 1996b; Buhrke et al., 2013; Butenhoff et al., 2014). An increase of chromosome aberrations was observed in another study in CHO cells in the presence of metabolic activation (US EPA, 1996c) (see Table 4.7).

In three in vitro studies, exposure to PFOS induced DNA strand breaks (assessed using the comet assay) and γH2AX foci in porcine ovary cells, sperm of boar, and mouse embryonic fibroblasts (Wang et al., 2015a; Oseguera-López et al., 2020; Chen et al., 2021), whereas, in a fourth study, exposure to the potassium salt of PFOS did

not alter the level of DNA strand breaks in Syrian hamster embryo cells (Jacquet et al., 2012b).

PFOS did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes (US EPA, 1999c). One study showed an increase in mutation frequency (*spi* mutation assay) in mouse embryonic fibroblasts (Wang et al., 2015a). In human–hamster (A_L) hybrid cells, exposure to PFOS did not alter the frequency of $CD59^-$ mutants (Wang et al., 2013). [The Working Group noted that a positive control group was not included in the study.]

No change in the frequency of micronuclei was reported in Chinese hamster lung fibroblasts (V79) (Jernbro et al., 2007). Lastly, Wei and colleagues observed aneuploidy in oocytes from mice after exposure to the potassium salt of PFOS, which was speculated to be caused by dysfunctions of spindle assembly and chromosome alignment in mitosis (Wei et al., 2021) [The Working Group noted that aneuploidy encompasses both gain and loss of chromosomes. However, the study had some limitations: the statistical analysis of aneuploidy appeared to be based on the number of oocytes (group sizes of 20 and 21 oocytes), rather than results from independent experiments, and the study did not include a positive control.]

(ii) Prokaryotes and other species

See Table 4.9 (PFOA) and Table 4.10 (PFOS).

Prokaryotes

PFOA has not been found to be mutagenic in various *Escherichia coli* and *Salmonella typhimurium* tester strains (Griffith and Long, 1980; US EPA, 1995a, 1996f; Oda et al., 2007; Fernández Freire et al., 2008; Buhrke et al., 2013; Butenhoff et al., 2014). PFOS has given negative results for mutagenicity in various *E. coli* and *S. typhimurium* tester strains (Simmon and Marx, 1978; US EPA, 1979, 1999a; Oda et al., 2007; NTP, 2019).

[The Working Group noted that PFOA and PFOS might not readily enter cells in the absence of appropriate transporters, which could be the case for the prokaryotic systems and for *Saccharomyces cerevisiae*.]

Lower eukaryotes

Exposure of *Paramecium caudatum* (unicellular freshwater protozoa) to PFOA was associated with increased levels of DNA strand breaks (Kawamoto et al., 2010). PFOA exposure did not alter the frequency of mitotic recombination in *S. cerevisiae* (Butenhoff et al., 2014).

Exposure to PFOS did not affect levels of DNA strand breaks in *P. caudatum* (Kawamoto et al., 2010) and did not induce mitotic recombination in *S. cerevisiae* (Simmon and Marx, 1978; US EPA, 1979).

Other species

Studies on PFOA have shown increased levels of DNA strand breaks in earthworms (*Eisenia fetida*) (Zheng et al., 2016; Wang et al., 2021a), green mussels (*Perna viridis*) (Liu et al., 2014a, b), planarians (*Dugesia japonica*) (Zhang et al., 2020a), and daphnia (*Daphnia carinata*) (Logeshwaran et al., 2021), whereas exposure to PFOA in the common carp (*Cyprinus carpio*) was not associated with changes in levels of DNA strand breaks (Kim et al., 2010). Increased micronuclei frequency was observed in haemolymph cells of PFOA-exposed *Perna viridis* (Liu et al., 2014b).

DNA strand breaks, measured using the comet assay, were increased after exposure to PFOS in *Cyprinus carpio* (Kim et al., 2010), zebrafish (*Dario rerio*) (Du et al., 2016), *Eisenia fetida* (Xu et al., 2013a; Zheng et al., 2016), *Perna viridis* (Liu et al., 2014a, b), *Dugesia japonica* (Shao et al., 2019), *Daphnia carinata* (Logeshwaran et al., 2021), and *Allium cepa* (Sivaram et al., 2021). One study showed unaltered levels of DNA strand breaks in brain and liver cells from

Table 4.9 End-points relevant to genotoxicity in non-mammalian systems exposed to PFOA

End-point (assay)	Species (cell type)	Result[a]	Concentration (LEC or HIC)	Comments	Reference
Prokaryotes					
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538)	–	5 µmol/plate	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	Buhrke et al. (2013)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and *Escherichia coli* WP2 *uvrA*	–	1000 µg/plate	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	Butenhoff et al. (2014)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA102, and TA104)	–	500 µM	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	Fernández Freire et al. (2008)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538)	–	500 µg/plate	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	Griffith and Long (1980)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537)	–	5 mg/plate	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	US EPA (1995a)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537)	–	5 mg/plate	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	US EPA (1996f)
Mutations	*Salmonella typhimurium* TA1535/pSK1002 (*umu* test)	–	1000 µM	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	Oda et al. (2007)
Lower eukaryotes					
Mitotic recombination	*Saccharomyces cerevisiae* D4	–	500 µM	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	Butenhoff et al. (2014)
DNA strand breaks (comet assay)	*Paramecium caudatum*	(+)	100 µM	Positive control groups included (2-aminoanthracene and MNNG)	Kawamoto et al. (2010)
Other species					
DNA strand breaks (comet assay)	*Eisenia fetida* (in the coelomocytes cells)	+	10 mg/kg soil	No positive control group included	Wang et al. (2021a)
DNA strand breaks (comet assay)	*Eisenia fetida* (in the coelomocytes cells)	+	600 mg/kg soil	No positive control included; there was uncertainty about whether the statistical analysis was based on all comets, independent experiments, or individual specimens	Zheng et al. (2016)
DNA strand breaks (comet assay)	*Perna viridis* (haemolymph)	+	1000 µg/mL	No positive control included	Liu et al. (2014a)

Table 4.9 (continued)

End-point (assay)	Species (cell type)	Result[a]	Concentration (LEC or HIC)	Comments	Reference
DNA strand breaks (comet assay)	*Perna viridis* (haemolymph)	+	1000 µg/mL	No positive control included	Liu et al. (2014b)
DNA strand breaks (comet assay)	*Dugesia japonica*	(+)	15 µg/mL	No positive control included; there was uncertainty about whether the statistical analysis was based on all comets, independent experiments, or individual specimens	Zhang et al. (2020a)
DNA strand breaks (comet assay)	*Daphnia carinata*	+	10 µg/mL	Positive control included (H_2O_2)	Logeshwaran et al. (2021)
DNA strand breaks (comet assay)	*Cyprinus carpio* (blood cells)	–	50 µg/mL	No positive control included	Kim et al. (2010)
Micronuclei	*Perna viridis* (haemolymph)	+	1000 µg/mL	No positive control included	Liu et al. (2014b)

H_2O_2, hydrogen peroxide; HIC, highest ineffective concentration; LEC, lowest effective concentration; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; PFOA, perfluorooctanoic acid.
[a] +, positive; –, negative; (+), positive in a study of limited quality.

Table 4.10 End-points relevant to genotoxicity in non-mammalian systems exposed to PFOS

End-point (assay)	Species (cell type)	Result[a]	Concentration (LEC or HIC)	Comments	Reference
Prokaryotes					
Mutations	*Salmonella typhimurium* (TA98 and TA100) and *Escherichia coli* WP2 *uvrA*/pKM101	–	10 mg/plate	Negative with and without metabolic activation; genotoxic effect by exposure to positive controls	NTP (2019)
Mutations	*Salmonella typhimurium* TA1535/pSK1002 (*umu* test)	–	1000 µM	Negative with and without metabolic activation; genotoxic effect by exposure to positive controls	Oda et al. (2007)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538)	–	5 mg/plate	Negative with and without metabolic activation; genotoxic effect by exposure to positive controls; report contains results from two compounds (T-2247 and T-2248) that were not identified by CAS No.[b]	Simmon and Marx (1978)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538)	–	2 mg/plate	Negative with and without metabolic activation; genotoxic effect by exposure to positive controls; results from one independent experiment, except TA100 (n = 2)	US EPA (1979)
Mutations	*Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and *Escherichia coli* WP2 *uvrA*	–	5 mg/plate	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls	US EPA (1999a)
Lower eukaryotes					
Mitotic recombination	*Saccharomyces cerevisiae* D3	–	5% (w/v or v/v)	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls; report contains results from two compounds (T-2247 and T-2248) that are not identified by CAS No.[b]	Simmon and Marx (1978)
Mitotic recombination	*Saccharomyces cerevisiae* D4	–	0.5 mg/plate	Negative with and without metabolic activation; genotoxic effect of exposure to positive controls; result from one experiment	US EPA (1979)
DNA strand breaks (comet assay)	*Paramecium caudatum*	–	100 µM	Positive control groups included (2-aminoanthracene and MNNG)	Kawamoto et al. (2010)
Other species					
DNA strand breaks (comet assay)	*Cyprinus carpio* (blood cells)	+	5 µg/mL	No positive control included	Kim et al. (2010)
DNA strand breaks (comet assay)	*Danio rerio* (blood cells)	+	0.4 µg/mL	No chemical positive control included (exposure to ZnO nanoparticles was associated with increased number of DNA strand breaks)	Du et al. (2016)

Table 4.10 (continued)

End-point (assay)	Species (cell type)	Result[a]	Concentration (LEC or HIC)	Comments	Reference
DNA strand breaks (comet assay)	*Eisenia fetida* (in the coelomocytes cells)	(+)	0.25 µg/cm^2	No positive control included; there was uncertainty about whether the statistical analysis was based on all comets, independent experiments, or individual specimens	Xu et al. (2013a)
DNA strand breaks (comet assay)	*Eisenia fetida* (in the coelomocytes cells)	(+)	470 mg/kg soil	No positive control included; there was uncertainty about whether the statistical analysis was based on all comets, independent experiments, or individual specimens	Zheng et al. (2016)
DNA strand breaks (comet assay)	*Perna viridis* (haemolymph)	+	10 µg/mL	No positive control included	Liu et al. (2014a)
DNA strand breaks (comet assay)	*Perna viridis* (haemolymph)	+	10 µg/mL	No positive control included	Liu et al. (2014b)
DNA strand breaks (comet assay)	*Dugesia japonica*	+	5 µg/mL	No positive control included	Shao et al. (2019)
DNA strand breaks (comet assay)	*Daphnia carinata*	+	1 µg/mL	Positive control included (H_2O_2)	Logeshwaran et al. (2021)
DNA strand breaks (comet assay)	*Allium cepa*	+	25 µg/mL	Positive control included (benzo[*a*]pyrene)	Sivaram et al. (2021)
DNA strand breaks (DNA precipitation assay)	*Larus michahellis* (brain and liver cells of embryos)	–	200 ng/g egg weight	No positive control included	Parolini et al. (2016)
Mutations	λ transgenic medaka (liver)	+	6.7 ng/mL	Mutation spectrum encompassed mainly +1 frameshift mutations	Chen et al. (2016)
Chromosomal aberrations	*Allium cepa*	+	25 µg/mL	Positive control included (benzo[*a*]pyrene)	Sivaram et al. (2021)
Micronuclei	*Danio rerio* (blood cells)	+	0.8 µg/mL	No chemical positive control included (exposure to ZnO nanoparticles was associated with increased number of DNA strand breaks)	Du et al. (2016)
Micronuclei	*Perna viridis* (haemolymph)	+	100 µg/mL	No positive control included	Liu et al. (2014b)

CAS No., Chemical Abstracts Service Registry Number; H_2O_2, hydrogen peroxide; HIC, highest ineffective concentration; LEC, lowest effective concentration; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; PFOS, perfluorooctanesulfonic acid; w/v, weight per volume; w/w, weight per weight; ZnO, zinc oxide.

[a] +, positive; –, negative; (+), positive in a study of limited quality.

[b] The compounds have been identified (OECD, 2002) as a 50% (by weight) solution of the diethanolammonium salt of perfluorooctanesulfonate in water (T-2247 CoC) and 22.5% of a reaction product of ethyl and methyl methacrylates and 22.5% of the pyridinium chloride salt of an *N*-methylperfluorooctanesulfonamidoethanol-based glutaryl amide (T-2248 CoC).

yellow-legged gull (*Larus michahellis*) embryos after exposure to PFOS (Parolini et al., 2016).

Increases in the frequencies of micronuclei and chromosome aberrations versus the control group were observed in haemolymph cells of PFOS-exposed *Perna viridis* (Liu et al., 2014b), in *Dario rerio* (Du et al., 2016), and in cells of *Allium cepa* (Sivaram et al., 2021). A higher frequency of +1 frameshift mutations was observed in the liver cells of λ transgenic medaka fish (Chen et al., 2016).

Synopsis

[The Working Group noted that there was a paucity of data in exposed humans regarding genotoxicity, especially investigating associations between PFOA exposure and DNA damage end-points such as mutations and chromosome aberrations. One study in exposed humans showed a positive association between the serum level of PFOA and DNA strand breaks in the leukocytes of adolescents in Menen, Belgium. This positive association was not statistically significant when corrected for multiple comparisons. Two studies on PFOS have shown inconsistent indices of DNA fragmentation in semen samples from exposed humans.

For both PFOA and PFOS, in vitro studies in human primary cells have shown a lack of genotoxicity. Studies in experimental systems in human cell lines and in non-human mammalian systems in vivo and in vitro have shown mixed results for various types of end-points, such as DNA strand breaks (measured with the comet assay or by counting γH2AX foci), micronuclei, and chromosome aberrations. PFOA and PFOS did not exert mutagenic effects in prokaryotes.]

4.2.3 Alters DNA repair or causes genomic instability

(a) Humans

No studies in exposed humans or in human cells in vitro were available to the Working Group.

(b) Experimental systems

Estefanía González-Alvarez et al. (2022) reported that oral treatment with PFOA at the dose of 2.5 mg/kg body weight (bw) for 15 days altered the ovarian contents of proteins that are involved in DNA damage sensing and repair in lean (4 increases and 12 decreases in protein contents) and obese (12 increases and 6 decreases in protein contents) female mice.

In vitro exposure of rhesus monkey trophoblast stem cells to PFOA at 100 nM for 4 weeks produced only subtle effects on gene expression related to DNA damage checkpoint signalling (Midic et al., 2018).

PFOS has been shown to reduce the activity of polymerase α from calf thymus: half-maximal inhibitory concentration (IC_{50}), 24.5 μM, and of the recombinant rat polymerase β, IC_{50} = 46.4 μM (Nakamura et al., 2007).

Synopsis

[The Working Group noted that there was a paucity of data on whether PFOA and PFOS altered DNA repair and caused genomic instability.]

4.2.4 Induces epigenetic alterations

See Tables 4.11 to 4.14.

DNA (CpG, 5′-C-phosphate-G-3′-dinucleotide) methylation, global DNA methylation, altered expression of microRNAs (miRNAs), and histone modifications are all forms of epigenetic change that have been associated with carcinogenesis (Sharma et al., 2010). The following sections detail studies that have investigated the

Table 4.11 End-points relevant to epigenetic alterations in humans exposed to PFOA or PFOS

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Epigenome-wide DNA methylation	Cord blood and peripheral leukocytes	Ohio, USA Prospective birth cohort with cross-sectional analysis	266 mother–child pairs (median: PFOS, 14 ng/mL; PFOA, 5.5 ng/mL) at birth	435 CpGs (PFAS); 2 CpGs PFOS + 12 CpGs PFOA + and –	Cell type composition, child age, child sex, annual household income, maternal race/ethnicity, and maternal smoking during pregnancy	Cohort replication included; comparison of methylation over time highlighting the persistence of epigenetic modifications	Liu et al. (2022a)
Epigenome-wide DNA methylation	Cord blood leukocytes	Taiwan, China Prospective birth cohort	Sapporo cohort of the Hokkaido (Japan) study (190 mother–child pairs from the general population; discovery cohort) (median: PFOS, 5.2 ng/mL; PFOA, 1.4 ng/mL) Taiwan, China Maternal and Infant Cohort Study (37 mother–child pairs from the general population; replication cohort (PFOS, 12.2 ng/mL; PFOA, 1.8 ng/mL)	4 CpGs for PFOS –; 3 CpGs for PFOA + and –	Maternal age, parity, maternal educational level, maternal blood sampling period, maternal pre-pregnancy BMI, maternal smoking during pregnancy, gestational age, infant sex, and cord blood cell estimates	Strengths of this study included the use of a replication cohort	Miura et al. (2018)
Epigenome-wide DNA methylation	Dried blood spots	New York, USA Cross-sectional study	597 neonates (median: PFOS, 1.74 ng/mL; PFOA, 1.12 ng/mL)	2 sex-specific associations for CpGs PFOS + and –; 1 CpG PFOA –	Sample plate and estimated cell count; infant sex, plurality, and epigenetically-derived ancestry (4 principal components); maternal age, race/ethnicity, education level, marital status, pre-pregnancy BMI, smoking during pregnancy, and history of pregnancy loss		Robinson et al. (2021)

Table 4.11 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Epigenome-wide DNA methylation	Cord blood leukocytes	Colorado, USA Cross-sectional study	583 mother–child pairs (median: PFOS, 2.4 ng/mL; PFOA 1.1 ng/mL)	1 DMR – for PFOA	Infant sex, gestational age (days), maternal age (years), education level (completed high school), smoking during pregnancy, race/ethnicity, BMI, previous pregnancies, cell type		Starling et al. (2020)
Epigenome-wide DNA methylation	Blood leukocytes	Shiyan Renmin Hospital Hubei Province, China Cross-sectional study	98 male and female patients (median: PFOS, 2.29 ng/mL; PFOA, 0.85 ng/mL)	87 CpGs and 11 DMRs for PFOS 63 CpGs for PFOA	Age, BMI, sex		Cheng et al. (2022)
Epigenome-wide DNA methylation	Placenta	USA Cross-sectional study	260 pregnant women (median in maternal plasma: PFOS, 4.74 ng/mL; PFOA, 2.2 ng/mL)	PFOS: 3 CpG sites (2 sites + and 1 site –) in placenta	Self-reported maternal race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, Asian), age (in years), offspring sex (male/female), pre-pregnancy BMI (kg/m^2), total plasma lipid concentration (ng/mL, except PFAS), log-transformed plasma cotinine concentration (ng/mL); methylation sample plate ($n = 5$); the first three methylation PCs and the first 10 genotype PCs were used to account for population structure	Included gene expression	Ouidir et al. (2020)
Epigenome-wide DNA methylation	Peripheral blood leukocytes	Ohio, USA Cross-sectional study	44 total (7.5 ng/mL); PFOA high ($n = 22$; 15 ng/mL) and low ($n = 22$; 2.4 ng/mL); median values	1 CpG –	Cell type, child sex, and income	Small sample size; nothing passed FDR	Kingsley et al. (2017)

Table 4.11 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Epigenome-wide DNA methylation	Blood leukocytes	Six European cohorts (France, Spain, Norway, Greece, UK, Lithuania) Cross-sectional study	1173 (PFOA, 1.51 µg/L; PFOS, 2.14 µg/L)	PFOS: 12 CpGs majority –; PFOA +/–	Cell type		Cadiou et al. (2020)
Epigenome-wide DNA methylation	Blood leukocytes	Dordrecht, Netherlands Cross-sectional study	34 (PFOS: 40 ng/g) median	PFOS: 29 CpGs, 38 DMRs	Age and leukocyte counts	Small sample size; did not differentiate between dioxins, PCBs, and PFOS; men only	van den Dungen et al. (2017a)
Epigenome-wide DNA methylation/ Epigenetic age	Whole blood leukocytes	Ronneby, Sweden Cross-sectional	63 participants (PFOS: controls, *n* = 32: 2.8 ng/mL, high exposure group, *n* = 31: 295 ng/mL) (PFOA: controls, *n* = 32: 1.4 ng/mL, high exposure group, *n* = 31: 19 ng/mL); medians	12 DMPs, PFAS +	Neutrophil fraction	Study did not differentiate between PFOS and PFOA; study design was unclear (case–control?)	Xu et al. (2022)
Global (Alu elements, LINE-1) DNA methylation	Cord blood leukocytes	Taipei, Taiwan, China Cross-sectional	363 participants (PFOS, 6.07 ng/mL; PFOA, 2.05 ng/mL)	Alu methylation: PFOS –; PFOA +/–; LINE-1 methylation +/–	Parental education level, maternal BMI, maternal age, delivery method (vaginal delivery or caesarean section), parity, infant sex, gestational age, and cotinine level		Liu et al. (2018a)
Global DNA methylation of LINE-1	Peripheral blood leukocytes	Ohio, USA Cross-sectional	685 participants (PFOS, 14.1 ng/mL; PFOA, 57.9 ng/mL)	LINE-1 methylation PFOS +; no association with PFOA	Age, sex, BMI, smoking status (ever/never), and current alcohol consumer (yes/no)		Watkins et al. (2014)
Global DNA methylation of LINE-1 and Alu methylation	Umbilical cord blood	Maryland, USA Cross-sectional	30 participants (PFOS, 5.8 ng/mL; PFOA, 1.8 ng/mL)	PFOS +/–; PFOA was marginally associated ($P = 0.06$) with a decrease in global DNA methylation	Maternal age or gestational age	Small sample size	Guerrero-Preston et al. (2010)

Table 4.11 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Global DNA methylation	Sperm	Greenland, Denmark; Poland; Ukraine Cross-sectional	262 participants (PFOS, 27.2 ng/mL; PFOA, 4.0 ng/mL)	DNA global methylation PFOS +/–; PFOA +/–	Age and smoking status	No results were significant when the entire sample was considered, but when broken down by sub-study, there were significant associations	Leter et al. (2014)
Global DNA methylation	Blood leukocytes	Taiwan, China Cross-sectional	1425 participants (mean PFOS, 4.95 ng/mL)	5mdC/dG +	Model 1: adjusted for age, sex, smoking, alcohol consumption, BMI, and household income; Model 2: adjusted for Model 1 parameters plus HTN, DM, or hyperlipidaemia	The overall epigenetic sampling was performed in a smaller sample	Lin et al. (2022)
Targeted DNA methylation	Cord blood leukocytes	Sapporo, Japan Cohort	177 mother–child pairs (PFOS, 5.2 ng/mL; PFOA, 1.3 ng/mL)	IGF2 methylation; PFOA –	Maternal age, maternal education, infant sex, maternal smoking during pregnancy, and blood sampling period		Kobayashi et al. (2017)
miRNA expression	Blood leukocytes	Ronneby, Sweden Cross-sectional	53 pregnant women (median: PFOS: low-exposure group, 3 ng/mL; high-exposure group, 230 ng/mL) (median: PFOA: low-exposure group: 2 ng/mL; high-exposure group, 8 ng/mL)	PFOS: ↓ miR-101-3p, ↓ miR-144-3p, ↓ miR-19a-3p	None	No covariates included; only women tested	Xu et al. (2020b)
Targeted DNA methylation	Cord blood leukocytes	Taiwan, China Cross-sectional	486 participants (PFOS, 6.09 ng/mL; PFOA, 2.04 ng/mL)	*Mest* promoter methylation PFOS –	Maternal age, infant sex, parental educational level, cotinine level in cord blood, maternal alcohol consumption, pre-pregnancy BMI, parity, type of delivery, and gestational age	Sex-specific findings (women higher than men)	Ku et al. (2022)

Table 4.11 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Targeted DNA methylation (*IGF2*, *NR3C1*), LINE-1 DNA methylation	Placenta	Hebei Province, China Cross-sectional	180 participants; median: PFOS, 1.21 ng/g; PFOA, 1.33 ng/g	LINE-1 methylation: PFOS –; PFOA –	Age, pre-pregnancy BMI, gestational weeks, GDM, parity, newborn sex, mean intakes of carbohydrate, protein, and fat during pregnancy, and physical activity		Wang et al. (2023a)
Epigenome-wide DNA methylation, targeted DNA methylation, epigenetic age	Blood leukocytes	Arizona, California, and Massachusetts, USA Firefighters Cross-sectional	197 firefighters (*n*-PFOS, 4.02 ng/mL; *sm*-PFOS, 2.06 ng/mL; PFOA, 1.79 ng/mL)	Epigenetic age: positive association with 2 epigenetic clocks for *sm*-PFOS; positive association with 6 epigenetic clocks for *n*-PFOA Targeted DNA methylation: *sb*-PFOA positively associated with CpG sites in *PPARG* and *CD36*; *n*-PFOA inversely associated with methylation at one CpG site in *ACOT2* Epigenome-wide DNA methylation: positively associated with a CpG site within *CAPN12*; *n*-PFOS was inversely associated with methylation near the transcription start site of *RAD1*	Age, sex, race/ethnicity, cell type estimates and PCs representing technical variation from the Infinium MethylationEPIC array, and ethnicity in site-specific analysis and EWAS		Goodrich et al. (2021)

Table 4.11 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Targeted miRNA expression	Blood leukocytes	Fluorochemical plant, Jiangsu Province, China Cross-sectional	55 workers: (PFOS, 33 ng/mL; PFOA, 1272 ng/mL), 132 nearby residents: PFOS, 30.92 ng/mL; PFOA, 249 ng/mL)	PFOA positively associated with the expression of miR-26b and miR-199-3p	Age, BMI, smoking, alcohol consumption status		Wang et al. (2012a)
Targeted miRNA expression	Blood leukocytes	China Cross-sectional	80 participants with MetS and 64 controls (PFOS, 3.3 ng/mL; PFOA, 2.1 ng/mL)	*n*-PFOA negatively associated with miR-140-5p	Age	All men	Yang et al. (2020)

BMI, body mass index; CpG, cytosine–guanosine dinucleotide; dG, 2′-deoxyguanosine; DM, diabetes mellitus; DMP, differentially methylated position; DMR, differentially methylated region; EWAS, epigenome-wide association analysis study; FDR, false discovery rate; GDM, gestational diabetes mellitus; HTN, hypertension; LINE-1, long interspersed nuclear element-1; 5mdC, 5-methyl deoxycytosine; MetS, metabolic syndrome; miRNA, microRNA; *n*-, linear isomer; PC, principal component; PCB, polychlorinated biphenyl; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; *sb*-, sum of branched isomers; *sm*-PFOS, sum of perfluoromethylheptane sulfonate isomers; UK, United Kingdom; USA, United States of America.

[a] +, increased methylation; –, decreased methylation, +/–, increase or decrease in methylation; ↑, increased gene expression; ↓, decreased gene expression; ↑↓ increased and decreased gene expression.

association between PFOA or PFOS exposure and these epigenetic modifications.

(a) Humans

(i) Exposed humans

Exposure to PFOA

Several epigenome-wide association analysis studies (EWASs) have examined the relation between maternal exposure to PFOA during pregnancy and DNA methylation in neonatal cord blood.

Noteworthy among these studies was that by Liu et al. (2022a), which focused on a prospective birth cohort of mother–child pairs from the Health Outcomes and Measures of the Environment (HOME) Study (2003–2006; Cincinnati, Ohio, USA). Pregnant women were enrolled in the study at around week 16 of gestation, and children were followed-up at age 4 weeks and at age 1, 2, 3, 4, 5, 8, and 12 years. Overall, 291 participants with data on PFOA exposure and DNA methylation were considered in the study (266 at baseline and 160 at follow-up). For this study, the median PFOA concentration in maternal serum was 5.5 ng/mL and the 25th and 75th percentile values were 3.9 and 7.9 ng/mL, respectively. After adjustment for potential confounders and multiple comparisons, the authors identified that the maternal concentration of PFOA was associated with differential methylation of 12 CpGs measured in cord blood. The CpGs displayed both hyper- and hypomethylation. Notably, several of these DNA methylation changes persisted up to age 12 years. The associations were consistent at birth and at age 12 years, having the same direction and comparable effect sizes (Liu et al., 2022a). In addition, several CpGs were annotated to genes that have been linked to cancer of the breast, prostate, pancreas, and/or brain, such as *MAGI1, KRT18, SRPRB, TNR,* and *SERPINA5* (Liu et al., 2022a). [The Working Group noted that a strength of this study was the comparison of differential DNA methylation in cord blood at birth and blood collected during adolescence in the same participants, highlighting the stability of the findings. This study also benefited from the inclusion of a replication cohort. The Working Group also noted that this study analysed the association between maternal exposure to other PFAS and CpG methylation and found that there were specific changes associated with PFOA that persisted over time, further supporting the chemical specificity of the finding. As a potential limitation, it is possible that exposure to PFAS during the postnatal period could have influenced CpG methylation in later life. However, the probability of the chance identification of common CpG methylation sites between the early (e.g. at birth) and adolescent time points is low. Finally, the Working Group noted that a potential limitation of this study was that serum PFAS were not measured during the same trimester of pregnancy in all participants. However, this would lead to non-differential exposure misclassification and thus bias towards the null.]

Miura et al. (2018) also performed an EWAS in 190 mother–child pairs from the prospective Sapporo cohort of the Hokkaido Study (discovery cohort) and from 37 mother–child pairs from the Taiwan Maternal and Infant Cohort Study (replication cohort), examining the relation between PFOA in maternal serum and cord blood DNA methylation. The median PFOA concentration was measured to be 1.4 ng/mL and exposure was associated with both hypo- and hypermethylation of CpGs (Miura et al., 2018). [The Working Group noted that a strength of this study was the inclusion of a replication cohort. As above, the Working Group noted a potential limitation of this study was that serum PFAS concentrations were not measured during the same trimester of pregnancy in all participants. As noted, this would lead to non-differential exposure misclassification that would bias towards the null.]

Robinson et al. (2021) examined associations between PFOA levels and DNA methylation in a

cohort study in 597 neonates in New York, USA, in which both were assessed in dried blood spots from newborns. The median PFOA concentration measured was 1.12 ng/mL. Overall, log-transformed values of PFOA were not related to site-specific DNA methylation. When comparing the participants in the top decile of PFOA concentration with the other participants, exposure was associated with decreased DNA methylation of one CpG methylation site annotated to genes *SCRT2* and *SRXN1* (Robinson et al., 2021).

Starling et al. (2020) performed an analysis of data from a prospective cohort study of mother–infant pairs in Colorado, USA (n = 583). The median PFOA concentration was 1.12 ng/mL, and the range was 0.1–15.4 ng/mL. The maternal serum level of PFOA was associated with decreased DNA methylation of a CpG site annotated to *TJAP* in the cord blood of infants (Starling et al., 2020). Additionally, PFOA was associated with altered methylation of 15 differentially methylated regions (DMRs).

In the study by Cheng et al. (2022), plasma PFOA was assessed in 98 patients and the median concentration was 0.85 ng/mL. There were 63 CpG sites and eight DMRs associated with the measured plasma PFOA levels. Among the identified CpGs were those that were annotated to the genes *AFF3*, *CREB5*, *NRG2*, and *USF2*, and one of the DMRs was annotated to *IRF6* (Cheng et al., 2022).

[The Working Group noted that, taken together, the EWAS studies relating maternal PFOA to cord blood-based DNA methylation identified statistically significant associations. Although the specific genes that were identified across studies were not the same, in numerous cases the CpG sites were located within cancer-associated genes.]

In addition to the EWAS described above, several studies have also investigated the relations between PFOA and gene-specific/targeted CpG methylation. For example, Kobayashi et al. (2017) examined prenatal exposure to PFOA in a cohort in Japan (177 participants). The mean level was 1.3 ng/mL and the concentrations ranged from below the detection limit to 5.3 ng/mL. Exposure to PFOA was associated with lower methylation of the imprinted gene *IGF2* in cord blood (Kobayashi et al., 2017).

Similarly, Goodrich et al. (2021) investigated the PFOA-associated DNA methylation in blood leukocytes in firefighters from three states in the USA, namely Arizona, California, and Massachusetts. The mean level of linear *n*-PFOA was 1.79 ng/mL, and the 25th and 75th percentile values were 1.40 and 2.20 ng/mL. [The Working Group noted that there were lower concentrations and detection frequencies for branched *sb*-PFOA in this cohort, with a detection frequency of 31%.] The results indicated that exposure to *n*-PFOA was associated with lower methylation at one CpG site annotated to *ACOT2*. The results also indicated that the sum of the branched isomers of perfluorooctanoate (*sb*-PFOA) was associated with greater methylation at two CpG sites annotated to *PPARG* and *CD36*. When assessed via an epigenome-wide approach, *sb*-PFOA was associated with greater methylation at a CpG site that was annotated to *CAPN12* (Goodrich et al., 2021).

The relation between PFOA and epigenetic ageing (i.e. a measure of biological ageing) has been assessed. Indicators of epigenetic age, referred to as epigenetic clocks, have been developed with the use of CpG methylation data (Li et al., 2022b). Epigenetic age is a predictor of age and/or mortality. Recent research in diverse cancer types has highlighted the crucial role of epigenetic ageing in the initiation of tumours and its potential utility in predicting cancer risk (Yu et al., 2020). Goodrich et al. (2021) highlighted a positive association between exposure to *n*-PFOA and epigenetic ageing, assessed using various epigenetic clocks. However, there were no associations identified between *sb*-PFOA and epigenetic ageing.

The relationship between exposure to PFOA and the expression levels of miRNAs has been assessed in several studies.

Wang et al. (2012a) performed a cross-sectional analysis of a cohort of 55 workers in a fluorochemical plant and 132 nearby residents (controls) in a suburban area of Changshu City, Jiangsu Province, China. The geometric mean levels of PFOA were 1272.31 ng/mL in the workers and 249.93 ng/mL in the residents. To explore the effect of PFOA on circulating miRNAs, serum samples from 10 workers and 10 residents were used for miRNA microarray analysis. The high serum level of PFOA (high PFOA group) was positively associated with the increased expression of miR-26b and miR-199–3p in blood leukocytes (Wang et al., 2012a).

Yang et al. (2020) performed a cross-sectional analysis in a cohort of male participants with (n = 80) and without (n = 64) metabolic syndrome from China in whom the mean level of PFOA was 2.1 ng/mL. The serum concentration of n-PFOA was found to be negatively associated ($\beta = -0.772$; 95% CI, −0.244 to −0.300; $P < 0.01$; $q < 0.05$) with the expression of miR-140-5p in blood leukocytes (Yang et al., 2020).

Two studies assessed PFOA in relation to the global methylation of long interspersed nuclear element 1 (LINE-1) and/or small dimeric elements ALU methylation. Specifically, Guerrero-Preston et al. (2010) performed a cross-sectional analysis of a cohort of newborns in Maryland, USA (30 participants). The mean PFOA concentration, measured in umbilical cord blood just after birth, was 1.8 ng/mL. PFOA was marginally associated (P = 0.06) with a low level of global DNA methylation in the umbilical cord blood (Guerrero-Preston et al., 2010).

Similarly, Watkins et al. (2014) examined the association between PFOA and LINE-1 in a subset of adults enrolled in the C8 Health Project in Ohio, USA (685 participants). The mean serum concentration of PFOA was 57.9 ng/mL. No association was observed between PFOA and LINE-1 (Watkins et al., 2014) (see Table 4.11).

Exposure to PFOS

EWAS have been used to investigate the relationship between exposure to PFOS during pregnancy and CpG methylation in neonatal cord blood. A prospective birth cohort study collected data from 266 mother–child pairs from the general population in Ohio, USA. The median maternal serum PFOS concentration was measured as 14 ng/mL and the 25th and 75th percentile values were 9.9 and 17.8 ng/mL. After adjusting for potential confounders and multiple comparisons, the study found that prenatal exposure to PFOS was associated with persistent hypermethylation of two CpGs, in both cord blood and peripheral blood later in life (at age 12 years) (Liu et al., 2022a). One of these CpGs was annotated to *HPSE2*, which has been linked to breast cancer (Zhang et al., 2021a).

[The Working Group noted two strengths of this study: (i) the comparison of DNA methylation at different time points in the same participants; and (ii) the inclusion of a replication cohort.]

Miura et al. investigated the relation between maternal serum PFOS and cord blood DNA methylation in mother–child pairs recruited from the Sapporo cohort of the Hokkaido Study (discovery cohort) and mother–child pairs from the Taiwan Maternal and Infant Cohort Study (replication cohort). A total of 190 mother–child pairs, with exposure and methylation data, were analysed. The median PFOS concentration was 5.2 ng/mL, and the exposure was associated with the hypomethylation of four CpGs, as well as one DMR (Miura et al., 2018). [The Working Group considered the inclusion of a replication cohort to be a strength of this study. However, the Working Group noted a potential limitation of this study, which was that the serum PFOS concentrations were not measured during the same trimester of pregnancy in all participants. This might lead to

non-differential exposure misclassification and thus bias towards the null.]

In a separate study, Cheng et al. (2022) examined the relation between plasma PFOS and DNA methylation in leukocytes sampled from both male and female patients (n = 98) from the Shiyan Renmin Hospital of Hubei Province in China. The patients were being treated for benign diseases or for cosmetic needs. The median plasma concentration was 2.29 ng/mL. A total of 87 CpG sites and 11 DMRs displayed associations with plasma PFOS concentrations (Cheng et al., 2022).

Ouidir et al. (2020) assessed the association between PFOS exposure (plasma median concentration, 4.74 ng/mL) in pregnant women (n = 260) enrolled in the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Fetal Growth Studies of a singleton cohort and epigenome-wide DNA methylation in the placenta. The authors identified three CpG sites with both hyper- and hypomethylation (Ouidir et al., 2020).

Robinson et al. (2021) investigated the association between PFOS exposure and DNA methylation in the Upstate KIDS cohort study, New York, USA, comprising 597 newborns, for whom the median PFOS concentration, measured in dried blood spots, was 1.74 ng/mL (25th to 75th quartile, 1.11 to 2.54 ng/mL). The results showed that PFOS was associated with lower DNA methylation, measured in dried blood spots, at one CpG site in boys, and higher DNA methylation at a different site in girls. In addition, the associations were observed only at the highest concentrations of PFOS, above the 90th percentile (Robinson et al., 2021).

Other studies have examined the relation between exposure to PFOS and epigenetic ageing. As reported above, DNA methylation can be used to estimate epigenetic age (Li et al., 2022b). Epigenetic ageing has been associated with tumour initiation and cancer risk prediction (Yu et al., 2020).

Goodrich et al. (2021) conducted a cross-sectional analysis of 197 firefighters from Arizona, California, and Massachusetts, USA. They observed that the mean serum concentration of n-PFOS was 4.02 ng/mL and the 25th and 75th percentile values were 3.00 and 5.80 ng/mL, respectively. The total concentration of perfluoromethylheptane sulfonate isomers (sm-PFOS) was 2.06 ng/mL and the 25th and 75th percentile values were 1.40 and 3.10 ng/mL, respectively. The results showed that for n-PFOS there was no association with epigenetic ageing, whereas for sm-PFOS there were associations with epigenetic ageing, specifically for two of the clocks (Goodrich et al., 2021).

Besides the EWAS approach, several studies have investigated the relationship between PFOS and gene-specific or targeted DNA methylation. For example, Ku et al. (2022) analysed 486 mother–infant pairs from the Taiwan Birth Panel Study cohort, China. The mean PFOS concentration was 6.09 ng/mL and the highest measured concentration was 67.92 ng/mL. The researchers identified that, in the multivariable model after adjustments, prenatal exposure to PFOS was associated with decreased methylation in the promoter region of *MEST* in the cord blood of infants (Ku et al., 2022). [The Working Group noted that *MEST* is an imprinted gene that encodes a protein belonging to the α/β hydrolase superfamily and has been found to be linked to adipocyte differentiation (Kamei et al., 2007).]

The associations of exposure to PFOS with global methylation, LINE-1, and/or ALU methylation have also been assessed. Wang et al. (2023a) performed a cross-sectional analysis of 180 pregnant women enrolled in a cohort study from Tangshan City, northern China, to examine the relation between PFOS and DNA methylation in the placenta. The median PFOS concentration in the placenta was 1.39 ng/g and ranged from 0.19 to 3.70 ng/g. The level of PFOS in the placenta was inversely associated with the overall methylation of LINE-1 (Wang et al., 2023a).

Similarly, Liu et al. (2018a) analysed 363 mother–infant pairs from the Taiwan Birth Panel birth cohort study in Taiwan, China. Maternal and cord blood samples were collected at birth. The mean PFOS concentration in the cord blood was 6.07 ± 1.93 ng/mL (geometric mean ± standard deviation, SD). Prenatal PFOS was associated with decreases in cord blood-derived ALU methylation (Liu et al., 2018a).

Guerrero-Preston et al. (2010) conducted a cross-sectional analysis of cohort of newborns (n = 30) in Maryland, USA. The mean PFOS concentration in the cord blood was 5.8 ng/mL. No association between PFOS and global DNA methylation was observed.

Another form of epigenetic modification is the altered expression of miRNAs. Several studies have examined the relation between PFOS and miRNA expression. For example, Xu et al. (2020b) performed a cross-sectional analysis of 53 women from the Ronneby area, Sweden, in whom the PFOS levels were up to 315 ng/mL because of contamination of drinking-water. The results highlighted that PFOS is associated with decreases in the expression of three miRNAs (miR-101-3p, miR-144-3p, and miR-19a-3p) in blood leukocytes (Xu et al., 2020b). These miRNAs are predicted to target genes annotated to cancer or endocrine dysfunction, such as *DNMT3A, EGFR, HMGCR, NR1H3, PPARA*, *PTGS2*, and *TGFA*.

[The Working Group noted several factors as strengths of the studies, including: (1) an examination of the persistence of the epigenetic mark over time; (2) the inclusion of gene expression measures; and (3) the use of replication cohorts. The Working Group noted that numerous studies deployed designs where chemical exposure was analysed in relation to epigenetic end-points in a cross-sectional manner. A potential limitation of this design is that a chemical measurement and/or epigenetic modification assessed at a single time point may not capture variation over time. In support of the above, it has been reported that PFAS levels can vary over the course of gestation: blood levels are higher in the first trimester of pregnancy than in later trimesters. Most of the association analyses presented here examined the relationship between PFAS exposure assessed later in pregnancy and the epigenetic end-point. This time frame of exposure assessment would thus be expected to lead to bias towards the null. The Working Group also noted that most studies assessed the epigenetic marks in blood leukocytes, and the relevance of the epigenetic marks to tumorigenesis has not been completely established. Despite the limitations of measurements in leukocytes, the Working Group noted features of the studies that have relevance to cancer, including PFOA and PFOS-associated DNA methylation of cancer-associated genes, altered expression of miRNAs that are known to be involved in carcinogenesis, and cancer-associated features, such as global hypomethylation.]

Other studies with inconclusive results or limitations in the quality of design were considered less informative. These studies investigated the associations of PFOA (Kingsley et al., 2017) or PFOS (van den Dungen et al., 2017a; Cadiou et al., 2020; Xu et al., 2022) with genome-wide methylation, global DNA methylation, or target DNA methylation (Leter et al., 2014; Lin et al., 2022).

(ii) Human cells in vitro

See Table 4.12.

The effects of in vitro exposure to PFOA and PFOS on DNA methylation have been assessed in a variety of human cells, including breast (MCF-7, MCF-10A), brain (SH-SY5Y), liver (HepG2), lung (A549), and placenta (HTR-8/SVneo) cell lines (Tian et al., 2012; Bastos Sales et al., 2013; Guo et al., 2017; van den Dungen et al., 2017b; Jabeen et al., 2020; Zhao et al., 2022).

Table 4.12 End-points relevant to epigenetic alterations in human cells in vitro exposed to PFOA or PFOS

End-point	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
DNA methylation	Brain, SH-SY5Y cells	PFOA –	PFOA: 0.4 or 4 µg/L in medium for 4 d		Zhao et al. (2022)
DNMT activity (DNMT1 and DNMT3A); global DNA methylation; *DNMT* expression	Breast, MCF7; liver, HepG2 cells	PFOA: global methylation –, ↓ *DNMT1* expression, ↑ *DNMT3A* in MCF-7	PFOA: 0, 20, 100, 200, or 400 µM for 48 h		Liu and Irudayaraj (2020)
Global DNA methylation	Brain, neuroblastoma cell line (SK-N-AS)	PFOS +/–; PFOA +/–	PFOS: 10 µM; PFOA 10 µM		Bastos Sales et al. (2013)
Global DNA methylation	Breast, MCF-10A cells	PFOS + and PFOA +	PFOS (10 µM) and PFOA (100 µM) for 72 h		Pierozan et al. (2020)
Global DNA methylation	Liver, L02 cells	PFOA +/–	PFOA: 5, 10, 25, 50, or 100 mg/L		Tian et al. (2012)
Global DNA methylation	Liver, HepG2	PFOA –	PFOA: 0–400 µM for 24 h		Wen et al. (2020)
Global methylation	Breast, MCF-10A	PFOS + and PFOA +	PFOS: 10 µM; PFOA: 100 µM		Pierozan et al. (2020)
mRNA expression of DNMTs and BDNF, miRNA-16, miRNA-22, and miRNA-30a-5p	Brain, SK-N-SH cells	↑ miRNA-16, ↑ miRNA-22, and ↑ microRNA-30a-5p, ↓ *DNMT1* mRNA, ↓ DNMT1 protein, ↑ *DNMT3A* mRNA, ↑ DNMT3A protein, ↑ *DNMT3B* mRNA, ↑ DNMT3B protein, ↓ BDNF protein, ↓ *BDNF* mRNA	PFOS: (0–150 µM)		Guo et al. (2017)
Targeted DNA methylation: DMRs, DMPs, 84 adipogenic genes	Human mesenchymal stem cells from bone marrow	DNA methylation –, 2 DMRs (*AXIN1*, *DKK1*) –, 45 DMPs (majority –)	PFOS: 0–30 µM, from day –1 to day 10; measurements on day 10	Treatment during cell differentiation	van den Dungen et al. (2017b)
Targeted gene expression (DNA methylation machinery (DNMTs, TETs)	Lung, A549 cells	Expression: PFOS: ↓ *TET1* mRNA, ↑ *TET2* mRNA, ↑ *TET3* mRNA, ↑↓ *DNMT1* mRNA, ↓ *DNMT3B* mRNA, ↓ *DNMT3A* mRNA, ↑↓ *CCNE1* mRNA, ↑↓ *CCNA2* mRNA, ↓ *CCNB1* mRNA PFOA: ↓ *TET1* mRNA, ↑ *TET3* mRNA, ↓ *DNMT1* mRNA, ↓*DNMT3B* mRNA, ↑ *DNMT3A* mRNA, ↑ *CCNE1* mRNA, ↓ *CCNA2* mRNA, ↑↓ *CCNB1* mRNA	PFOS: 0–400 µM; PFOA: 0–400 µM	DNA methylation was not assessed	Jabeen et al. (2020)
Targeted miRNA expression (miR-155 expression)	Liver, HepG2 cells	↑ miR-155	PFOS: 0–50 µM for 24 h		Wan et al. (2016)

Table 4.12 (continued)

End-point	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
Targeted miRNA expression and gene expression (MEG3)	HTR-8/SVneo cells	PFOS: ↓ miR-770; ↓ *MEG3*, ↑ *PTX3*	PFOS: 0–10 μM		Li et al. (2022b)
Targeted miRNA expression: miR-19a and miR-19b expression; targeted DNA methylation (H19 methylation)	HTR-8/SVneo cells	↓ miR-19a; ↓ miR-19b; ↑ *H19* expression	PFOS: 0, 0.1, 1, or 10 μM for 24 or 48 h		Li et al. (2020b)
Targeted miRNA expression (miRNA-22)	Brain, SH-SY5Y cells	↓ miR-16 expression; ↑ miR-22 expression, ↓ *BDNF*, ↓ *CREB* (100 μM), ↑ *TrkB*	PFOS: 0–100 μM for 48 h		Li et al. (2015)
Targeted gene expression (DNMTs, SIRTs), global DNA methylation, targeted miRNA expression (miR-29b)	HTR-8/SVneo cells	PFOS: ↓ *DNMT1*, ↓ *DNMT3A*, ↓ *DNMT3B*, ↓ *SIRT1*, ↓ *SIRT3*; global DNA methylation; expression: ↑ miR-29b	1, 10, and 50 μM		Sonkar et al. (2019)

BDNF, brain-derived neurotrophic factor; d, day(s); DMP, differentially methylated position; DMR, differentially methylated region; DNMT, DNA methyltransferase; miRNA, microRNA; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; SIRT, sirtuin; TET (enzymes), ten eleven translocation, alias for tet methylcytosine dioxygenases.

[a] +, increased methylation; –, decreased methylation, +/–, increase or decrease in methylation; ↑, increased gene expression; ↓, decreased gene expression; ↑↓, increased and decreased gene expression.

Exposure to PFOA

Pierozan et al. (2020) examined the effects of PFOA at a concentration of 100 μM for 72 hours and found increased global DNA methylation in breast MCF-10A cells. In another study in which PFOA was tested at a range of 0 to 400 μM, exposure was associated with a dose-dependent decrease in global DNA methylation in HepG2 cells (Wen et al., 2020).

Liu and Irudayaraj (2020) exposed breast MCF7 and liver HepG2 cells to PFOA at concentrations ranging from 0–400 μM. PFOA was associated with reduced global DNA methylation and altered expression of *DNMT1* in both cell types. *DNMT3A* displayed increased expression in MCF7 cells. An inconclusive trend in the expression level of *DNMT3B* was observed in both cell types (Liu and Irudayaraj, 2020).

Exposure to PFOS

Pierozan et al. (2020) examined the effects of PFOS exposure on MCF-10A cells using a concentration of 10 μM. PFOS was associated with increases in global DNA methylation in breast MCF-10A cells (Pierozan et al., 2020).

In relation to miRNAs, Wan et al. studied PFOS at concentrations ranging from 0–50 μM. They found that PFOS was associated with increased expression of miR-155 in HepG2 cells (Wan et al., 2016).

Li et al. (2020b) examined the effects of PFOS in HTR-8/SVneo cells at concentrations ranging from 0–10 μM. They found that PFOS altered the expression of several miRNAs in HTR-8 cells, including the reduction of the expression of miR-19a and miR-19b (Li et al., 2020b). PFOS was subsequently shown to reduce the expression of miR-770 in HTR-8 cells (Li et al., 2022c). The effects of PFOS were tested in SH-SY5Y at concentrations ranging from 0–100 μM; PFOS caused decreased expression of miR-16 and increased expression of miR-22 (Li et al., 2015).

PFOS treatment for 24 or 48 hours, even at 10 μM, decreased gene and protein expression of the DNA methyltransferases, and significantly increased reactive oxygen species (ROS) production in the first-trimester human HTR-8/SVneo trophoblast cell line. In addition, PFOS reduced global DNA methylation and increased protein lysine acetylation (Sonkar et al., 2019).

(b) Experimental systems

(i) Non-human mammalian systems in vivo

See Table 4.13.

Exposure to PFOA

In relation to histone modifications, Li et al. (2019a) exposed female pregnant Kunming mice to PFOA doses of 1, 2.5, 5, 10, or 20 mg/kg bw per day from pregnancy day 0, i.e. gestational day (GD)1, to GD17, and found that PFOA was associated in a dose-dependent manner with decreased histone acetylation in the liver: the histone acetyltransferase activity of the female offspring was reduced significantly up to the dose of 5 mg/kg, and the histone deacetylase activity was increased significantly up to the highest dose of 10 mg/kg. The expression of both acetyl-histone H3 and acetyl-histone H4 proteins was reduced significantly (Li et al., 2019a).

Rashid et al. (2020a) exposed CD-1 mice (age 30 days) to PFOA at 1, 5, 10, or 20 mg/kg per day for 10 days and found that PFOA exposure was associated with 879 DMRs and increased *DNMT1* expression in the kidney (Rashid et al., 2020a).

Ahmad et al. (2021) exposed CD-1 mice to PFOA at 5 or 20 mg/kg per day for 10 days and observed decreases in the methylation of the gene encoding transmembrane serine protease *Tmprss2* (a prognostic marker for lung adenocarcinoma) in lung tissue where also PFOA accumulated (Ahmad et al., 2021). The gene expression of *Dnmts* and *Tets* was also decreased (Ahmad et al., 2021).

Table 4.13 End-points relevant to epigenetic alterations in non-human mammals in vivo exposed to PFOA or PFOS

End-point	Species, strain	Tissue	Result[a]	Concentrations or dosing regimen	Route, duration	Comments	Reference
Global DNA methylation level, targeted gene expression (histone demethylases *Kdm1a* and *Kdm4c*)	Mouse, CD-1	Kidney	Global DNA methylation level –, gene expression of histone demethylases Kdm1a and Kdm4c ↑	PFOS, 5, 10, 20 mg/kg per day	Oral, 14 d		Wen et al. (2022)
Global DNA methylation, LINE-1 methylation, *GSTP* promoter region methylation	Rat, Sprague-Dawley	Liver of offspring, on PND21	Global DNA methylation –, LINE-1 methylation –, *GSTP* promoter region methylation changes +	PFOS, 0.1, 0.6, or 2.0 mg/kg bw per day	Oral gavage to dams from GD2 to GD21	Prenatal treatment	Wan et al. (2010)
Histone acetylation	Mouse, Kunming	Liver of female offspring on PND21	PFOA: ↓ histone acetylation, ↓ HAT activity, ↑ HDAC activity	PFOA, 1, 2.5, 5, or 10 mg/kg/bw (0.2 mL per day) to dams	Gavage, solution in deionized water, dams, GD1 to GD17	Prenatal treatment; liver specimens collected from female offspring killed on PND21	Li et al. (2019a)
Histone acetylation	Rat, Wistar	Testis	PFOS: H3K9me2 +, H3K9ac +, H3K18ac +, H3K9me3 –	PFOS, 0.015 and 0.15 mg/kg per day	Oral gavage, 60 d		Alam et al. (2021)
Histone acetylation	Mouse, ICR	Ovary	PFOS: histone H3K14 acetylation of *StAR* promoter –	PFOS, 0.1 mg/kg per day	Drinking-water, 4 mo		Feng et al. (2015)
Targeted DNA methylation, targeted gene expression	Mouse, CD-1	Kidney	879 differentially methylated regions; ↑ *Dnmt1* expression, ↓ *Dnmt3a* expression, ↑ *Dnmt3b* expression, ↓ *Tet1* expression, *Tet2* expression +/–, ↑ *Tet3* expression; global DNA methylation +/–; ↑ Hdac1, Hdac3, Hdac4, Hdac 2–10 +/–; ↓ *RASAL1* mRNA expression, ↑ *Acta2* mRNA expression, *Lrnf2* and *Dlg2* mRNA expression +/–, ↑ *Tgfb* mRNA expression	PFOA, 1, 5, 10, or 20 mg/kg per day	Oral, 10 d		Rashid et al. (2020a)

Table 4.13 (continued)

End-point	Species, strain	Tissue	Result[a]	Concentrations or dosing regimen	Route, duration	Comments	Reference
Targeted gene and miRNA expression (angiogenesis-related mRNA, miRNA, and lncRNA)	Pregnant mouse, CD-1	Placenta of dams	↓ lncRNA Xist expression	PFOS, 0.5, 2.5, 12.5 mg/kg bw per day	Oral gavage, GD1 to GD17	Analysis performed on GD18	Chen et al. (2018)
Targeted gene expression (*Dlk1–Dio3* imprinted cluster)	Mice, Kunming	Testes of offspring	↓ *Dlk1–Dio3* on PND21	PFOA, 1, 2.5, or 5 mg/kg per day	Gavage during gestation, GD1 to GD17	Prenatal treatment	Song et al. (2018)
Targeted gene expression (Dnmts and Tets); targeted CpG methylation	Mouse, CD-1	Lung tissue	PFOA: ↓ Dnmts and ↓ Tets expression: – CpG *Tmprss2*	PFOA, 5 or 20 mg/kg per day	Oral gavage, 10 d		Ahmad et al. (2021)
Targeted gene expression (epigenetic machinery)	Mouse, CD-1	Small intestine, colon	Small intestine: mRNA: ↓ *Dnmt1*, ↓ *Dnmt3a* (↑↓), ↓ *Dnmt3b*; ↑ *Tet1*, ↑ *Tet2*, ↓ *Tet3*, ↓ *Cldn2*, ↓ *Cldn8*, ↓ *Cldn12*, ↑ *Cld4*, *Cldn3* (↑ and ↓), ↑ *Cldn15*, *Cldn7* (↑↓), *Tjp1* (↑↓), *Tjp2* (↓↑), *Ocln* +/– Colon: mRNA: ↓ *Dnmt3b*, ↓*Dnmt3a*, *Dnmt1* +/–; ↓ *Tet1*, *Tet2* +/–, *Tet3* +/–, ↑ *Cldn2*, ↑ *Cldn3*, ↑ *Cldn8*, ↓ *Cldn7*, *Cldn4* +/–, *Cldn12* +/–, *Cldn15* +/–, ↓ *Tjp1*, ↓ *Ocln*, *Tjp2* +/–	PFOA, 1, 5, 10, or 20 mg/kg per day	Oral gavage, 10 d		Rashid et al. (2020b)
Targeted gene expression (H19)	Pregnant mouse, CD-1	Placenta of dams	↑ *H19* expression, H19 methylation –	PFOS, 0, 0.5, 2.5, or 12.5 mg/kg per day	Oral, GD1 to GD17	Analysis performed on GD18	Li et al. (2020b)
Targeted miRNA and gene expression (miR-770 expression; *Meg3* expression)	Pregnant mouse, CD-1	Placentas of dams	PFOS: ↓ miR-770 expression, ↓ *Meg3* expression, *Meg3* methylation +, ↑ *Ptx3* expression	PFOS, 0.5, 2.5, or 12.5 mg/kg per day	Gavage, GD0 to GD17	Analysis performed on GD18	Li et al. (2022c)
Targeted miRNA expression	Rat, Wistar	Brain	↓ miR-466b, ↓ miR-672, ↓ miR-297	PFOS, 0–3.2 mg/kg per day	Feed, GD1 to PND7		Wang et al. (2012b)

Table 4.13 (continued)

End-point	Species, strain	Tissue	Result[a]	Concentrations or dosing regimen	Route, duration	Comments	Reference
Targeted miRNA expression	Chicken, Plymouth Rock	Heart	PFOA: ↑ miR-490-5p	PFOA, 0–2 mg/kg per day	Cell injection, ED0 to ED21		Guo et al. (2022a)
Targeted miRNA expression	Mouse, BALB/c	Testes	9 ↓miRNAs, 8 ↑ miRNAs, including miR-133b-3p	PFOA, 5 mg/kg per day	Oral gavage, 28 d		Lu et al. (2017)
Targeted miRNA expression	Mice, BALB/c	Serum	miR-28-5p, miR-32-5p, miR-122-5p, miR-192-5p, and miR-26b-5p (all ↑)	PFOA, 1.25 or 5 mg/kg per day	Oral gavage, 28 d		Yan et al. (2014)
Targeted MiRNA expression	Mice, ICR	Sertoli cells and Leydig cells	↑ miR-9-3p, ↑↓ miR-1954, ↑↓ miR-710	PFOS, (0.5–10 mg/kg per day)	Oral gavage, 4 wk		Huang et al. (2022a)
Targeted miRNA expression (387)	Rat, Wistar	Liver	Significantly altered miRNAs included ↑ miR-19b, miR-21*, miR-17-3p, miR-125a-3p, miR-16, miR-26a, miR-1, miR-200c, and miR-451. PND1: 35 miRNAs ↑, 11 ↓ miRNAs; PND7: 8 ↑ miRNAs, 1 ↓ miRNA; 4 miRNAs on both PND1 and PND7	PFOS, 3.2 mg/kg per day	Feed (dam), GD1 to PND7 Pups, until PND7		Wang et al. (2015b)
Targeted miRNA expression (miR-34a)	Mice, BALB/c	Liver	miR-34a ↑	PFOA, 5 mg/kg per day	Gavage, 28 d		Cui et al. (2019)
Targeted gene expression, histone modifications	Rat, Wistar	Testes	PFOA: *Lhr* ↑, *Star*↑, *Hsd3b* ↓, *Hsd17b* ↓, *Arom* ↓, *Cyp11a1* +/–, *Cyp17a1* +/–; histone modification: ↓ H3K9me1, H3K9me2, H3K9me3, H3K9ac, H3K18me1, H3K18ac, H3K23me1, and H3K23ac	PFOA, 0.015 or 0.15 mg/kg per day	Gavage, 60 d		Han et al. (2022)

bw, body weight; d, day(s); CpG, cytosine–guanosine dinucleotide; DMP, differentially methylated position; ED, embryonic day; GD, gestational day; HAT, histone acetyltransferase; HDAC, histone deacetylase; HIC, highest ineffective concentration; lncRNA, long non-coding RNA; LEC, lowest effective concentration; LINE-1, long interspersed nuclear element-1; mo, month(s); miRNA, microRNA; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PND, postnatal day; wk, week(s).

[a] +, increased methylation; –, decreased methylation; +/–, increase or decrease in methylation; ↑, increased gene expression; ↓, decreased gene expression; ↑↓, increased and decreased gene expression.

Targeted gene expression analyses have shown that PFOA exposure was associated with an increase in *DNMT* expression in the mouse kidney (Rashid et al., 2020a). PFOA was associated with decreases in the expression of *Dnmt1*, *Dnmt3a*, and *Dnmt3b* in the intestines of CD-1 mice exposed to PFOA at 1, 5, 10, or 20 mg/kg per day (Rashid et al., 2020b). Targeted gene expression analysis has shown that *Dlk1 and Dio3* have decreased expression in the testes of Kunming offspring mice exposed prenatally to PFOA at doses of 1, 2.5 or 5 mg/kg during gestation (Song et al., 2018).

In relation to miRNAs, in male BALB/c mice exposed to PFOA at 0.08, 0.31, 1.25, 5 or 20 mg/kg per day for 28 days, increases in the expression of miR-28-5p, miR-32-5p, miR-122-5p, miR-192-5p, and miR-26b-5p were identified in mouse serum at the doses of 1.25 and 5 mg/kg (Yan et al., 2014).

Developmental exposure of fertile hatchling chicken eggs (incubated to hatch) at PFOA doses of 0.5, 1, or 2 mg/kg per egg weight was also associated with increased expression of miR-490-5p in heart tissues compared with the vehicle control group (Guo et al., 2022a).

Exposure to PFOS

Wen et al. (2022) exposed CD-1 mice to PFOS at doses of 5, 10, or 20 mg/kg per day for 14 days and found decreases in global DNA methylation levels in the kidney.

Wan et al. (2010) exposed Sprague-Dawley rats to PFOS at doses of 0.1, 0.6, or 2.0 mg/kg per day from GD2 to GD21. PFOS was found to accumulate in the kidney in a dose-dependent manner and was associated with increased expression of the kidney injury markers *Acta2* and *Bcl2l1*. In addition, PFOS was found to be associated with decreased global DNA methylation and decreased LINE-1 methylation in the livers of the offspring (Wan et al., 2010). These authors also found that PFOS was associated with increased methylation the *Gstp* promoter region in the livers (Wan et al., 2010). [The Working Group noted the relevance of this finding, because it represents DNA methylation in a region of the *Gstp* gene, which is a member of the glutathione *S*-transferase (GST) gene family, involved in carcinogenesis.]

In relation to targeted gene expression, Wen et al. (2022) exposed CD-1 mice to PFOS at doses ranging from 5–20 mg/kg per day for 14 days. PFOS was associated with increased expression of the histone demethylases *Kdm1a* and *Kdm4c* in the kidney (Wen et al., 2022).

In relation to miRNAs, Wang et al. (2012b) observed decreased expression of miR-466b, miR-672, and miR-297 in the brains of neonatal albino Wistar rats on postnatal day (PND) 1 and PND7 that were born from mothers fed with PFOS at a dose of 3.2 mg/kg per day from GD1 to PND7, compared with neonatal brain tissue derived from mothers treated with vehicle (Wang et al., 2012b).

Li et al. (2022c) observed an inverse association between PFOS, at the highest dose, and miR-770 expression in the placenta sampled on GD18 from CD-1 pregnant mice treated with PFOS at doses ranging from 0.5 to 12.5 mg/kg per day by gavage from GD0 to GD17. Similarly, the expression of *MEG3*, a cancer suppressor gene, was significantly decreased in the placenta, and there was hypermethylation in a CpG site in its promoter region (Li et al., 2022c).

Wang et al. (2015b) fed pregnant albino Wistar rats with chow containing PFOS at a dose of 3.2 mg/kg per day from PND1 to PND7 and reported that 35 miRNAs were highly expressed on PND1, eight miRNAs were highly expressed on PND 7, and four miRNAs (miR-125a-3p, miR-23a*, miR-25*, and miR-494) were significantly expressed on both PND1 and PND7.

Related to histone modifications, PFOS was found to be associated with increased H3K9me2, H3K9ac, and H3K18ac, and decreased H3K9me3, in the testes of male Wistar rats treated with PFOS at a dose of 0.015 or 0.15 mg/kg per day for 60 days (Alam et al., 2021).

PFOS was also associated with decreases in histone acetylation of the StAR promoter in the ovaries of ICR mice exposed to PFOS at a dose of 0.1 mg/kg per day for 4 months (Feng et al., 2015).

PFOS was associated with decreases, observed on GD18, in the expression of lncRNA Xist in the placentas of CD-1 mouse dams exposed to PFOS at doses of 0.5, 2.5, or 12.5 mg/kg from GD1 to GD17 (Chen et al., 2018) (see Table 4.13).

(ii) Non-human mammalian systems in vitro

See Table 4.14.

In vitro testing has been carried out in cells that represent the mouse brain (mHypoE-N46), mouse liver, embryonic stem cells, and macrophages; as well as in rat mitochondria and kidneys. In addition, zebrafish embryos and bovine tissues have been assessed.

Exposure to PFOA

Kim et al. (2021) examined the effects of PFOA exposure at concentrations of 0.25–250 µmol/L on the embryonic hypothalamic cell line N46 (mHypoE-N46). PFOA was found to increase global DNA methylation.

Mouse fibroblast preadipocytes (3T3-L1) exposed to PFOA at concentrations of 0.01–100 µg/mL showed decreases in global DNA methylation compared with control (Ma et al., 2018).

No apparent DNA methylation was observed in a study by Starkov and Wallace performed in mitochondria isolated from the livers of Sprague-Dawley rats and exposed to 100 µM PFOA (Starkov and Wallace, 2002), or in the kidneys of Balb/c mice treated with PFOS at 0.1 or 1 mg/kg intraperitoneally every other day for 3 months, and rat renal tubular epithelial NRK-52E cells treated with PFOS (0–500 nM) for 6, 12 or 24 hours (Chou et al., 2017). However, Chou et al. (2017) observed increases in the upregulation of Sirt1, and in the deacetylation of peroxisome proliferator-activated receptor gamma (PPARγ) that mediated epithelial–mesenchymal transition-associated renal fibrosis.

Exposure to PFOS

PFOS exposure was associated with increased expression of miR-9-3p in Sertoli cells and Leydig cells of male ICR mice treated orally with PFOS at doses ranging between 0.5 and 10 mg/kg bw per day for 4 weeks. PFOS exposure was not associated with changes in miR-1954 or miR-710 in Sertoli cells (Huang et al., 2022a).

Blanc et al. (2019) exposed zebrafish embryos and liver (ZF-L) cells to PFOS at a concentration equivalent to its EC_{10} (93 µM) for 96 hours and showed an increase in global DNA methylation of 13% versus control. Hallberg et al. exposed bovine oocytes complexes to PFOS at concentrations of 2 ng/g or 53 ng/g for 22 hours during their maturation and found both increases and decreases in target DNA methylation on day 8 after fertilization (Hallberg et al., 2021). The authors identified that the most altered pathways were those involved in cell death and survival, with the p53 pathway the most altered (see Table 4.15).

Synopsis

[The Working Group noted that numerous studies investigating epigenetic alterations in humans exposed to PFOA and PFOS were in cohorts with background exposures representative of the general population. Many of these studies were prospective birth cohort studies that evaluated the relation between maternal/in utero exposure and epigenetic alterations in the neonate. These studies are of great importance, because they investigate the potential for developmental reprogramming that may influence cancer susceptibility. Numerous studies in exposed humans have identified associations between exposure to PFOA or PFOS and altered DNA methylation in cancer-associated genes. For PFOA and PFOS, the specific gene targets identified in these studies differed. The Working

Table 4.14 End-points relevant to epigenetic alterations in non-human mammalian systems in vitro exposed to PFOA or PFOS

End-point	Species	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
Global DNA methylation	Mouse	Brain, embryonic hypothalamic cell line N46 (mHypoE-N46)	Global DNA methylation +	PFOA, ranging study of 0–250 µmol/L for 24 h or 48 h; cells were then exposed to PFOA at EC_{50} = 27.5 µmol/L, for 24 h		Kim et al. (2021)
Global DNA methylation; DNA methyltransferase gene expression	Mouse	Fibroblasts, preadipocytes (3T3-L1)	Global DNA methylation –; DNA methyltransferase genes +	PFOA, 0.01–100 µg/mL for 4–8 days		Ma et al. (2018)
Permeability of mitochondrial membranes	Rat	Liver, mitochondria	+	PFOA, 100 µM; PFOS: 10 µM; concomitant measurements	No apparent DNA methylation or miRNA	Starkov and Wallace (2002)
Targeted DNA methylation	Cow	Egg, bovine cumulus oocyte	PFOS +/–; gene-dependent	PFOS, 2 or 53 ng/g for 22 h		Hallberg et al. (2021)
Targeted gene expression	Rat	Kidney, renal tubular epithelial cells (NRK-52E)	+ expression of EMT and renal injury biomarkers (e.g. *N*-cadherin, vimentin, *Snai1*, *Kim1*, and *Lcn2*); – expression of *Tjp1*; + expression of *Sirt1*	PFOA, 0–500 nM for 24 h	No apparent DNA methylation or miRNA	Chou et al. (2017)
Targeted gene expression (Sirt1–7)	Mouse	Macrophages (RAW 264.7 cells)	SIRT1–7 mRNA: PFOS +/–; PFOA +/–	PFOS or PFOA, 0, 0.5, 5, or 50 µM for 24 h		Park et al. (2019)
Targeted gene expression, histone modifications; global DNA methylation	Zebrafish	Embryo, liver, ZFL cells	Global DNA methylation: PFOS +	PFOS at EC_{10} = 93 µM, for 48 h		Blanc et al. (2019)
Targeted miRNA expression (miR-145 and miR-490-3p) and mRNA and protein expression	Mouse	Embryonic stem cells	PFOS: miR-145 expression +, miR-490-3p expression +, *Sox2* mRNA –, Sox2 protein –, *Nanog* mRNA –, Nanog protein –, *Oct4* mRNA +/–, Oct4 protein +/–, *Chrm2* expression +	PFOS, 0.2, 2, 20, or 200 µM for 24 h		Xu et al. (2013b)

Table 4.14 (continued)

End-point	Species	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
Targeted miRNA expression (miR-134, miR-145, miR-490-3p)	Mouse	Embryonic stem cells, D3	miR-134 ↓, miR-145 ↓, miR-490-3p ↓	PFOS, 0.2, 2, 20, or 200 μM; medium changed on days 0, 2, and 4; measurement on day 6		Xu et al. (2015)

EMT, epithelial–mesenchymal transition; h, hour(s); mRNA, messenger RNA; miRNA, microRNA; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid.
[a] +, increased methylation; –, decreased methylation; +/–, increase or decrease in methylation; ↑, increased gene expression; ↓, decreased gene expression; ↑↓, increased and decreased gene expression.

Table 4.15 End-points relevant to oxidative stress in humans exposed to PFOA or PFOS

End-point Assay	Biosample type	Location, setting Study design	Exposure level No. of participants	Response[a]	Covariates adjusted for	Comments	Reference
MDA, by TBARS 8-OHdG, by HPLC-MS/MS	Urine	Seoul, Republic of Korea Placebo-controlled crossover trial	PFOS, 10.04 ng/mL; PFOA, 4.61 ng/mL; 141 participants, age > 60 years	↑ MDA and 8-OHdG in a dose-dependent response with PFOS, but not PFOA	Age, sex, BMI, cotinine level, PM_{10}, outdoor temperature, dew point, treatment arm, and treatment	Vitamin C did not significantly reduce MDA or 8-OHdG in the PFOA or PFOS group	Kim et al. (2016b)
8-oxodG, 15-F2t-isoP, by ELISA	Urine, plasma	Ceske Budejovice, Prague, and Ostrava, Czechia Cross-sectional study	PFOA means: 1.05 (Ceske), 0.96 (Prague), 0.98 (Ostrava) ng/mL; PFOS means: 3.49 (Ceske), 3.23 (Prague), 3.35 (Ostrava) ng/mL in plasma 126 healthy non-smoking policemen; mean age, 38–40 years	↑ 8-OHdG and ↓ 15-F2t-isoP. Statistically significant ($P < 0.05$) association only between PFOS and 8-OHdG	Sampling period and locality, non-smoking policemen	Plasma PFOS and PFOA concentrations did not differ between the three different areas	Ambroz et al. (2022)
8-OHdG 8-Nitrosoguanine by HPLC-ESI-MS/MS	Urine	Taipei, Taiwan, China Cross-sectional study	*n*-PFOA, 3.77 ng/mL; branched PFOA, 0.08 ng/mL; *n*-PFOS, 12.92 ng/mL; branched PFOS, 0.44 ng/mL 597 participants (519 men and 78 women, aged 22–63 years; mean, 45.8 years)	↑ in a dose-dependent manner across the four quartiles of linear PFOS, but not PFOA Positive association with linear PFOS using 3 models	Model 1 was adjusted for age and sex; Model 2 was adjusted for the Model 1 parameters plus smoking status, alcohol intake, education level, BMI, hypertension and diabetes mellitus; Model 3 was adjusted for the Model 2 parameters plus LDL-C and urinary creatinine as covariates (multiple linear regression)	Recruited participants were controls and patients with acute coronary heart disease from another study; therefore, few women were enrolled in this study Both branched and linear PFOS and PFOA were measured in serum	Lin et al. (2020b)

Table 4.15 (continued)

End-point Assay	Biosample type	Location, setting Study design	Exposure level No. of participants	Response[a]	Covariates adjusted for	Comments	Reference
8-OHdG, by LC-MS/MS	Urine	Taiwan, China Cross-sectional study	Geometric mean and 95% CI, 3.21 (3.00–3.46) ng/mL for PFOA, 6.44 (6.05–6.89) ng/mL for PFOS 848 participants (331 men and 517 women, aged 12–30 years	No association with PFOA or PFOS using Models 1 and 2; Model 1 was adjusted for age and sex; Model 2 was adjusted for age, sex, and other risk factors, such as smoking status, BMI, systolic blood pressure, LDL-C, HDL-C, and hs-CRP	Age, sex, systolic blood pressure, LDL-C, HDL-C, insulin resistance, serum hs-CRP, history of medication, income, smoking, alcohol consumption, BMI, hypertension, diabetes mellitus	Sufficient sample size; serum PFC and microplastics were analysed	Lin et al. (2016)
8-OHdG, by ELISA	Urine	Flanders, Belgium	PFOA, 2.55 µg/L; 596 adolescents (324 males and 282 females, aged 14–15 years)	Serum PFOA concentration (n = 197) weakly associated (not statistically significant) with 8-OHdG (n = 195), and associated with increased DNA damage (alkaline comet assay, n = 598)	Sex, age, BMI, smoking habits, alcohol consumption, education level, season of sample collection	Study limitation: use of ELISA method; the presence of contaminants, including lead, chromium, cadmium, arsenic, methyl mercury, and PAH complicated the interpretation	Franken et al. (2017)
Bilirubin (an antioxidant) Albumin (extracellular antioxidant) NHANES 2007–2008 Laboratory Data Overview (National Center for Health Statistics, 2023)	Serum	USA (NHANES 2005–2012) Cross-sectional study	Serum 95th percentile of PFOA, 8.90 (max. 104.0) ng/mL; PFOS, 49.40 (max. 281.0) ng/mL 6652 participants (3246 men, 3406 women, aged 49.48 ± 18.07 years)	Positive associations with PFOA and PFOS; dose-dependent	Age, sex, education, ethnicity, income level, cotinine, and BMI	Large sample size. However, the classical and most informative oxidative end-points were not measured. These two end-points are not considered to be specific to oxidative stress	Omoike et al. (2021)

Table 4.15 (continued)

End-point Assay	Biosample type	Location, setting Study design	Exposure level No. of participants	Response[a]	Covariates adjusted for	Comments	Reference
α-klotho antioxidant, by ELISA	Serum	USA (NHANES 2007–2016) Cross-sectional study	3981 participants (1940 men, 2041 women, aged 40–79 years)	Positive associations of PFOA and PFOS with α-klotho in participants with GF-3B/4 stage, without albuminuria. Inverse associations between PFOS and α-klotho, with healthy kidney	Age, sex, ethnicity, obesity status, hypertension, diabetes, smoking, anaemia, alcohol consumption, glomerular filtration stage, and albuminuria	Large sample size. However, the classical oxidative biomarkers were not measured	Jain and Ducatman (2022)
Metabolome biomarkers of oxidative/ nitrosative stress: hydroxybutyric acid, pyroglutamic acid, oxoglutaric acid, D-glucurono-6,3-lactone, deoxyarabinohexonic acid, tetrahydrobiopterin, α-carboxyethyl hydroxychromanol, and arachidonic acid, by LC/orbitrap-MS	Serum	China	Median concentration: PFOA, 7.56 nM; PFOS, 12.78 nM 181 male participants, aged 22–48 years	PFOA and PFOS were associated with direct or indirect biomarkers of oxidative/nitrosative stress	Age, BMI, smoking, alcohol consumption	Small sample of men in a single region with incomplete demographic data; a metabolomic approach, involving the measurement of biomarkers directly or indirectly involved in the oxidative/nitrosative pathways using a state-of-the-art instrument. The metabolism biomarkers were not specific for oxidative/nitrosative stress	Wang et al. (2017)

Table 4.15 (continued)

End-point Assay	Biosample type	Location, setting Study design	Exposure level No. of participants	Response[a]	Covariates adjusted for	Comments	Reference
ROS, by OxiSelect ROS/RNS assay kit	Umbilical cord plasma	Shanghai, China Prospective study	PFOA: girls, 0.70–29.97 ng/mL; boys, < LOD to 25.99 ng/mL PFOS: girls, 0.39–18.68 ng/mL; boys,0.62–65.61ng/mL Newborns (299 boys, 282 girls)	Positive association with PFOS in female newborns; however, in male newborns, there were no relations with PFOA or PFOS	Maternal and paternal age, maternal education, maternal pre-pregnancy BMI, mode of delivery, gestational age at birth, infant sex, infant birth weight, and antepartum obstetric risk	It was impossible to distinguish the separate effects of PFOA and PFOS	Liu et al. (2018b)
8-isoprostane-PGF2α, PGF2α, 2,3-dinor-8-iso-PGF2α, and 2,3-dinor-5,6-dihydro-8-iso-PGF2α, by LC-MS/MS	Urine	Illinois, USA Prospective birth cohorts	Geometric means: PFOA, 0.75 ng/mL; PFOS, 2.03 ng/mL 428 pregnant (15-week) mothers, aged 18–40 years	Association with PFOS but not PFOA	Sociodemographic backgrounds and geographical locations; clinical characteristic and trimesters	Relatively small sample size	Taibl et al. (2022)
HO-1, by ELISA kit	Plasma	Shanxi, China Case–control study	PFOS median, 1.79 ng/mL; PFOA median, 0.79 ng/mL 144 spontaneous preterm births, and 375 full-term deliveries as controls	No association with HO-1 observed	Demographic characteristics	A nested case–control study, minimizing selection and recall bias; however, subgroups were small	Liu et al. (2020a)

BMI, body mass index; h, hour(s); CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; ESI, electrospray ionization; GF-3B/4 stage, glomerular function stage of kidney disease; HDL-C, high-density lipoprotein-cholesterol; HO-1, haem oxygenase 1; HPLC, high-performance liquid chromatography; hs-CRP, high-sensitivity C-reactive protein; LC, liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LDL-C, low-density lipoprotein-cholesterol; LOD, limit of detection; LTL, leukocyte telomere length; MDA, malonaldehyde; NHANES, National Health and Nutrition Examination Survey; *n*-, linear isomer; 8-NO2Gua, 8-nitrosoguanine; 8-OHdG, 8-hydroxydeoxyguanosine; 8-oxodG, 8-oxo-2′-deoxyguanosine; PAH, polycyclic aromatic hydrocarbon; PFC, perfluorochemicals; PFOA, perfluorooctanoic acid; PFDA, perfluorodecanoic acid; PFNA, perfluorononanoic acid; PFOS, perfluorooctanesulfonic acid; PG, prostaglandin; PM_{10}, particulate matter of < 10 μm in diameter; RNS, reactive nitrogen species; ROS, reactive oxygen species; SD, standard deviation; TBARS, thiobarbituric acid-reactive substance; USA, United States of America; yr, year(s).

[a] ↓, decrease; ↑, increase.

Group analysed the strengths and limitations of individual studies and considered the relevance of the KC-associated end-points across systems. The Working Group noted that the significant findings in humans are unlikely to be attributable to chance. The Working Group noted that the data in experimental systems corroborate the evidence observed in exposed humans. Specifically, in vivo and in vitro studies using rodents and human- and rodent-derived cells suggest that PFOA and PFOS alter DNA methylation, LINE methylation, histone modifications, and miRNA expression.]

4.2.5 Induces oxidative stress

(a) Humans

See Table 4.15.

(i) Exposed humans

Eleven studies on PFOA and PFOS (or both) relevant to exposed humans were identified in which oxidative stress-related end-points were measured.

Kim et al. (2016b) conducted a double-blind, randomized, placebo-controlled cross-over trial to study the effect of vitamin C on PFOA and PFOS-induced insulin resistance in 141 healthy elderly participants aged > 60 years from the Korean Elderly Environmental Panel (KEEP) study in Seoul, Republic of Korea. Serum PFOA and PFOS concentrations were 4.61 ± 1.86 ng/mL (11.1 ± 4.5 µM) and 10.04 ± 4.12 ng/mL (20.1 ± 8.2 µM), respectively. PFOS, but not PFOA, was positively associated with increased urinary end-points of oxidative stress malondialdehyde (MDA, P = 0.02) and 8-hydroxy-2′-deoxyguanosine (8-OHdG, P = 0.001) levels. Vitamin C treatment (1000 mg/day for 4 weeks) did not significantly alter MDA or 8-OHdG levels induced by PFOA or PFOS. The measurement of both end-points included the use of radiolabelled internal standards. MDA was measured using the thiobarbituric acid-reactive substance (TBARS) assay. The urinary 8-OHdG concentration was measured using HPLC-tandem mass spectrometry (MS/MS). [The Working Group noted that the TBARS method was not specific for malonaldehyde but, nevertheless, the MDA results complemented those for 8-OHdG.]

In a recent cross-sectional study (Ambroz et al., 2022), 126 healthy, non-smoking adult policemen from three areas of Czechia were sampled for their plasma PFOA, PFOS, and 15-F_{2t}-isoprostane (IsoP), and urinary 8-oxo-2′-deoxyguanosine (8-oxodG). 15-F_{2t}-IsoP, and urinary 8-oxodG were measured using enzyme-linked immunosorbent assay (ELISA) kits. The PFOA and PFOS concentrations correlated with elevated urinary 8-oxodG levels and reduced plasma 15-F_{2t}-IsoP, but only the association between PFOS and 8-OHdG was statistically significant.

A cross-sectional study (Lin et al., 2020b) was conducted in 597 adult (519 men and 78 women) from a middle-aged cohort (mean age, 45.8 years) from the National Taiwan University Hospital, China, to assess the associations of serum isomers of PFOA and PFOS (branched and linear) with urinary 8-OHdG and 8-nitroguanine (8-NO_2Gua). The levels of urinary 8-OHdG and 8-NO_2Gua were measured using HPLC-electrospray ionization (ESI)-MS/MS with satisfactory accuracy. Branched PFOA and PFOS were 2.1% and 3.2% of the total PFOA and PFOS concentrations, respectively. The geometric means of urinary 8-OHdG and of 8-NO_2Gua significantly increased across the four quartiles (< 8.39 and > 22.3 ng/mL for n-PFOS; from 6.82 to 8.65 µg/mL for 8-OHdG, P for trend = 0.016; and from 0.78 to 1.21 µg/mL for 8-NO_2Gua; P for trend, 0.041) in multiple linear regression analysis, after controlling for potential confounders; however, this was not true for PFOA. The results indicate that n-PFOS in serum was significantly associated with urine oxidative/nitrative stress end-points in a relatively large cohort in Taiwan.

Lin et al. (2016) investigated the relation between serum PFOA and PFOS and urinary 8-OHdG in 848 participants aged 12–30 years (331 men and 517 women) in a cross-sectional study in Taiwan, China. Urinary 8-OHdG concentrations were measured using liquid chromatography (LC)-MS/MS, with the inclusion of a suitable internal standard ($^{15}N_5$8-OHdG). The geometric means of serum PFOA and PFOS were 3.21 ng/mL (95% CI, 3.00–3.46 ng/mL) and 6.44 ng/mL (95% CI, 6.05–6.89 ng/mL) (i.e. 7.8, 7.2–8.4 µM and 12.9, 12.1–13.8 µM), respectively. There were no associations of serum PFOA and PFOS concentrations with 8-OHdG levels in the urine. [The Working Group noted that the cohort was younger, and the exposure level was lower than those in the most 8-oxodG studies in humans exposed to PFOS.]

In a cross-sectional study of a cohort of approximately 600 adolescents (both males and females aged 14–15 years) from the Flanders region of Belgium, urinary 8-OHdG as a measure of DNA damage was assessed by ELISA in 596 adolescents (see also Section 4.2.4) (Franken et al., 2017). In addition, damage to DNA was assessed with alkaline comet assay, and specifically, oxidative damage to DNA was also assessed with the Fpg-modified comet assay in a subpopulation of the cohort. Increased serum PFOA levels were associated with a 9% increase in DNA damage, as measured by the alkaline comet assay (95% CI, 1.5–17.0%; n = 196); however, in 195 participants the PFOA level was only weakly and not significantly associated with increased 8-OHdG.

In another cross-sectional study, Omoike et al. (2021) analysed data from the National Health and Nutrition Examination Survey (NHANES) cohort (2005–2012; n = 6652, 3246 men and 3406 women; age, 49.40 ± 18.07 years) that included the measurement of serum antioxidants bilirubin and albumin (extracellular antioxidants) as indicators of oxidative stress. The 95th percentile values for PFOA and PFOS were 8.90 (maximum, 104) ng/mL (i.e. 21.5, max. 251.2 µM) and 42.70 (max. 281) ng/mL (i.e. 85.4, max, 561.9 µM), respectively. PFOA and PFOS were found to be positively associated with bilirubin and albumin levels. [The Working Group noted that the actual serum levels of bilirubin and albumin were not reported in this study. Also, although the study included a substantial number of participants, the most informative oxidative stress-related end-points were not assessed; those measured were not specific for oxidative stress.]

Another NHANES cross-sectional study (Jain and Ducatman, 2022) analysed data for 3981 US adults (aged 40–79 years), 3461 with and 530 without albuminuria. The study authors hypothesized that PFAS may adversely affect the antioxidant response of the normal kidney. Serum PFAS, including PFOA and PFOS, and α-klotho, an anti-ageing protein that plays a key role in the production of antioxidant enzymes in the kidney, were measured. A positive association between PFOA or PFOS and α-klotho was observed in participants without albuminuria and kidney function in glomerular function (GF) stage 3B/4 ($15 \leq$ eGFR < 45 mL/min/1.73 m^2). In stage GF-1 (eGFR ≥ 90 mL/min/1.73 m^2), an inverse association between PFOS and α-klotho was observed in individuals without albuminuria (Jain and Ducatman, 2022).

Wang et al. (2017) studied serum PFAS, including PFOA and PFOS, and serum metabolome markers related to oxidative/nitrosative stress in 181 Chinese men (aged 22–48 years; mean ± SD, 33.2 ± 6.4 years). Metabolism end-points included hydroxybutyric acid, pyroglutamic acid, oxoglutaric acid, D-glucurono-6,3-lactone, deoxyarabinohexonic acid, tetrahydrobiopterin, α-carboxyethyl hydroxychromanol, and arachidonic acid, and were measured using LC-Orbitrap-MS. The metabolism markers directly or indirectly correlated with lipid oxidation. Associations between PFOA or PFOS and oxidative/nitrosative stress-related end-points were observed. The authors suggested that low environmental levels of PFAS, including PFOA

and PFOS, may increase the early risk of metabolic diseases, including diabetes and cardiovascular diseases. [The Working Group noted that this study included a small male cohort from a single region with incomplete demographics, which complicates the interpretation of the observations. It was noted that the metabolic markers measured in the study might not all be specific for oxidative/nitrosative stress. However, they are involved and/or result from an oxidative stress process.]

Liu et al. (2018b) studied umbilical cord plasma PFAS, including PFOA and PFOS, and ROS and fetal leukocyte telomere length (LTL) in 299 newborn boys and 282 newborn girls from a prospective cohort in Shanghai, China. The ROS/reactive nitrogen species (RNS) ratios in the cord plasma were measured using a commercial kit. Shorter LTL and high levels of ROS were observed in female newborns, and these were associated with PFOS. No association with PFOA or PFOS was observed in male newborns. [The Working Group noted that in this study the umbilical cord plasma PFOA level was higher than that for PFOS, in contrast to what is normally observed in adults. It was impossible to distinguish the effect of individual PFOA and PFOS exposure, because they are strongly positively correlated, as acknowledged by the authors.]

Taibl et al. (2022) tested the hypothesis that excess ROS might be a contributor to preterm birth (at less than 15 weeks of gestation) in 428 pregnant mothers (aged 18–40 years), not pregnant with multiple fetuses, from the Illinois Kids Development Study (IKIDS) and Chemicals in Our Bodies (CIOB) prospective birth cohorts between 2014 and 2019 in the USA. Serum PFAS levels (second trimester) and urinary levels of end-points of oxidative stress (second and third trimesters), including prostaglandin-F2α (PGF2α), 8-isoprostane-prostaglandin F2α (8-iso-PGF2α), 2,3-dinor-8-iso-PGF2α, and 2,3-dinor-5,6-dihydro-8-iso-PGF2α were measured using LC-MS/MS. The 95th percentiles and geometric means ± SD of PFOA and PFOS were 2.17 (0.75 ± 2.14) ng/mL (5.2, 1.7 ± 5.2 μM) and 7.01 (2.03 ± 2.47) ng/mL (14, 4.1 ± 4.9 μM), respectively. PFOS was found to have a modest positive association with increases in the oxidative end-points measured.

Liu et al. (2020a) conducted a nested case–control study in Shanxi, China, that included 144 women who underwent spontaneous preterm birth and 375 who underwent full-term delivery as controls. Among the 17 PFAS measured in the maternal plasma, the median values of PFOA and PFOS were 0.79 ng/mL (1.9 μM) and 1.79 ng/mL (3.6 μM), respectively. No significant differences in haem oxygenase 1 (HO-1) in the maternal plasma were observed between the spontaneous preterm birth group and the controls, with no association between HO-1 level and PFOA or PFOS. [The Working Group noted that the nested case–control study was designed to minimize selection and recall bias. However, the small sample size was considered a limitation.]

Of the eleven human studies evaluated, six were in Asian countries including China (including Taiwan) and the Republic of Korea; three studies were in the USA; one in Belgium; and one in Czechia. Among the studies, eight demonstrated a positive association between PFOS exposure and oxidative stress, and four showed that PFOA might cause oxidative stress in humans. Five studies included the measurement of the 8-OHdG or 8-oxodG form of the oxidative product, a relevant end-point of oxidative DNA damage. Three of the five studies reported a positive association for PFOS, and only one a positive association for PFOA, with 8-OHdG.

Among the studies that reported a positive association for PFOS, only two measured 8-oxodG with a highly specific analytical method. The evidence for PFOA is weak compared with that for PFOS. It was also noted that the serum/plasma PFOS concentrations were generally higher than the PFOA concentrations, except

in the umbilical cord plasma, as reported by Liu et al. (2018b).

[The Working Group noted that four prospective studies reported associations of the urinary excretion of 8-oxodG with the risks of specific cancers, although they did not evaluate any PFAS with this effect. Two of the studies showed associations with lung cancer (Loft et al., 2006, 2012) and the other two studies showed associations with breast cancer (Loft et al., 2013; Broedbaek et al., 2015). It was noted that the study by Broedback et al. was in patients with type 2 diabetes. In these studies few cancer cases were included; however, the studies were considered relevant because the authors reported on effects on this particular oxidative damage-related end-point.]

[The Working Group also noted there was a recent systematic review article (Chen et al., 2023a) that analysed the associations of exposure to persistent organic pollutants (POPs) in humans, including PFAS (PFOA and PFOS were included), with oxidative stress end-points. The review included typical oxidative stress end-points, such as 8-OHdG, ROS, MDA, reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), and some clinical diagnostic markers (gamma-glutamyl transferase, GGT), uric acid, and bilirubin). The meta-analysis of Chen et al. (2023a) acknowledged that the association of PFOA or PFOS with oxidative stress in exposed humans would require further studies.]

(ii) Human cells in vitro

See Table 4.16.

Human primary cells

There have been fewer studies using human primary cells in vitro compared with immortalized cells to investigate oxidative stress induced by PFOA or PFAS. Alterations of oxidative stress end-points were observed in cells treated with non-cytotoxic concentrations ranging from submicromolar to under 100 μM in most studies. Most studies set $P < 0.05$ for significant differences.

Han et al. (2020) exposed human epidermal keratinocytes and human dermal fibroblasts within a human full-thickness skin model (EpiDermFT (EFT-400), MatTek Corporation, Ashland, MA, USA) to PFOA at 250 μM or 2500 μM for 6 days. MDA lipid peroxidation marker levels, measured using TBARS, were significantly increased at both concentrations in the treated cells, but no significant changes in 8-OHdG were observed, as measured using an ELISA kit (Han et al., 2020).

For experiments using human primary cells, Orbach et al. (2018) used primary liver cells to assemble a multicellular organotypic culture model in 96-well plates (μOCMs) and collagen sandwich (CS) culture. Commercially available primary hepatocytes, derived from two adult males, were exposed to ½ or 1 median lethal concentration (LC_{50}) (250 or 500 μM) of PFOA for 24 hours. GSH was measured to evaluate PFOA-induced oxidative stress, and PFOA was found to reduce GSH to < 10% of control (Orbach et al., 2018). [The Working Group noted that GSH was measured using the commercial HTS GSH-Glo assay kit and that GSH reduction because of binding or oxidation did not occur because cytochrome P450 (CYP) enzymes do not oxidize PFOA. It was also noted that the doses of ½ LC_{50} and 1 LC_{50} were considered high.]

In a study from Pan et al. (2018), human erythrocytes were isolated from blood samples collected from six healthy, non-smoking adults from China. Cells were exposed to three PFAS compounds, including PFOA, at 0, 5, 10, 50, or 100 μM for 3 hours, then MDA, GSH, glutathione peroxidase (GPx), SOD, and CAT were measured using the TBARS, 2,3-naphthalenedicarboxaldehyde, 5,5-dithiobis (2-nitrobenzoic acid), SOD assay kit, and ammonium molybdate methods, respectively. PFOA at 100 μM induced a significant increase in MDA level, GSH levels were reduced by 10 and 100 μM, and the CAT and

Table 4.16 End-points relevant to oxidative stress in human cells in vitro exposed to PFOA or PFOS

End-points	Assay	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
Primary cells						
8-oxodG, MDA	ELISA for 8-oxodG, TBARS for MDA	EpiDermFT skin model (human epidermal keratinocytes and human dermal fibroblasts cells)	No change in 8-oxodG ↑ MDA	PFOA: 250 or 2500 µM, 6 days of treatment every other day	No positive control; levels of 8-oxodG in unexposed controls (0.3 ng/µg DNA, corresponding to 1740 lesions/10^6 dG); significant increase in MDA	Han et al. (2020)
GSH	HTS GSH-Glo glutathione assay	Primary human hepatic cells	↓ GSH at 250 µM	PFOA: 250 or 500 µM (1/2 LC_{50} and LC_{50}), 24 h	PFOA reduced GSH to < 10%; CYP enzymes did not oxidize PFOA, GSH depletion due to binding or oxidation did not occur; doses of ½ LC_{50} and 1 LC_{50} were considered to be high	Orbach et al. (2018)
MDA, GSH, GPx, SOD, CAT	TBA, 2, 3-NDA, DTNB, SOD by assay kits with ammonium molybdate	Erythrocytes	↑ MDA, ↓ GSH, ↓ GPx, ↓ CAT, no change in SOD	PFOA: 10 or 100 µM, 3 h for GSH; 5, 10, 50, or 100 µM for MDA, GPx, CAT, SOD	MDA: (100 µM) significant increase; GSH (10 and 100 µM), GPx (100 µM), and CAT (100 µM): significant decrease; SOD: no change. The results suggest PFOA induces oxidative stress in erythrocytes	Pan et al. (2018)
ROS	Muse Oxidative Stress Kit, followed by flow cytometry	Embryonic stem cell system – primary spermatocytes, secondary spermatocytes, and spermatids	No increase in ROS	PFOA: 11, 25, or 100 µM; PFOS: 24, 48, or 126 µM on days 1–10 of the differentiation process	No significant effect on ROS generation PFOS (all concentrations) and PFOA (11 and 25 µM) exposure resulted in significantly lower ROS levels	Steves et al. (2018)
ROS	ROS kit	Sperm	↑ ROS	PFOA 0.25, 2.5, or 25 µg/mL (0.6, 6, or 60 µM) for 0.5 or 4 h	60 µM significantly induced ROS production after 4 h	Yuan et al. (2020)
ROS, MDA, GSH, GSSG	DCFH-DA, TBARS, *o*-phthalaldehyde fluorescence detection	Lymphocytes	↑ ROS, ↑ MDA, ↓ GSH, ↑ GSSG	PFOS, 75, 150, or 300 µM for 2, 4, 6, 8, 10, or 12 h	20 healthy adults: aged 18–30 yr, sex not reported ↑ ROS in a time- and dose–dependent manner ↑ MDA after 6 h at 150 and 300 µM; ↓ GSH and ↑ GSSG after 4, 8, 10, and 12 h, but not after 6 h Biomarker data supportive of oxidative stress	Zarei et al. (2018)

Table 4.16 (continued)

End-points	Assay	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
ROS	Electron spin resonance (ESR) spectroscopy, DHE-fluorescence–confocal microscopy, DHE–fluorescence microplate reader	Microvascular endothelial (HMVEC) cells	↑ ROS	PFOS, 10, 20, 50, or 100 µM for 1 h; 2 µM for 1, 2, 3, or 5 h (time study)	↑ ROS at 50 or 100 µM by DHE method; ↑ ROS at 2 µM at all time points (1–5 h)	Qian et al. (2010)
ROS, GPx, HO-1	DCFH-DA, western blotting	Proximal tubular epithelial (HK-2) cells	↑ ROS, ↓ GPx4, ↓ HO-1	PFOS, 200 µM for 12 h	Significant ↑ ROS, ↓ GPx4, and ↓ HO-1 provided evidence of oxidative stress Only a single concentration and time point	Wang et al. (2022b)
ROS	DCFH-DA	Corneal epithelial (HCEpiC) cells	↑ ROS	PFOA 100, 200, or 400 ppm (mg/kg) for 6 h	The solubility of the PFOA in the culture medium and hence its bioavailability to cells was unknown	Tien et al. (2020)
ROS	DCF-DA	Insulin-producing EndoC-βH1 cells	No change in ROS	PFOA 1 nM or 1 µM for 24 h	No change in ROS	Dos Santos et al. (2022)
ROS	DCFH-DA	Placental trophoblast (HTR-8/SVneo) cells	↑ ROS	PFOA 0, 100, 200, 400, 600, 800, or 1000 µM for 24 h	Increased ROS in a dose–response manner	Du et al. (2022)
ROS	DCFH-DA	Placental trophoblast (HTR-8/SVneo) cells	↑ ROS	PFOS, 1, 10, or 50 µM for 24 h or 48 h	Significant ↑ ROS at 10 and 50 µM in a time- and dose-dependent manner	Sonkar et al. (2019)
ROS	DCFH-DA	Umbilical vein endothelial (HUVEC) cells	↑ ROS	PFOS, 100 mg/L (200 µM) for 1, 5, 12, 24, or 48 h	ROS increased in a time-dependent manner	Liao et al. (2012)
ROS	DCFH-DA	Umbilical vein endothelial (HUVEC) cells	↑ ROS	PFOS, 100 mg/L (200 µM) for 24 h or 40 h	PFOS increased ROS production after 24 or 40 h of exposure. In cells co-treated with anti-oxidant *Flos lonicerae* extract or chlorogenic acid for 40 h, ROS production was reduced to levels comparable to those of cells exposed to PFOS for 24 h. ROS levels were not reduced by co-treatment of cells exposed to PFOS for 24 h only	Liao et al. (2013)

Table 4.16 (continued)

End-points	Assay	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
ROS	ROS sensor (CellROX)	Mesenchymal stem (hMSC) cells	↑ ROS	PFOS, 0.1 or 10 μM at 12, 24, 36 and 48 h; 7 and 14 days	↑ ROS at 10 μM at all time points, ↑ ROS at 7 days at 0.1 μM	Gao et al. (2020)
Cell lines						
Oxidatively damaged DNA	Fpg-modified comet assay	HepG2 cells	No change	PFOA or PFOS, 100 or 400 μM	Positive control (Ro19-8022 + light) increased DNA damage	Eriksen et al. (2010)
8-oxodG	Immunostaining	HaCaT cells	↑ ROS	PFOA, 50 μM for 24 h + 8 days recovery	No positive control; increased 8-oxodG at 8 days recovery	Peropadre et al. (2018)
8-oxodG	HPLC-MS/MS	TK6 cells	↑ ROS	PFOA, 125 or 250 μg/mL for 2 h	No positive control	Yahia et al. (2016)
ROS, 8-OHdG	DCFH-DA for ROS, immunocytochemical detection for 8-OHdG	HepG2 cells	↑ ROS, ↑ 8-OHdG	PFOA, 100, 200, or 400 μM for 3 h	ROS production increased in a dose–response manner; no positive control for ROS; 8-OHdG increased in a dose–response manner with H_2O_2 used as a positive control	Yao and Zhong (2005)
ROS (H_2O_2 and superoxide anions)	Flow cytometry: DCFH-DA for H_2O_2; DHE for superoxide anion	HepG2 cells	↑ ROS	PFOA, 200 or 400 μM for 3 h	Minimum 3 replicates per treatment; ROS measured after 1.5, 3, 5, and 24 h and was found to peak at 3 h	Panaretakis et al. (2001)
ROS	DCFH-DA by flow cytometry; GSH, SOD, CAT by ELISA	Hep2G cells	↑ ROS, ↑ GSH, ↑ CAT, no change in SOD	PFOA, 10, 25, or 50 μM for 24 h	PFOA at 10, 25, or 50 μM significantly increased ROS (≤ 5.3-fold at 50 μM); GSH (1.7-fold), and CAT (1.4-fold) only at 10 μM; no change in SOD	Abudayyak et al. (2021b)
ROS	DCFH-DA	HepG2 cells	↑ ROS, ↓ GSH, ↓ GPx, ↑ SOD, ↑ CAT, GST	PFOA and PFOS 50, 100, 150, or 200 μM for 5, 10, or 15 h (ROS) and 48 h (GSH, GPx, SOD, CAT)	Significant increase in ROS at ≥ 100 μM after 5, 10, or 15 h; GSH and GPx increased at ≥ 100 μM; SOD and CAT increased at ≥ 150 μM; GST increased at 200 μM	Hu and Hu (2009)
ROS	DCFH-DA	HepG2 cells	↑ ROS	PFOA or PFOS at 0.4, 4, 40, 200, 400, 1000, or 2000 μM; every 15 min for 3 h	Significant increases at all time points, but no dose-dependency	Eriksen et al. (2010)
ROS, TAC	Carboxy-DCFH-DA, antioxidant assay kit	HepG2 cells	↑ ROS, ↓ TAC	PFOA or PFOS: ROS: 0.2, 2, or 20 μM for 24 h; TAC: 0.02, 0.2, 2, 20, or 200 μM for 24 h	PFOA or PFOS significantly induced ROS; PFOA significantly reduced TAC; PFOS reduced TAC, but not significantly	Wielsøe et al. (2015)

Table 4.16 (continued)

End-points	Assay	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
ROS	DCFH-DA	HepG2 cells	↑ ROS	PFOA or PFOS: 0, 5, 25, 50, 100, 200, 400, or 800 μM for 3 or 24 h	PFOA or PFOS induced ROS in a dose-dependent manner	Amstutz et al. (2022)
ROS, NO	DCF, and Greiss reagent	HepG2 cells	↑ ROS, ↑ NO	PFOA: 100 or 200 μM for 24 h	PFOA increased ROS and NO levels and *NOS2A* mRNA expression	Yarahalli Jayaram et al. (2020)
ROS	CellRox green reagent	HepaRG and HepG2 cells	↑ ROS	PFOA: 10, 100, or 1000 nM for 72 h	Generated PFOA-resistant cells; conducted acute (1–3 days) and chronic (30–60 days) exposure experiments for the steatosis and fibrosis study	Qi et al. (2023)
ROS, NOx	DCFH-DA or fluorescent MAK145 for ROS; 2, 3-diaminonaphthalene (fluorometric assay kit for NOx)	HepG2 cells and keratinocyte (HaCaT) cell line	↑ ROS, ↑ NOx in both cells	PFOA: 10 μM for 24 h	The DCFH-DA assay yielded higher intensity compared with the MAK14 assay	Magnifico et al. (2022)
Nrf2–ARE	Luciferase assay	ARE reporter HepG2 cell line	↑ Nrf2–ARE	PFOA or PFOS: 1, 2, 3, 4, 5, or 6 μM for 24 h	PFOA and PFOS induced Nrf2–ARE activation; the effective concentration of induction ratio 1.5 ($EC_{IR1.5}$) was 1.38 μM for PFOA and 1.17 μM for PFOS; supportive of oxidative stress being involved	Ojo et al. (2022b)
ROS	DCFH-DA	HepG2 cells	↑ ROS	PFOS: 10, 20, 30, 40, or 50 μM for 24 h; PFOA: 50 μM for 1, 3, 6, 12, or 24 h	Significant ↑ ROS at PFOS concentrations ≥ 30 μM after 24 h of treatment; when treated with 50 μM, ↑ ROS gradually, peaking after 12 h, then lower after 24 h	Wan et al. (2016)
MDA, ROS, superoxide anion, SOD, CAT, GSH	TBARS for MDA, assay kits for other biomarkers	HepG2 cells	↑ ROS, no significant changes in other biomarkers	PFOS: 50, 100, or 200 μM for 24 or 72 h	Significant ↑ ROS with PFOA ≥ 100 μM after 24 h, but not 72 h; no significant changes in the other biomarkers measured; cells pretreated with NAC showed a reduction in PFOA-induced ROS production	Yan et al. (2015a)
ROS	DCFH-DA	HepG2 cells	No change in ROS	PFOA: 5, 10, 50, 100, 200, or 400 μM; PFOS: 5, 10, 50, 100, 200, or 300 μM; 1 or 24 h	No significant increase at any time point Avoided additional stress by not trypsinizing cells before adding DCFH-DA	Florentin et al. (2011)

Table 4.16 (continued)

End-points	Assay	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
ROS, GSH	DCFH-DA for ROS, GSH-Glo assay kit	HepG2 cells	No change in ROS, ↓ GSH	PFOA: 0.2, 2, or 20 μM for 24 h PFOS: 0.2, 2, or 20 μM for 24 h	No increase in ROS but a decrease in GSH as the PFOA or PFOS concentration increased; 20 μM PFOA or PFOS induced a significant decrease in GSH; oxidative stress implicated, but unclear; the avoidance of additional physical stress by not trypsinizing cells before adding DCFH-DA may have been a factor	Ojo et al. (2021)
ROS, GSH/GSSG ratio, MDA	DCFH-DA for ROS, HPLC/fluorescence detector for GSH and GSSG, assay kit for MDA	HepG2 cells	No change in ROS, MDA, GSH/GSSG	PFOA or PFOS at 100 μM for 3 h	No change in ROS, MDA, or GSH/GSSG; only a single level of exposure; no detailed data were provided	Shan et al. (2013)
ROS, MDA, GSH, SOD, CAT	ROS by DCFH-DA/flow cytometry; MDA, GSH, SOD, CAT by ELISA	Pancreatic epithelioid carcinoma (PANC-1) cell line	No changes in ROS, ↑ GSH, ↑ CAT, ↑ SOD, ↑ MDA	PFOA at 10, 50, or 100 μM for 24 h	No increase in ROS levels; GSH levels increased only at 10 μM; MDA and SOD increased to similar levels regardless of the dose; the involvement of oxidative stress was unclear; CAT increased at all doses in a non-dose-dependent manner	Abudayyak et al. (2021a)
ROS	ROS-Glo H_2O_2 assay; DCFH-DA assay	Differentiated neuroblastoma (SH-SY5Y) cells	No changes in ROS	PFOA, 1, 10, 100, 150, 200, or 250 μM for 4, 24, 48, or 72 h	DCFDA detected ROS increases at 100, 200, and 250 μM after 4 h and at 250 μM after 24 h, but no change after 48 h at any concentration	Souders et al. (2021)
ROS, MDA, GSH, SOD	DCFH-DA followed by fluorescence photography, TBARS, GSH assay kit, SOD assay kit	Lung carcinoma (A459) cells	↑ ROS, ↑ MDA, ↑ SOD, ↓ GSH	PFOS, 25, 50, 100, or 200 μM for 24 h	PFOS at 50, 100, or 200 μM significantly increased ROS, MDA, and SOD, and reduced GSH; ROS increase was measured using fluorescence photography, but not quantified	Mao et al. (2013)
mtROS	Immunofluorescence microscopy	Ovarian granulosa-like tumour (KGN) cells	↑ mtROS	PFOA, 250, 500, or 750 μM for 24 h	Significant increase in mtROS at all doses	Zhang et al. (2023a)

Table 4.16 (continued)

End-points	Assay	Tissue, cell line	Result[a]	Concentrations or dosing regimen	Comments	Reference
ROS	DCFH-DA	Embryonic kidney (HEK293) cells	↑ ROS	PFOS, 10–60 μM for 24 h	Significant increase in ROS production; CBD (2–80 μM) partially restored PFOS-induced ROS; however, the concentrations of PFOS and CBD that had their effects were unclear	Du et al. (2023)

ARE, antioxidant responsive element; CAT, catalase; CBD, cannabidiol; CYP, cytochrome P450; DCFH-DA, 2′,7′-dichlorodihydrofluorescein diacetate; DCF, dichlorofluorescein; dG, 2′-deoxyguanosine; DHE, dihydroethidium; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); ELISA, enzyme-linked immunosorbent assay; Fpg, formamidopyrimidine DNA glycosylase; GPx, glutathione peroxidase; GSH, glutathione; GSSG, oxidized glutathione; GST, glutathione *S*-transferase; h, hour(s); H_2O_2, hydrogen peroxide; HK-2, human kidney proximal tubular epithelial cells; HMVEC, human microvascular endothelial cells; HO-1, haem oxygenase 1; HPLC, high-performance liquid chromatography; HUVEC, human umbilical vein endothelial cells; LC_{50}, median lethal concentration; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOD, limit of detection; MDA, malondialdehyde; min, minute(s); NAC, *N*-acetylcysteine; 2,3-NDA, 2,3-naphthalenedicarboxaldehyde; NOx, nitrogen oxides; NRf2, NF-E2-related factor 2; 8-OHdG, 8-hydroxydeoxyguanosine; 8-oxodG, 8-oxo-2′-deoxyguanosine; PANC-1, human pancreatic cells; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; ROS, reactive oxygen species; mtROS, mitochondrial reactive oxygen species; SD, standard deviation; SOD, superoxide dismutase; TAC, total antioxidant content; TBA, thiobarbituric acid; TBARS, thiobarbituric acid-reactive substances; yr, year(s).

[a] ↑, increase; ↓, decrease.

GPx levels were reduced by 100 µM. No change was observed for SOD (Pan et al., 2018). [The Working Group noted that the authors concluded that PFOA induces oxidative stress in human erythrocytes.]

Steves et al. (2018) used a human stem cell-based model of spermatogenesis to assess the effects of PFOA and PFOS individually or in mixtures. Cells were treated with PFOS (24, 48, or 126 µM) or PFOA (11, 25, or 100 µM), starting on day 1, for the entire 10-day cell differentiation process. ROS production was measured using the Muse Oxidative Stress Kit, followed by flow cytometry. No significant changes in ROS production in the cells exposed to PFOA at 100 µM were observed, whereas PFOS at 126 µM significantly reduced ROS compared with the control group (0.25% dimethyl sulfoxide only). Both PFOA and PFOS significantly reduced ROS production at the two lowest concentrations. The authors concluded that ROS production induced by PFOA or PFOS is unlikely to influence the viability of spermatogenic cells in vitro.

In another study, Yuan et al. (2020) treated human sperm with PFOA at 0, 0.25, 2.5, or 25 µg/mL (0, 0.6, 6, or 60 µM) for 30 minutes or 4 hours. ROS was measured using a ROS assay kit. The highest exposure concentration (60 µM) after 4 hours induced a significant increase in ROS generation. Sperm have high levels of polyunsaturated fatty acids and low antioxidant enzyme levels. Hence, they are especially vulnerable to oxidative stress, and high levels of ROS may disrupt sperm function.

Human lymphocytes for a PFOS exposure study were isolated from blood samples of 20 healthy adults aged 18–30 years (sex not reported) (Zarei et al., 2018). The cells were treated with PFOS at 75, 150, or 300 µM for 2, 4, 6, 8, 10, or 12 hours. ROS production was measured using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), MDA using TBARS, and GSH and oxidized glutathione (GSSG) using the *o*-phthalaldehyde fluorescence method. PFOS significantly ($P < 0.05$) induced ROS production in a time– and dose–responsive manner: the levels of ROS (measured by DCFH-DA) increased at concentrations of 150 and 300 µM PFOS at 2 hours and at all concentrations and later time points (4–12 hours), in comparison with the control. MDA levels were also increased after 6 hours of treatment with 150 or 300 µM PFOS. A reduction in GSH and increase in GSSG were observed at 4, 8, 10, and 12, but not at 6 hours, at all concentrations. Butylated hydroxytoluene (50 µM), an antioxidant, was found to inhibit PFOS-induced oxidative stress.

In another study (Qian et al., 2010), human microvascular endothelial cells (HMVEC) were exposed to PFOS at 10, 20, 50, or 100 µM for 1 hour, or 2 µM for 1, 2, 3, or 5 hours. Dihydroethidium-fluorescence-confocal microscopy and dihydroethidium-fluorescence microplate reader methods were used to measure ROS. PFOS increased ROS production at all concentrations (10–100 µM for 1 hour) and time points (2 µM for 1–5 hours). The low concentrations of PFOS applied to HMVEC cells matched occupational and environmental levels.

Wang et al. (2022a) treated human L02 liver cells with 0, 8, 64, or 256 µM PFOA for 24 hours. PFOA significantly increased ROS production at 64 and 256 µM. The authors concluded that PFOA may induce endoplasmic reticulum stress (ERS) and oxidative stress. [The Working Group noted that the L02 cells used in the study might be contaminated with HeLa cells (SIB, 2024). The HeLa cell line is derived from cervical cancer cells. Therefore, contamination of L02 cells with HeLa cells would complicate the interpretation of the results of this study.]

In another study (Wang et al., 2022b), human proximal tubular epithelial cells (HK-2) were exposed to PFOS at 200 µM for 12 hours. PFOS significantly increased the level of ROS production and reduced expression of the antioxidants GPx4 and HO-1. In this study, the authors proposed that PFOS may cause ferroptosis because of an

intracellular increase in iron, which would lead to an increase in ROS production and a reduction in GSH concentration.

Tien et al. (2020) identified consistently high concentrations of PFOA in the particulate matter ($PM_{2.5-1,}$) fraction of indoor dust to which people can be exposed and assessed the effects of PFOA on human corneal epithelial (HCEpiC), endothelial (HCEC), and retinal pigment epithelial (RPE) cells. HCEpiC cells were treated with PFOA at concentrations of 100, 200, or 400 ppm for 6 hours. PFOA at 200 and 400 ppm increased ROS production in HCEpiC cells, and a significant increase was observed for 400 ppm exposure. The authors concluded that PFOA in dust might induce ROS production in the retina and that this may have a risk implication for age-related macular degeneration (Tien et al., 2020). [The Working Group noted that the response could have been because of the dust particles as well as the PFOA in the dust.]

Elumalai et al. (2023) reported that PFOS-induced oxidative stress contributed to apoptosis in human pancreatic 1.1b4 β cells. Human 1.1b4 cells were treated with 100 μM PFOS for 48 or 36 hours. PFOS was found to increase mitochondrial ROS generation with significantly decreased GSH/GSSG ratios through NOX2-gp91Phox activation and the inhibition of cyclic adenosine monophosphate-protein kinase A (cAMP-PKA).

However, in another study, Dos Santos et al. (2022) treated the human insulin-producing EndoC-β-cell line (a human pancreatic β-cell model) with relatively low concentrations of PFOA (1 nM or 1 μM). No significant changes in ROS production, as measured as 2′,7′-dichlorofluorescein diacetate (DCF-DA) fluorescence, were observed.

Du et al. (2022) treated human placental trophoblast cells immortalized with SV40T antigen (HTR-8/SVneo cells) with PFOA at 0, 100, 200, 400, 600, 800, or 1000 μM for 24 hours. PFOA was found to increase ROS production in a dose-dependent manner. The study also showed that PFOA induced ERS, which triggered both the proliferation and apoptosis of trophoblasts via ROS generation or activation of the unfolded protein response (UPR) signalling pathway.

Sonkar et al. (2019) also studied PFOS-induced ROS production in HTR-8/SVneo cells. The cells were exposed to PFOS (1, 10, or 50 μM) for 24 or 48 hours. ROS production in the cells was significantly induced at concentrations ≥ 10 μM at both 24 and 48 hours.

Liao et al. (2012) exposed human umbilical vein endothelial cells (HUVECs) to PFOS (100 mg/L; 200 μM), and ROS production was measured after 1, 5, 12, 24, and 40 hours using the DCFH-DA fluorescence assay. A significant increase in ROS production was observed in a time-dependent manner.

In a follow-up study, Liao et al. (2013) reported that PFOS (at 100 mg/L, i.e. 200 μM) significantly increased ROS production, as measured by DCFH-DA, in HUVECs. The study showed that PFOS treatment alone significantly increased ROS production. Co-treatment with PFOS and either *Flos lonicerae* extract containing chlorogenic acid (CGA) or CGA for 40 hours reduced ROS production to levels comparable to those in cells exposed to PFOS only for 24 hours. However, the increased ROS levels of cells exposed to PFOS for just 24 hours were not affected by co-treatment with either *Flos lonicerae* or CGA.

Gao et al. (2020) studied the effects of PFOS on human mesenchymal stem cells (hMSCs) by treating the cells with 0.1 or 10 μM PFOS for 12, 24, 36, or 48 hours. Treatment with 10 μM PFOS was found to significantly increase ROS production at all time points. The lower concentration of PFOS only increased ROS production significantly in cells treated for 7 days. The results suggest an accumulation effect of low-dose PFOS in hMSC cells.

[The Working Group noted that primary hepatic cells were the most-used human cells for the study of PFOA and PFOS induction of oxidative stress in vitro, with studies also using

primary cells derived from other organ systems. PFOA and PFOS exposure concentrations typically ranged from low doses (submicromolar) to medium doses (up to 100–200 μM) and high doses (above 500 μM). Except for a few studies reporting inverse associations between the exposure and response, both PFOA and PFOS were shown to have varying potencies to induce ROS production in primary liver cells and cells derived from other organ systems.]

Human cell lines

Among immortalized cell lines, HepG2 cells are the most widely used. This cell line was used for exposure experiments involving exposure to a wide range of concentrations of PFOA or PFOS for different periods of time.

Two studies examining the effect of PFOA and/or PFOS on the induction of oxidative DNA damage found no effects. The first study used the Fpg-modified comet assay, which included a Ro19-8022 + light as a positive control for the generation of 8-oxodG, to examine the effect of PFOA and PFOS treatment on HepG2 DNA. PFOA and PFOS treatment did not generate oxidative damage to DNA (Eriksen et al., 2010). The authors observed an increase in ROS production. Specifically, HepG2 cells were treated with PFOA or PFOS at concentrations ranging from 0.4 μM to 2 mM (0.4, 4, 40, 200, 400, 1000, or 2000 μM), and ROS production was measured every 15 minutes up to 3 hours. PFOA and PFOS induced a moderate, non-significant increase in ROS production in the cells; however, the response was not concentration-dependent (Eriksen et al., 2010). The second study (Han et al., 2020) exposed a human skin equivalent to PFOA for 6 days. A slight but non-significant increase in 8-oxodG, measured by ELISA, was observed at 0.25 mM.

In three other studies, PFOA exposure was shown to increase the levels of 8-oxodG in human hepatoma cells (HepG2), in lymphoblastoid cells (TK6), and in epidermal keratinocytes (HaCaT) [although the Working Group considered the reliability of the measurements to be questionable] (Yao and Zhong, 2005; Yahia et al., 2016; Peropadre et al., 2018). The first study reported relatively high background levels of 8-oxodG, measured by HPLC-MS/MS (i.e. 8.3 lesions/10^6 dG) (Yahia et al., 2016), which is almost one order of magnitude higher than the generally accepted background level of 8-oxodG in mammalian cells (1 lesion/10^6 dG) and exceeds the level at which methodological artefacts become a serious concern (5 lesions/10^6 dG) (ESCODD, 2002). [The Working Group considered that the results from three studies with antibody-based detection of 8-oxodG in cells were inconclusive because of a lack of positive controls to verify the specificity of the assay (Yao and Zhong, 2005; Peropadre et al., 2018). The detection of 8-oxodG by an antibody-based system was considered questionable, because of non-specific binding to other biomolecules in cells or biological matrices (Chao et al., 2021).]

Studies demonstrating the induction of ROS production are summarized below.

PFOA (200 or 400 μM) was found to significantly increase ROS production in HepG2 cells after a 3-hour exposure (Panaretakis et al., 2001). ROS were measured using flow cytometry as hydrogen peroxide (H_2O_2) and the superoxide anion O_2^- after reaction with DCFH-DA and dihydroethidine, respectively.

HepG2 cells were exposed to PFOA at concentrations of 10, 25, or 50 μM for 24 hours (Abudayyak et al., 2021b). In this study, ROS production was increased by all concentrations (≤ 5.3-fold at 50 μM), GSH was increased (1.7-fold at 10 μM), and CAT was increased (1.4-fold at 10 μM), but changes in SOD activity were not observed at any concentration. Fluorescein isothiocyanate-labelled DCFH-DA was used to measure ROS, whereas ELISA kits were used for the measurement of other end-points.

Hu and Hu (2009) measured the following end-points of oxidative stress – ROS, GSH, GPx, SOD, and CAT – in HepG2 cells exposed to PFOA or PFOS (50, 100, 150, or 200 μM) for 5, 10, or 15 hours. PFOS significantly increased ROS production in a time- and dose-dependent manner (at 100, 150, and 200 μM). PFOA induced ROS production in a similar manner, although no data were shown. At 48 hours, significant changes in antioxidant activity were observed: both PFOA and PFOS significantly reduced GSH and GPx at 100, 150, and 200 μM and increased SOD and CAT activities at the two highest concentrations, 150 and 200 μM. PFOA and PFOS induced an increase in GST only at 200 μM.

Wielsøe et al. (2015) studied the effects of exposure to seven different PFAS, including PFOA and PFOS, on oxidative stress end-points, such as ROS production and the total antioxidant capacity (TAC) in HepG2 cells. The cells were exposed to concentrations of 0.02, 0.2, 2, 20, or 200 μM for 24 hours. ROS production was significantly induced by 0.2, 2, and 20 μM of PFOA and PFOS. TAC was reduced at all the tested concentrations of PFOA and PFOS; however, the reduction only reached significance with PFOA exposure.

Amstutz et al. (2022) exposed HepG2 cells to 5, 25, 50, 100, 200, 400, or 800 μM of PFOA, PFOS, or several other PFAS for 3 or 24 hours. PFOA and PFOS induced concentration-dependent increases in ROS generation. Yarahalli Jayaram et al. (2020) exposed HepG2 cells to PFOA at 100 or 250 μM for 24 hours. PFOA exposure at 250 μM significantly increased ROS production and NO levels, measured using dichlorofluorescein (DCF) and Greiss reagent, respectively. The mRNA expression of *NOS2A* also increased upon exposure to PFOA, providing further evidence for oxidative effects of PFOA.

Qi et al. (2023) studied the pro-steatotic and fibrotic effects of PFOA in HepG2 and HepaRG cells. Cells were exposed to PFOA at 10, 100, or 1000 nM for 72 hours, which led to increased ROS production. Additionally, PFOA was found to increase steatosis and fibrosis in both cell lines, as indicated by the upregulation of specific genes involved in UPR signalling and non-alcoholic fatty liver disease (NAFLD).

HepG2 cells and HaCaT cells were treated with 10 μM PFOA for 24 hours. Afterwards, ROS levels were measured using two different fluorescent probes, MAK145 (red fluorescence) and DCFH-DA, with the DCFH-DA probe being more sensitive than the MAK145 probe (Magnifico et al., 2022). In addition, levels of nitrogen oxides (NO_x) were measured using the fluorescent probe 2,3-diaminonaphthalene from a nitrate/nitrite fluorometric assay kit. Fluorescence intensity, proportional to the total NO production, was measured using a fluorometer. The results showed that PFOA significantly increased ROS production and nitrosative stress in both cell lines.

One study by Ojo et al. (2022b) investigated the effects of several PFAS, including PFOA and PFOS, on the Nrf2–antioxidant responsive element (ARE) pathway, individually or in combination, using ARE reporter–HepG2 cells. The induction of Nrf2–ARE is indicative of an event of cellular oxidative stress. The cells were exposed to PFOA or PFOS at 1, 2, 3, 4, or 5 μM for 24 hours. The results showed a significant induction of oxidative stress. The 1.5-fold induction ratios ($EC_{IR} = 1.5$) for PFOA and PFOS were determined to be 1.38 μM and 1.17 μM, respectively.

Wan et al. (2016) treated HepG2 cells with PFOS at concentrations of 0, 10, 20, 30, 40, or 50 μM for 24 hours or 50 μM for 1, 3, 6, 12, or 24 hours. ROS levels increased significantly at PFOS concentrations ≥ 30 μM. When treated with 50 μM, ROS levels gradually increased over time, peaking at 12 hours, and then decreased to 24 hours. GSH levels significantly decreased at PFOS concentrations ≥ 20 μM and at all time points after 6 hours during the time-course experiment.

Yan et al. (2015a) exposed HepG2 cells to PFOA at concentrations of 0, 50, 100, or 200 µM for 24 or 72 hours. ROS production was significantly increased in cells treated with PFOA concentrations ≥ 100 µM at 24 hours but was decreased at 72 hours. ROS production was decreased in cells pre-treated with *N*-acetylcysteine (NAC).

Several studies have reported no effects of PFOA or PFOS exposure on the production of ROS in HepG2 cells. For example, Florentin et al. (2011) assessed the induction of ROS production after exposure to PFOA or PFOS in HepG2 cells. The cells were exposed to PFOA at 0, 5, 10, 50, 100, 200, or 400 µM, or 0, 5, 10, 50, 100, 200, or 300 µM PFOS for 1 or 24 hours. No increase in oxidative stress was observed. No effect on ROS production was also reported by Ojo et al. (2021) after they exposed HepG2 cells to 0.2, 2, or 20 µM PFOA or PFOS for 24 hours. It was noted that the doses used in this study were lower and more relevant to environmental exposure levels.

In another study, Shan et al. (2013) exposed HepG2 cells to a single concentration (100 µM) of PFOA or PFOS for 3 hours. No significant differences in ROS production, GSH, GSSG, or MDA were observed between the PFAS test groups and the negative control. [The Working Group noticed that the study focused on the combined effects of PFOA or PFOS with pentachlorophenol. No tabulated or graphical data were provided for PFOA and PFOS alone, only descriptive text. Nevertheless, this was another study available in the literature that used relatively low concentrations of PFOA and PFOS and with no effects on the cell oxidative status.]

The concentrations of PFAS and the treatment conditions of the cells before the addition of the ROS-detection probe were considered of relevance (Ojo et al., 2021). It was noted that the cell trypsinization stage included in the ROS measurement method could have induced additional stress in the cells, as noted by Florentin et al. (2011). Exposure concentrations may also affect ROS production. It was reported that low concentrations may protect against ROS generation, and high concentrations may induce ROS production (Steves et al., 2018).

Yao and Zhong (2005) reported that 8-OHdG content, measured using immunocytochemical staining, was significantly increased in a dose-dependent manner when HepG2 cells were exposed to PFOA (100, 200, or 400 µM) for 3 hours. Hydrogen peroxide was used as a positive control. ROS, measured using DCFH-DA, was also increased in a dose-dependent manner. No positive control was included in the ROS assay. As mentioned above, Eriksen et al. (2010) also did not observe a significant increase in ROS.

Fewer studies have examined the effects of PFOA and PFOS on the induction of oxidative stress in human cells other than HepG2. Abudayyak et al. (2021a) exposed the human pancreatic epithelioid carcinoma cell line PANC-1 to PFOA (0, 10, 50, or 100 µM) for 24 hours. ROS levels were not increased by any tested concentration. However, MDA, SOD, and CAT were significantly increased upon PFOA treatment, although not in a dose-dependent manner. It was noted that GSH was increased in cells treated with 10 µM PFOA, but not higher concentrations.

Souders et al. (2021) exposed differentiated human SH-SY5Y neuroblastoma cells to PFOA for a metabolic profiling study. Cells were treated with 1, 10, 100, 150, 200, or 250 µM PFOA, and ROS production was measured at 4, 24, and 48 hours using the ROS-Glo H_2O_2 and DCFH-DA assays. The ROS-Glo assay did not detect changes in ROS production, except a reduction after 4 hours at 250 µM, whereas the DCFH-DA assay detected a significant increase in ROS levels at 4 hours for 100, 200, and 250 µM and at 24 hours for 250 µM, but no increase at 48 hours.

In another study (Mao et al., 2013), human lung carcinoma A459 cells were treated with PFOS (25, 50, 100, or 200 µM) for 24 hours. ROS were measured using DCFH-DA, MDA

using TBARS, and the GSH and SOD levels were measured using assay kits. PFOS significantly increased ROS, MDA, and SOD, and reduced GSH at all concentrations ≥ 50 μM. ROS was measured qualitatively using fluorescence photography, and an increase in fluorescence levels was observed with increasing PFOS concentrations. PFOS-induced ROS generation was also inhibited by pre-treatment with the thiol antioxidant NAC.

More recently, Zhang et al. (2023a) exposed human ovarian granulosa-like tumour cells (KGN) to PFOA at concentrations ranging from 250 to 750 μM for 24 hours. Mitochondrial ROS (mt-ROS), determined by the superoxide indicator fluorescence ratio of red MitoSOX to MitoTracker Green was significantly increased in a dose-dependent manner across all tested concentrations.

Du et al. (2023) observed PFOS-induced ROS production (DCFH-DA method) in human embryonic kidney cells (HEK293) exposed for 24 hours to a medium containing PFOS at 40 μM and/or cannabidiol (CBD) at 20 μM. Co-exposure with CBD reduced the level of PFOS-induced ROS production.

(b) Experimental systems

(i) Non-human mammalian systems in vivo

See Table 4.17.

Some studies have shown mixed results regarding 8-oxodG formation in the kidneys, liver, and testes of mice and rats after oral exposure to PFOA (Takagi et al., 1991; Abdellatif et al., 2003–2004; Zou et al., 2015; Ma et al., 2023). [The Working Group noted that some of the studies did not include positive controls. In addition, there was uncertainty about the reliability of the results, because of high background levels of 8-oxodG in rat tissues (Takagi et al., 1991; Abdellatif et al., 2003–2004) and the measurement of 8-oxodG by a non-specific antibody-based method in the mouse tissues (Zou et al., 2015; Ma et al., 2023).]

Abdellatif et al. (2003–2004) studied the effects of dietary PFOA exposure on peroxisomal enzymes and 8-OHdG in male Wistar rats (minimum 15 rats per group), following a biphasic initiation procedure involving dosing with diethylnitrosamine (DEN) or a triphasic initiation involving dosing with DEN, 2-acetylaminofluorene, and carbon tetrachloride (CCl_4) (Abdellatif et al., 2003–2004). For the rats that underwent biphasic initiation, basal rodent diet or diet containing 0.005% or 0.02% PFOA (daily dose in mg/kg per day was not estimated) was ingested for 14 or 25 weeks. Those that underwent triphasic initiation ingested a diet without PFOA or a diet containing 0.015% PFOA for 25 weeks. CAT, measured spectroscopically, was significantly increased in the rat livers after 14 or 25 weeks compared with the basal diet group. To study the effect of PFOA on 8-OHdG induction, male Wistar rats underwent the initiation treatment and were fed either a basal diet or a basal diet containing 0.02% PFOA for 5 or 9 weeks. No significant increase in 8-OHdG was observed in the liver after 5 or 9 weeks compared with the basal diet group. [The Working Group noted that the study authors suggested that PFOA was a liver cancer promoter that may not require significant DNA damage. It was also noted that the study did not include a positive control group and that the background 8-OHdG was relatively high in the livers from the basal diet group.]

Ma et al. (2023) investigated the potential of rutin to ameliorate the oxidative effects of PFOA in mice (10 mice per group). Male ICR mice were orally dosed with PFOA (20 mg/kg bw) daily for 28 days in the presence or absence of rutin. The levels of 8-OHdG, GPx, SOD, and MDA were measured. 8-OHdG was significantly increased in the serum and testes; and MDA, GPx, and SOD were significantly increased in the testes of

Table 4.17 End-points relevant to oxidative stress in in non-human mammalian systems in vivo exposed to PFOA or PFOS

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
8-OHdG, CAT, by HPLC-ECD, spectroscopic method	Rat, Wistar, male	Liver	No change in 8-OHdG, significant increase in CAT	PFOA 0.02% in feed for 8-OHdG, 0.005% or 0.02% with DEN biphasic initiation, or 0.015% PFOA after DEN, 2-acetylaminofluorene and CCl_4 triphasic initiation	Oral, 5 or 9 wk for 8-OHdG, 14 or 25 wk for CAT	No positive control; tissues from unexposed rats have high background levels of 8-oxodG (i.e. 0.126 ng/μg DNA, corresponding to 730 lesions/10^6 dG)[a]	Abdellatif et al. (2003–2004)
8-OHdG, MDA, GPx, SOD 8-OHdG by ELISA kit; MDA, SOD, and GPx by microplate reader	Mouse, ICR, male	Serum and testicular tissue	Serum and testis: ↑ 8-OHdG Testis: ↑ MDA, ↑ GPx, ↑ SOD	PFOA (20 mg/kg per day)	Daily oral gavage for 28 days	Rutin ameliorates PFOA-induced oxidative stress; no positive control group; results on 8-oxodG reported as pg/mg protein	Ma et al. (2023)
8-OHdG, by HPLC-ECD	Rat, Fischer 344, male	Kidney, liver	↑ 8-OHdG (liver) ≥ 3 days No changes (kidney)	PFOA, 100 mg/kg	Peritoneum single injection, dosing at 1, 3, 5, or 8 days post-injection	No positive control group; tissues from unexposed rats had high background levels of 8-oxodG (> 30 lesions/10^6 dG)	Takagi et al. (1991)
8-OHdG, by HPLC-ECD	Rat, Fischer-344, male	Kidney, liver	↑ 8-OHdG (liver) No changes (kidney)	PFOA, 0.02% in feed	Oral, 2 wk, feed	No positive control group; tissues from unexposed rats had high background levels of 8-oxodG (i.e. 17 lesions/10^6 dG)	Takagi et al. (1991)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
8-OHdG, MDA, H_2O_2, SOD, CAT by ELISA for 8-OHdG, commercial kits for other biomarkers	Mouse, Kunming, male	Liver	↑ 8-OHdG, ↑ H_2O_2, ↑ MDA, ↓ SOD, ↓ CAT	PFOA, 10 mg/kg per day	Oral, daily for 14 days	No positive control group; results for 8-oxodG were reported as pg/mg protein; commercial kits may not be specific for the particular end-points Quercetin, a flavonoid, given at 75 mg/kg per day for 14 days limited the oxidative effects of PFOA	Zou et al. (2015)
8-OHdG, by HPLC (UV and electrochemical detectors)	Mouse, wildtype mice (129S4/SvlmJ) and Pparα-null mice (129S4/SvJae-Ppartm1Gonz/J), male	Liver	↑ 8-OHdG (only in Pparα-null mice)	PFOA, 12.5, 25, or 50 µmol/kg per day	Oral, daily for 4 wk	No change in 8-OHdG in the wildtype mice; the ablation of Pparα exacerbated PFOA-induced oxidative stress	Minata et al. (2010)
8-OHdG, MDA, 8-OHdG by ELISA, MDA by HPLC	Rat, Sprague-Dawley, male	Urine	↑ 8-OHdG, ↑ MDA	PFOA, 10, 33, or 100 mg/kg per day	Oral gavage on days 4, 5, and 6	Urine 8-OHdG and MDA tested from day 1 to day 10; both biomarkers increased from day 3 or 4, peaked on day 5–7, then returned to baseline by day 8 or 10	Rigden et al. (2015)
ROS, MDA, GSSG, CAT, SOD, and GSH, by DCFH-DA for ROS, assay kits for other biomarkers	Rat, Sprague-Dawley, male	Liver	↑ 8ROS, ↑ MDA, ↑ GSSG, ↓ SOD, ↓ CAT, ↓ GSH, ↓ GSH/GSSH	PFOS, 1 or 10 mg/kg per day	Oral, daily for 28 days	Significant dose-dependent changes in biomarkers; the observed induction of oxidative stress was also supported by increases in serum NO and liver *Nos2* mRNA expression	Han et al. (2018a)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
MDA, GSH, SOD, and CAT, by TBARS assay, GSH ELISA, SOD kit, CAT kit	Mouse, Balb/c, male	Liver	↓ MDA, ↑ GSH, ↓ SOD, ↑ CAT	PFOA, 0, 0.08, 0.31, 1.25, 5, or 20 mg/kg per day	Gavage, daily for 28 days	Multiple groups and daily dosing; PFOA disturbed the antioxidant defence system in the liver, but did not significantly induce oxidative stress	Yan et al. (2015a)
8-iso-pg-PGF2α; *Sod1*, *Sod2*, *Gpx2*, *Cat*, *Nqo1* expression, by LC/MS for 8-iso-pg-PGF2α, qRT-PCR for the other biomarkers	Mouse, C57BL/6, male	Liver, pancreas	Pancreas: ↑ all biomarkers, except for *Cat* Liver: ↑ 8-iso-pg-PGF2α, ↑ *Sod1*, ↑ *Sod2*, ↑ *Cat*, and ↑ *Nqo1* dosed with 2.5 or 5 mg/kg; no change in *Gpx2* expression	PFOA, 0, 0.5, 2.5, or 5 mg/kg per day	Gavage, daily for 7 days	LC-MS is a robust method for 8-iso-pg-PGF2α measurement; qRT-PCR is considered to be reliable	Kamendulis et al. (2014)
MDA, SOD, CAT, TrxR, by commercial kits	Mouse (conditional $Kras^{G12D}$ mouse model [*LSL-*$Kras^{G12D}$ and Pdx-1 Cre mice])	Pancreas	↑ MDA, ↑ SOD, ↑ CAT, ↑ TrxR	PFOA, 5 ppm in drinking-water	Drinking-water, 4 mo or 7 mo exposure; mice were of age 6 or 9 mo at the end of the dosing period	MDA protein level increased only at age 6 mo; SOD enzyme activity increased at both 6 and 9 mo, whereas CAT and TrxR enzyme activities were increased only at age 9 mo	Kamendulis et al. (2022)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
GSH, CAT, GPx, GR, GST, by spectrometry	Rat, Wistar, male and female	Liver	↑ CAT, ↑ GPx, No change in GSH, ↑ GR, ↓ GST (male), GST – no effect (female), ↑ peroxisomal β-oxidation, ↑ microsomal NADP-dependent lipid peroxidation (male, age 26 weeks)	PFOA, 0.01% of diet	Oral, 2 or 26 wk	Increases in peroxisomal β-oxidation in male and female rats after 2 or 26 wk of PFOA exposure, plus an increase in microsomal NADP-dependent lipid peroxidation, and changes in other biomarkers supported the induction of oxidative stress in male and female rats by PFOA	Kawashima et al. (1994)
Oxidized lipid products (15-F_{2t}-IsoP, 5-F_{2t}-IsoP, 8-F_{3t}-IsoP, 4(RS)-4-F_{4t}-NeuroP, 17(*RS*)-F_{2t}-dihomo-Iso-P, Iso-F, NeuroF, 17(*RS*)-SC-Δ^{15}-11-dihomo-IsoF, 7β-hydroxycholesterol, 7-ketocholesterol, 27-hydroxycholesterol, 9(*S*)-HETE, 11(*S*)-HETE, 9(*S*)-HETE, 20-HETE, 5(S)-HETE, 8(S)-HETE, 12(*S*)-HETE, 15(*S*)-HETE), SOD, CAT, by LC-MS/MS, and assay kits	Mouse, CD-1, pregnant and adult females and fetuses	Liver, brain, kidney	No changes in SOD or CAT, significant increases in various oxidized lipid products in fetal liver, brain, and kidney	PFOS, 3 mg/kg per day	Oral, pregnant mice from GD1 to GD17, adult female mice daily for 14 days	Comprehensive evaluation of oxidized lipid products derived from the lipid peroxidation of polyunsaturated fatty acid; both non-enzymatic and enzymatic oxidation (CYP and LOX) products were measured. Changes in oxidative stress biomarkers were more significant in the fetuses	Lee et al. (2015)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
MDA, H_2O_2, SOD, CAT, by TBARS, assay kits	Mouse, Kunming, male	Testis	↑ MDA, ↓ SOD, ↓ CAT	PFOA, 0, 2.5, 5, or 10 mg/kg per day	Oral, daily for 14 days	Oxidative stress was further supported by a reduction in Nrf2 expression	Liu et al. (2015a)
MDA, H2O2, SOD, CAT, by assay kits	Mouse, Kunming, male	Liver	↑ MDA, ↑ H_2O_2, ↓ SOD, ↓ CAT	PFOA, 10 mg/kg per day	Oral, daily for 14 days	Oxidative stress was further supported by a reduction in Nrf2 expression	Liu et al. (2015b)
MDA, RONs, NO, GPx, GST, SOD, CAT, GSH, By TBARS, DCFH-DA, Griess reaction, 1-chloro-2,4-dinitrobenzene, autoxidation of epinephrine, H_2O_2, *o*-phthaldialdehyde	Rat, Wistar, male	Liver, kidney	↑ RONs, ↑ NO, ↓ GPx, ↓ GST, ↓ SOD, ↓ CAT, ↓ GSH, ↑ MDA	PFOA, 5 mg/kg per day	Oral, daily for 28 days	Evidence of oxidative stress is further supported by increases in xanthine oxidase and myeloperoxidase. NAC alleviated the level of oxidative stress	Owumi et al. (2021a)
MDA, RONs, NO, GPx, GST, SOD, CAT, GSH, by TBARS, DCFH-DA, Griess reaction, 1-chloro-2,4-dinitrobenzene, autoxidation of epinephrine, H_2O_2, *o*-phthaldialdehyde	Rat, Wistar, male	Testis, epididymis	↑ MDA, ↑ RONs (testes only), ↑ NO, ↓ GPx (epididymis only), ↓ GST (testes only), ↓ SOD, ↓ CAT, ↓ GSH	PFOA, 5 mg/kg per day	Oral, daily for 28 days	Evidence of oxidative stress is further supported by increases in xanthine oxidase and myeloperoxidase; NAC alleviated the level of oxidative stress	Owumi et al. (2021b)
MDA, H_2O_2, by assay kits	Mouse, Kunming, male	Liver	↑ MDA, ↑ H_2O_2	PFOA, 0, 2.5, 5, or 10 mg/kg per day	Oral, daily for 14 days	Significant dose-dependent increases in biomarkers	Yang et al. (2014)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
MDA, GSH, GSSG, by assay kits	Mouse, C57BL/6, male	Liver	↑ MDA, ↓ GSH/GSSG	PFOS, 0, 0.003%, 0.006%, or 0.012% of diet	Oral, daily for 14 days 0.003% for 6 wk	Increase in MDA and decrease in GSH/GSSG ratio mice consuming either a normal diet or a marginal methionine/choline-deficient diet containing PFOS; oxidative stress biomarker data were only available for the 0.003% PFOS groups; choline reduced oxidative stress	Zhang et al. (2016a)
ROS, by CM-DCF-DA	Mouse, ICR, female	Oocyte	↑ ROS	PFOA, 0, 1, or 5 mg/kg per day	Oral, daily for 28 days	ROS significantly increased in a dose-dependent manner; increase in γH_2AX, a DNA damage marker provided supportive evidence for oxidative stress	Zhang et al. (2022a)
MDA, SOD, GPx, ROS, by TBARS assay for MDA, nitroblue tetrazolium-illumination method for SOD, benzoic acid method for GPx, DCFH-DA for ROS	Mouse, Balb/c, male	Epididymis, sperm cells	↑ MDA, No change in SOD, ↓ GPx, ↓ SOD/MDA	PFOA, 0, 1.25, 5, or 20 mg/kg per day	Gavage, daily for 28 days Sperm cells were exposed to PFOA at 0, 100 or 400 μM for 1, 2, 4, or 8 h	No available method for ROS measurement in epididymis; therefore, sperm cells were isolated from mice for in-vitro PFOA exposure	Lu et al. (2016b)
MDA, SOD, GPx, by TBARS for MDA, xanthine oxidase assay for SOD, DTNB assay for GPx	Mouse, Kunming, pregnant female	Liver	↑ MDA, ↓ SOD, ↓ GPx	PFOA, 0, 1, 5, 10, 20, or 40 mg/kg per day	Gavage, daily from GD1 to GD7, killed on GD9	Dose-dependent effects observed for all biomarkers tested in the liver; uterine cell apoptosis was observed	Zhang et al. (2021b)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
MDA, H_2O_2, SOD, CAT, by commercial kits	Mouse, Kunming, pregnant female	Ovary	↑ MDA, ↑ H_2O_2, ↓ SOD, ↓ CAT	PFOA, 0, 2.5, 5, or 10 mg/kg per day	Gavage, daily from GD1 to GD7 or GD13	Significant increases in MDA and H_2O_2, and decreases in SOD and CAT on GD13 (dose-dependent); significant increase in MDA on GD7 (not dose-dependent); mixed and non-significant results for H_2O_2, SOD, and CAT on GD7	Chen et al. (2017)
mtROS, by immunofluorescent microscopy (MitoSOX Red/MitoTracker Green)	Mice, unspecified strain, female	Granulosa cells/ oocytes	↑ mtROS	PFOA, 4 mg/kg per day for 30 days	Oral, in drinking-water		Zhang et al. (2023a)
MDA, SOD, GPx, by ELISA kits	Rat, Sprague-Dawley, male	Testis	↑ MDA, ↓ SOD, ↓ GPx	PFOA, 0.01 g/kg per day	Oral gavage daily for 30 days	Both low and high doses of lipoic acid protected rats against reproductive damage by reducing oxidative stress biomarkers induced by PFOA	Zhang et al. (2023c)
MDA, SOD, GPx, by commercial kits	Rats, strain not specified (albino), male	Jejunum	↑ MDA, ↓ SOD, ↓ GPx,	Potassium salt of PFOS, 5 mg/kg per day	Oral gavage daily for 28 days	Co-exposure with lemongrass essential oil (100 mg/kg per day) restored the levels of the biomarkers in rats dosed with 5 mg/kg per day	Shalaby et al. (2023)
MDA, SOD, GPx, NOX4, by commercial kits and immunofluorescence staining	Mouse, C57BL/6, male	Serum (MDA, SOD, GPx); kidney (NOX4)	↑ MDA, ↓ SOD ↓ GPx	PFOS, 5 mg/kg per day	Oral gavage, 28 days	Cannabidiol partially restored the levels of markers	Du et al. (2023)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
MDA, GPx, ROS, by assay kits (A003-1, A005-1) for MDA and GPx, DCFH-DA for ROS (in vitro)	Rat, Sprague-Dawley, male	Kidney	↑ MDA, ↓ GPx	PFOS, 1 or 10 mg/kg	Intraperitoneal injections every other day for 15 days	↑ MDA and ROS (significant at either dose), ↓ G-Px (significant only at 10 mg/kg); approx. 12% apoptosis at 10 mg/kg dose	Tang et al. (2022)
HO-1, SOD, Nrf2, by gene expression (mRNA) assays	Mouse, C57BL/6, male	White adipose cells	↑ Nrf2 expression, HO-1 and SOD: non-significant increases	PFOS, 100 µg/kg per day	Oral gavage for 36 days	Gene expression of oxidative stress biomarkers was measured	Xu et al. (2016)
MDA, H_2O_2, SOD, GPx, by TBARS, hydrogen peroxide kit, ELISA kits	Mouse, DBA/1J, male	Paw tissue	↑ MDA, ↓ GPx, ↓ SOD, H_2O_2 non-significant increase	PFOS, 10 mg/kg	Rheumatoid arthritis was induced on days 1 and 21; daily oral gavage of PFOS on days 21–35	Paw tissue was extracted for analyses; PFOS induced oxidative stress in normal mice; the effect was enhanced in mice with rheumatoid arthritis	D'Amico et al. (2022)
MDA, CAT, GSH, by commercial kits	Mouse, C57BL/6, male	Ileum and colon	↑ MDA (colon and ileum), no changes in CAT, ↑ GSH versus PFBS	PFOS, 500 µg/L in drinking-water	PFOS in drinking-water for 28 days	TNF-α and IL-1β expression was also significantly increased in ileum but not in colon	Chen et al. (2023b)
MDA, H_2O_2, SOD, by assay kits	Mouse, Kunming, male	Liver	↑ MDA, ↑ H_2O_2, ↓ SOD	PFOS, 10 mg/kg per day	Daily oral gavage for 21 days	Grape seed proanthocyanidin extract was shown to be protective against PFOS-induced oxidative stress	Huang et al. (2020)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
MDA, SOD, CAT, GPx, by assay kits	Mouse, C57BL/6J male	Liver	↑ MDA, ↓ SOD, ↓ CAT, ↓ GPx	PFOA, 5 mg/kg per day	Daily oral gavage for 6 wk, followed by 0.5 mL saline, with or without gastrodin, daily for 8 wk	Gastrodin, isolated from the root of *Gastrodia elata* Blume, partially protected against the oxidative stress caused by PFOS	Ma et al. (2021)
MDA, GSH, SOD, MPO, by assay kits	Rat, Sprague-Dawley, pregnant female	Lung	↑ MDA, ↑ MPO ↓ SOD, ↓ GSH	PFOS, 0, 0.1, or 2 mg/kg per day	Daily from GD0 to GD21	2 male and 2 female pups were randomly selected for the oxidative biomarker assays; significant oxidative stress was observed in the pups' lungs	Chen et al. (2012)
Peroxidation (MDA), by TBARS	Mouse, C57BL/6, male	Liver	No change in MDA	PFOA, 0, 0.1, 1, or 5 mg/kg	Oral, via drinking-water containing 0.55, 5.5, or 28 mg/L PFOA	No significant increase in liver MDA in PFOA-treated mice; liver MDA significantly increased in CCl_4-treated mice (positive control)	Crebelli et al. (2019)
mtROS, by DCFH-DA	Mouse, NMRI, pregnant female	Liver, brain, heart of fetus and placenta	↑ ROS in liver, brain, and heart of fetus but not placenta	PFOA, 0, 1, 10, or 20 mg/kg	Intraperitoneal injections, GD5–GD9	Significant increase in mtROS production with PFOA at 10 and 20 mg/kg (brain), and 20 mg/kg (liver, heart)	Salimi et al. (2019)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
MDA, SOD, T-AOC, by TBARS, xanthine oxidase, Fe^{3+} reduction methods	Mouse, Kunming, male and female pups	Liver, brain	No change in MDA, ↓ SOD, ↓ T-AOC	PFOA, 50 mg/kg	0 or 50 mg/kg by subcutaneous injection on PND7, 14, 21, 28, and 35	No changes in MDA levels in liver or brain at any time point; ↓ SOD in male pup brain on PND7 and PND21 and in female pup liver on PND14; ↓ T-AOC in male pup brain on PND21, in female pup liver on PND21, and in male pup liver on PND7 and PND14; ↑ T-AOC in male pup liver on PND21	Liu et al. (2009)
GSH, GR, by biochemical assay kits	Mouse, Balb/c, male	Liver	↑ GSH, ↑ GR	PFOS, effect at 5 mg/kg per day only	0.2, 1, or 5 mg/kg per day; oral gavage, 28 days		Wang et al. (2022c)
MDA, GSH, GPx, CAT, Cu-Zn-SOD, by TBARS for MDA, DTNB for GSH, assay kits for other biomarkers	Mouse, Balb/c, male	Liver, brain	Liver (no change in MDA, ↑ GSH, ↑ CAT, ↓ Cu-Zn-SOD, ↓ GPx). Brain (↑ MDA only for 30 mg/kg, ↓ GPx, no changes in other biomarkers)	PFOA, 15 or 30 mg/kg per day	Oral, 10 days One group received PFOA 30 mg/kg for another 10 days	Depletion of the antioxidant system suggested potential oxidative stress in the liver and brain	Endirlik et al. (2022)

Table 4.17 (continued)

End-point, assay	Species, strain, sex, cell line	Tissue	Result[a]	Dose	Route, duration, dosing regimen	Comments	Reference
Hepatic peroxisomal β-oxidation and CAT, by spectrophotometry at 340 nm for β-oxidation and 240 nm for CAT activity	Rat, Fischer 344, male	Liver	↑ β-oxidation ↓ CAT	PFOA, 150 mg/kg	150 mg/kg bw PFOA in corn oil given to rats of age 4, 10, 20, 50, and 100 wk	Rats were killed at pre-set time points between days 0 and 28 after exposure for oxidative stress determination; β-oxidation significantly increased in all age groups; CAT significantly reduced only in the rats aged 100 wk, probably due to senescence	Badr and Birnbaum (2004)
β-oxidation, by [1–^{14}C]palmitoyl-CoA as the substrate	Rat, Crl:CD BR, male	Liver	↑ β-oxidation	PFOA (ammonium salt): 0.2, 2, 20, or 40 mg/kg per day	0.2, 2, 20, or 40 mg/kg per day PFOA, daily oral gavage for 14 days	No other oxidative stress biomarkers were measured	Liu et al. (1996)
MDA, H_2O_2, GSH, SOD, by assay kits	Mouse, strain not specified, male	Liver	↑ MDA, ↑ H_2O_2, ↓ SOD, ↓ GSH	PFOS, 10 mg/kg per day	10 mg/kg per day PFOS, oral gavage daily for 3 wk	The study also showed the protective effects of naringin against PFOS-induced oxidative stress	Lv et al. (2018)
mtROS, by fluorescent microscopy	Mouse, strain not specified, female	Cumulus–oocyte complexes	↑ mtROS at 250, 500, and 750 μM	PFOA, 50–2000 μM	PFOA for 24 h	Significant dose-dependent increases in mtROS	Zhang et al. (2023b)

bw, body weight; CAT, catalase; CCl_4, carbon tetrachloride; CoA, coenzyme A; CYP, cytochrome P450; DCF, dichlorofluorescein; DCFH-DA, 2′,7′-dichlorodihydrofluorescein diacetate; DEN, diethylnitrosamine, dG, 2′-deoxyguanosine; ECD, electrochemical detection; ELISA, enzyme-linked immunosorbent assay; ESR, electron spin resonance; GD, gestational day; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GSSG, oxidized glutathione; GST, glutathione *S*-transferase; h, hour(s); γH_2AX, γ-H2A histone family member X; HETE, hydroxyeicosatetraenoic acid; H_2O_2, hydrogen peroxide; HPLC, high-performance liquid chromatography; IsoP, isoprostane; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOX, lipoxygenase; MDA, malondialdehyde; mo, month(s); MPO, myeloperoxidase; mRNA, messenger RNA; mtROS, mitochondrial reactive oxygen species; NAC, *N*-acetylcysteine; NeuroP, neuroprostane; NO, nitrogen oxide; NR, not reported; NRf2, NF-E2-related factor 2; 8-OHdG, 8-hydroxydeoxyguanosine; 8-oxodG, 8-oxo-2′-deoxyguanosine; PFBS, perfluorobutanesulfonate; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PGF, prostaglandin; PND, postnatal day; ppm, parts per million; qRT-PCR, quantitative reverse transcription–polymerase chain reaction ; RONS, reactive oxygen and nitrogen species; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TBARS, thiobarbituric acid-reactive substances; wk, week(s).

[a] +, positive; ↑, increase; ↓, decrease.

the PFOA-treated mice. Rutin was able to reduce the oxidative stress induced by PFOA.

Takagi et al. (1991) measured 8-OHdG in the DNA of liver and kidney from rats dosed with PFOA. In the first experiment, male Fischer 344 rats were exposed to a single i.p. dose of PFOA (100 mg/kg) in corn oil (5 rats per group) and killed 1, 3, 5, or 8 days after dosing. In a second experiment (a feeding trial), rats were fed a powdered diet ad libitum containing 0.02% PFOA for 2 weeks (5 rats per group; the dose as mg/kg per day was not estimated). After necropsy, liver and kidney DNA was isolated. Significant increases in 8-OHdG levels were found in the livers of rats injected with a single dose of PFOA on days 3, 5, and 8. No effect of PFOA on 8-OHdG level was observed in the kidney. In the feeding trial, 8-OHdG was significantly increased in the liver but not in the kidney. [The Working Group noted that relatively high 8-OHdG levels were reported in both liver and kidney DNA of the control group rats. In addition, HPLC with electrochemical detection, as performed in this study, was considered to be a better technique than assay kits for 8-OHdG measurement.]

Zou et al. (2015) orally dosed male mice ($n = 8$) with PFOA at 10 mg/kg per day for 14 days. Oxidative end-points including ROS, 8-OHdG, H_2O_2, MDA, SOD, and CAT were measured in the liver using commercial kits. The levels of 8-OHdG, H_2O_2, and MDA were significantly increased in PFOA-treated mice compared with control mice, whereas SOD and CAT levels were significantly decreased. When the mice were co-treated with PFOA (10 mg/kg per day) and the flavonoid quercetin (75 mg/kg per day) for 14 days, quercetin was found to be able to reverse the PFOA-induced effects on these end-points.

In a study by Minata et al. (2010), 39 wildtype (129S4/SvlmJ) and 40 PPARα-null (129S4/SvJae-Pparαtm1Gonz/J) male mice were randomly divided into four groups and orally dosed with PFOA (0, 12.5, 25, or 50 μmol/kg per day) for 4 weeks. The level of 8-OHdG in the liver was measured by HPLC using ultraviolet and electrochemical detectors. No changes in 8-OHdG levels were observed in wildtype mice, whereas the 8-OHdG levels were increased in a dose-dependent manner in the PPARα-null mice, with a significant increase in the group at 50 μmol/kg.

Rigden et al. (2015) dosed male Sprague-Dawley rats with PFOA and measured urinary 8-OHdG and MDA. Groups of five rats were dosed by gavage with PFOA (0, 10, 33, or 100 mg/kg bw once per day on days 4, 5, and 6). Urine samples were collected from day 1 to day 10. The two highest doses of PFOA induced significant increases in urinary MDA. The urinary 8-OHdG levels were significantly increased at a PFOA dose of 100 mg/kg. The levels of 8-OHdG started rising from day 3 and peaked from day 4 to day 7, depending on the dose, then decreased to baseline on days 8–10. The MDA levels started to increase from day 4, peaked on day 5, then decreased to baseline on day 8. The rises and peaks in 8-OHdG and MDA levels seemed to occur at about the same time after PFOA dosing.

[The Working Group noted that, altogether, the results support oxidative effects of PFOA, although the commercial kits may not be specific for the particular end-points measured, and the studies did not include a positive control group.]

Groups of six Sprague-Dawley male rats were dosed with PFOS at 0, 1, or 10 mg/kg per day for 28 days (Han et al., 2018a). The oxidative stress end-points measured in the liver included ROS production (measured by DCFH-DA), MDA, GSSG, CAT, SOD, and GSH (measured with assay kits). The MDA and GSSG levels were found to be significantly increased in a dose–response manner, whereas GSH, SOD, CAT, and GSH/GSSG were significantly reduced. An increase in serum NO, coupled with an increase in mRNA expression of inducible nitric oxide synthase (iNOS) in the liver, provided additional evidence for oxidative stress induction.

Yan et al. (2015a) dosed male Balb/c mice (age 6–8 weeks) with PFOA by oral gavage (0, 0.08, 0.31, 1.25, 5, or 20 mg/kg per day) daily for 28 days (the second gavage was used for the administration of additional chemicals, but the mice that yielded the results described here only received water in this second gavage). The MDA, GSH, SOD, and CAT levels were measured in the liver after necropsy. The levels of MDA in the liver were found to be significantly decreased at all doses compared with the control. The liver GSH protein levels decreased, but not significantly, at the three lowest doses but significantly increased when the mice were dosed with 5 or 20 mg/kg. The authors also reported increased CAT (≥ 3 mg/kg) and decreased SOD (≥ 5 mg/kg) enzyme activities in the livers of the mice. However, the dosing regimen was not provided in the manuscript. The study also analysed the mRNA expression of oxidative stress-responsive genes, including *Cat*, *Sod1*, *Sod2*, and *Sesn1*, in the livers of mice after exposure to PFOA for 28 days. *Cat* mRNA expression was unchanged after PFOA exposure. *Sesn1* mRNA expression was significantly increased at the highest dose of 20 mg/kg, *Sod1* mRNA was significantly increased at doses of 1.25 and 5 mg/kg, and *Sod2* mRNA was significantly increased at 5 mg/kg. However, the mRNA expression changes for these three genes were considered moderate. This study suggested that the oxidative stress defence system was affected by repeated doses of PFOA, although oxidative stress, as indicated by the MDA concentration, did not increase in the mouse liver.

Kamendulis et al. (2014) dosed male C57BL/6 mice with PFOA at 0.5, 2.5, or 5 mg/kg for 7 days. The lipid peroxidation product 8-iso-PGF2α (by LC-MS) and the expression of the antioxidation response genes *Sod1*, *Sod2*, *GPx2*, *Cat*, and *Nqo1* (by quantitative reverse transcription-polymerase chain reaction, qRT-PCR) were measured in the liver and pancreas. Cerulein was used as a positive control. The levels of 8-iso-PGF2α were significantly increased in the liver and pancreas upon treatment with PFOA. In the pancreas, all end-points, except for CAT, were significantly and positively correlated with PFOA exposure. In the liver, *Sod1*, *Sod2*, *Cat*, and *Nqo1* expression was significantly increased by PFOA doses of 2.5 and 5 mg/kg. PFOA treatment did not have an effect on *Gpx2* expression in the liver. It was noted that cerulein treatment did not significantly induce any biomarkers, except for 8-iso-PGF2α, compared with the negative control. In another study (Kamendulis et al., 2022), PFOA exposure was thought to promote pancreatic cancer in a conditional $Kras^{G12D}$ mouse model (*LSL-*$Kras^{G12D}$ and *Pdx-1 Cre* mice). Drinking-water containing PFOA at 5 ppm was administered to mice (age 2 months) for 4 or 7 months. The mice, which were aged 6 or 9 months at the end of the dosing period, were examined for levels of MDA, and the antioxidant enzymes SOD, CAT, and thioredoxin reductase in the pancreas were measured using commercial kits. The MDA protein levels were significantly increased in mice only at age 9 months, whereas SOD enzyme activity was significantly increased at age 6 and 9 months. The CAT and thioredoxin reductase enzyme activities were only significantly increased in mice aged 6 months.

Male and female Wistar rats were fed a rodent diet containing PFOA at 0.01% w/w for 2 or 26 weeks (Kawashima et al., 1994). Microsomal NADPH-dependent lipid peroxidation, GSH, GPx (with H_2O_2 or cumene hydroperoxide), glutathione reductase (GSR), GST, CAT (H_2O_2 assay), and peroxisomal β-oxidation were measured in the liver. Peroxisomal β-oxidation was significantly increased in both male and female rats after 2 and 26 weeks. CAT levels were also increased compared with the controls at that time. In the livers of male rats, no changes in GSH were observed after 2 or 26 weeks, whereas microsomal NADPH-dependent lipid peroxidation, GPx, and GSR were increased, but were only significantly higher in male rats after 26 weeks of exposure. The GST activities

towards 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene were significantly reduced in male rats after both 2 and 26 weeks of PFOA exposure, whereas in female rats, the GST activities towards 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene were unchanged.

Lee et al. (2015) studied the effects of prenatal PFOS exposure on the fetal development of CD-1 mice. Pregnant mice were orally dosed with PFOS at 3 mg/kg per day from GD1 until GD17. In another group, non-pregnant female adult mice were dosed with PFOS at 3 mg/kg per day for 14 days. Oxidative stress biomarkers were measured in the mothers, fetuses, and non-pregnant mice. Oxidized lipid products derived from the lipid peroxidation of polyunsaturated fatty acids were measured by LC-MS/MS, and SOD and CAT were measured using assay kits. CAT was not measured in the fetuses because of insufficient sample sizes. No significant changes in SOD or CAT levels were observed in the livers or placentas of the pregnant mice. Levels of non-enzymatically oxidized lipid products, including isoprostanes (IsoP) 15-F_{2t}-IsoP, 5-F_{2t}-IsoP, and 8-F_{3t}-IsoP, and neuroprostane (NeuroP) 4(*RS*)-4-F_{4t}-NeuroP, 7β-hydroxycholesterol, and 7-ketocholesterol, were significantly increased in fetal livers compared with levels in livers of control mice. Effects on enzymatically oxidized lipid products of the lipoxygenase (LOX) and CYP450 pathways were also examined. Upon PFOS treatment, the level of 15(*S*)-hydroxyeicosatetraenoic acid (HETE) decreased in fetal livers, whereas the level of 5(*S*)-HETE increased in maternal livers. As for products of the CYP450 pathway, increased levels of 27-hydroxycholesterol were only observed in fetal livers. In a second experiment, the effects of PFOS exposure on adult female brains and kidneys were compared with those on fetuses. With respect to the non-enzymatic pathway, arachidonic acid, adrenic acid, and neurofuran levels were significantly elevated in PFOS-dosed adult brains compared with non-exposed adults. However, only 8-F_{3t}-IsoP levels were significantly increased in the brains of PFOS-exposed fetuses compared with controls. With respect to the enzymatic pathway, oxidized lipid products were significantly increased in fetal brains, but not in adults. These included 9(*S*)-HETE (CYP-mediated), 5(*S*)-HETE, and 8(*S*)-HETE (LOX-mediated). These results indicate that oxidative stress due to PFOS exposure is more severe in fetuses than in their dams. In the kidneys of PFOS-dosed fetuses, significant increases in all the measured non-enzymatically oxidized lipid products, including 15-F_{2t}-IsoP, 5-F_{2t}-IsoP, 8-F_{3t}-IsoP, 4(RS)-4-F_{4t}-NeuroP, 17(*RS*)-F_{2t}-dihomo-IsoP, isofuran (IsoF), neurofuran, and 17(*RS*)-SC-Δ^{15}-11-dihomo-IsoF, were observed; whereas in PFOS-dosed adult female mice, only 15-F_{2t}-IsoP was increased, and a decrease in levels was observed for 5-F_{2t}-IsoP, 4(RS)-4-F_{4t}-NeuroP, 17(*RS*)-F_{2t}-dihomo-IsoP, and 17(*RS*)-SC-Δ^{15}-11-dihomo-IsoF. The results indicated that oxidative stress induced by PFOS is more severe in fetuses than in their dams or adult female mice.

In a study by Liu et al. (2015a), adult male Kunming mice were dosed with PFOA at 2.5, 5, or 10 mg/kg per day for 14 days. The testicular levels of MDA, measured by TBARS, were significantly increased in mice exposed to PFOA at 5 or 10 mg/kg. Additionally, a significant increase in H_2O_2, measured using the reaction with molybdenum acid, was observed. The induction of oxidative stress was further indicated by the reduction in *Nrf2* gene and protein expression, as well as reduced SOD and CAT activities (assay kits) in all the PFOA-treated mice.

Liu et al. (2015b) exposed male Kunming mice to PFOA orally at 10 mg/kg per day for 14 days. A second group of PFOA-dosed mice was co-treated orally with grape seed proanthocyanidin extract at 150 mg/kg per day. MDA, H_2O_2, SOD, and CAT were measured in liver homogenates using assay kits (Jiancheng Institute of Biotechnology, Nanjing, China). The hepatic levels of MDA and H_2O_2 were significantly

increased in PFOA-treated mice, whereas the SOD and CAT activities were reduced. In addition, *Nrf2* mRNA expression and protein levels were also decreased. The co-treatment with grape seed proanthocyanidin extract was able to restore the oxidative status of the livers of PFOA-dosed mice.

Another study investigated both the hepatic and renal effects of PFOA in male Wistar rats (Owumi et al., 2021a). Groups of 10 rats were dosed orally with PFOA at 0 or 5 mg/kg per day for 28 days. Oxidative biomarkers were measured in the liver and kidney. The levels of reactive oxygen and nitrogen species (RONS) (measured with DCFH-DA), NO (Griess reaction), xanthine oxidase, and myeloperoxidase were significantly increased in both the liver and kidney upon PFOA exposure. The levels of GPx (measured using H_2O_2 and Ellman reagent), GSH (H_2O_2), GST (1-chloro-2,4-dinitrobenzene), SOD (autoxidation of epinephrine), and CAT (H_2O_2) were significantly decreased in the liver and kidneys of PFOA-exposed rats. Co-treatment with NAC was found to alleviate the oxidative stress of the PFOA-dosed rats. The effects of oxidative stress on testicular function in male Wistar rats were reported in another study (Owumi et al., 2021b). Groups of 10 rats were dosed orally with PFOA at 0 or 5 mg/kg per day for 28 days. The testes and epididymides were removed for the same suite of biomarker measurements to that described above (Owumi et al., 2021a). The levels of MDA, NO, xanthine oxidase, and myeloperoxidase were significantly increased in both the testes and epididymides of PFOA-exposed rats, whereas RONS levels were significantly increased only in the testes. The antioxidants SOD, CAT, and GSH were all significantly decreased upon PFOA treatment in both the testes and epididymides, the GPx levels were significantly decreased in the epididymides, and the GST levels were significantly decreased in the testes. Co-treatment with NAC alleviated the oxidative stress observed in the PFOA-dosed rats.

Yang et al. (2014) demonstrated oxidative stress in male Kunming mice dosed with PFOA at 2.5, 5, or 10 mg/kg per day for 14 days. The biomarkers MDA and H_2O_2 were measured using assay kits from the Jiancheng Institute of Biotechnology, Nanjing, and were found to be significantly increased in liver tissue after 2.5, 5, and 10 mg/kg, and 5 and 10 mg/kg doses, respectively.

Zhang et al. (2016a) fed male C57BL/6 mice a normal diet or a marginal methionine/choline-deficient (mMCD) diet, both containing 0.003%, 0.006%, or 0.012% PFOS, for 23 days or 21 days (second replicate). A potential modulatory mechanism affecting hepatic steatosis and oxidative stress was investigated. Both PFOS-containing diets caused weight loss in the mice, and this weight loss was more severe with the mMCD diet. In a choline-supplementation experiment, the levels of MDA, GSH, and GSSG (assay kits) were measured in the livers of mice exposed to 0.003% PFOS in their diet for 6 weeks. The levels of MDA significantly increased, whereas the GSH/GSSG levels significantly decreased in mice fed either the 0.003% PFOS-normal diet or the 0.003% PFOS-mMCD diet. Choline supplementation alleviated the oxidative stress effects of PFOS. No biomarker data were available for the other dosage groups.

Zhang et al. (2022a) studied the effects of PFOA on oocyte maturation in ICR mice dosed with PFOA at 1 or 5 mg/kg per day for 28 days. ROS production measured by chloromethyl derivative of DCFH-DA was significantly increased in oocytes in a dose-dependent manner. PFOA-mediated induction of oxidative stress was further supported by an increase in the DNA damage marker γH2AX.

Another study investigated the effects of PFOA on the mouse epididymis (Lu et al., 2016b). Male BALB/c mice (age 6–8 weeks) were dosed by oral gavage with PFOA at 0, 1.25, 5, or 20 mg/kg per day for 28 days. At necropsy, the epididymides were extracted, and SOD, MDA, and

GPx levels were measured using the nitroblue tetrazolium-illumination, TBARS, and benzoic acid methods, respectively. Because there was no available method for measuring ROS in the epididymis, the assessment of ROS production was performed in sperm cells isolated from the epididymides and treated in vitro with PFOA at 0, 100, or 400 µM for 1, 2, 4, or 8 hours. Rosup was used as a positive control. ROS production in the sperm cells was measured spectroscopically using the DCF-DA method. The study reported significantly increased ROS production in sperm cells after 1 and 2 hours of treatment with 100 µM PFOA and at all time points after treatment with 400 µM PFOA. In the epididymides of the mice, MDA levels were significantly elevated when the mice were treated with PFOA at concentrations of 1.25 or 5 mg/kg per day, but not 20 mg/kg per day. No significant changes in SOD levels were observed upon PFOA exposure. However, the SOD/MDA ratio significantly decreased in the epididymides of mice exposed to PFOA at 1.25 or 5 mg/kg per day. Additionally, GPx levels were significantly decreased when mice were treated with PFOA at 5 or 20 mg/kg per day. In this study, the authors also demonstrated that PFOA disturbed the profile of polyunsaturated fatty acids in the epididymides of PFOA-treated mice. This observation, coupled with the increased oxidative stress in the epididymis, may explain the negative effects of PFOA on the reproductive function of male mice.

Zhang et al. (2021b) studied the mechanisms of PFOA toxicity in the uterus and liver of Kunming mice during early pregnancy. PFOA (1, 5, 10, 20, or 40 mg/kg per day) was administered to pregnant mice by gavage from GD1 to GD7, and they were killed on GD9. Their liver MDA levels significantly increased with increasing PFOA concentration, and the SOD and GPx levels significantly decreased in a dose-dependent manner. [The Working Group noted that the findings of this study suggest that oxidative damage may be involved when PFOA induces liver toxicity and uterine cell apoptosis, leading to possible embryo loss or damage.]

Chen et al. (2017) also studied the effects of PFOA on pregnant mice. Adult female Kunming mice were dosed by oral gavage with PFOA at 2.5, 5, or 10 mg/kg per day from GD1 to GD7 or GD13. Significant dose-dependent elevations in MDA and H_2O_2 and significant dose-dependent decreases in CAT and SOD levels were observed in the ovaries of PFOA-exposed mice on GD13. On GD7, MDA levels also significantly increased in the ovaries of mice at all the tested PFOA concentrations; however, no dose-dependent effects were observed. The levels of H_2O_2, SOD, and CAT were not significantly changed on GD7. Biomarkers were measured using commercial kits.

In a recent study (Zhang et al., 2023a), female mice (strain not reported) were treated with PFOA at 4 mg/kg per day for 30 days. The levels of mt-ROS were significantly increased in granulosa cells upon treatment with PFOA, as assessed using the fluorescence ratio of MitoSOX Red/MitoTracker Green.

In another study on the reproductive effects of PFOA, male Sprague-Dawley rats were dosed by oral gavage with PFOA at 0.01 g/kg bw for 30 days (Zhang et al., 2023c). Levels of MDA, SOD, and GPx in the testes were measured by ELISA kits. Levels of MDA were significantly increased, whereas levels of SOD and GPx were significantly decreased upon PFOA exposure. In addition, in PFOA-treated rats that were subsequently treated with lipoic acid at a dose of 0.1 g/kg per day for 42 days, levels of the markers were restored to those in the control rats. Lipoic acid at the low daily dose of 0.05 g/kg was less effective than the higher dose. Shalaby et al. (2023) reported that when rats were dosed with the potassium salt of PFOS by oral gavage at 5 mg/kg per day for 28 days, jejunal MDA levels were significantly increased, whereas jejunal SOD and GPx levels were significantly decreased. When rats were co-treated with lemongrass

essential oil at 100 mg/kg, 30 minutes before the PFOS dose (5 mg/kg per day) every day for 28 days, the lemongrass essential oil restored the levels of oxidative stress biomarkers.

Du et al. (2023) reported that CBD partially alleviated PFOS-induced apoptosis via the NOX4/ROS/JNK pathway. In this study, one group of male C57BL/6 mice was dosed by oral gavage with PFOS alone at 5 mg/kg per day for 4 weeks. Another group of PFOS-dosed mice was co-treated with CBD at 5 mg/kg by oral gavage daily, 4 hours after the PFOS treatment. The levels of MDA were significantly increased, whereas the SOD and GPx levels were significantly decreased in the serum of PFOS-dosed mice, as was the expression of NOX4, assessed using immunofluorescence staining, in kidney tissue. Co-treatment with CBD partially restored the levels of these markers. In addition, the authors observed alterations of MDA, SOD and GPx (oxidative stress end-points) in mouse kidney HEK293 cells in vitro.

Intraperitoneal injections of PFOS (1 or 10 mg/kg bw) were given to male Sprague-Dawley rats every other day for 15 days (Tang et al., 2022). The kidney MDA levels significantly increased in all PFOS-dosed rats compared with the control group. A significant decrease in GPx level was only observed in the group dosed with 10 mg/kg bw.

Xu et al. (2016) proposed that PFOS induces adipogenesis and glucose uptake in association with an activation of the Nrf2 signalling pathway. Male C57BL/6 mice (age 21 weeks) were given PFOS (100 µg/kg bw) by oral gavage for 36 days. The expression of SOD and HO-1 did not increase, but *Nrf2* mRNA expression was significantly increased upon PFOS exposure, implying an upregulation of the Nrf2 signalling pathway and adipogenic gene expression in epididymal white adipose tissue. [The Working Group considered that the activation of the Nrf2 pathway suggested an oxidative stress-mediated effect of PFOS on adipogenesis.]

D'Amico et al. (2022) studied the oxidative effects of PFOS exposure on healthy DBA/1J mice and mice with rheumatoid arthritis (induced by type II chicken collagen and complete Freund's adjuvant administered intradermally on days 0 and 21). PFOS (10 mg/kg bw) and other endocrine disruptors were given by oral gavage from day 21 to 35. Paw tissue was analysed for oxidative stress biomarkers, including MDA, H_2O_2, SOD, and GPx. PFOS exposure significantly induced oxidative stress in healthy mice, and this effect was more severe in mice with rheumatoid arthritis.

The induction of metabolic disturbances by PFOS in different regions of the mouse gut was studied by Chen et al. (2023a). Male C57BL/6 mice (age 6 weeks) were exposed to PFOS at 500 µg/L in drinking-water for 28 days. CAT, GSH, and MDA levels were measured using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China). The MDA levels significantly increased in the ileum and colon of mice exposed to PFOS, and this effect was more marked in the ileum than in the colon. However, no significant changes in CAT or GSH levels were observed in the ileum and colon of PFOS-exposed mice compared with control mice. The levels of tumour necrosis factor alpha (TNF-α) and interleukin-1-beta (IL-1β) were also significantly increased in the ileum but not in the colon of mice treated with PFOS.

In a study by Huang et al. (2020) in mice, the authors investigated the protective effects of grape seed proanthocyanidin extract against PFOS-induced oxidative stress. Male Kunming mice were dosed with PFOS (10 mg/kg bw) via gastric gavage daily for 21 days. Levels of MDA, H_2O_2, and SOD were measured in the liver. PFOS-induced oxidative stress was indicated by an increase in MDA and H_2O_2 levels and a decrease in SOD level. The extract was shown to be protective against PFOS-induced oxidative stress.

In another study (Ma et al., 2021), gastrodin, a water-soluble organic compound isolated from the root of *Gastrodia elata* Blume, was shown to reduce the oxidative stress induced by PFOA. C57BL/6J mice were dosed with PFOA (5 mg/kg bw) by oral gavage daily for 6 weeks, which was followed by treatment with gastrodin (20 mg/kg bw) or vehicle (saline) daily for 8 weeks. After PFOA administration, the liver MDA levels significantly increased, whereas the CAT, SOD, and GPx levels significantly decreased. The administration of gastrodin significantly reduced PFOA-induced oxidative stress.

Ma et al. (2023) also investigated the potential for rutin to reduce the PFOA-induced oxidative effects in mice. As reported above, male ICR mice were dosed with PFOA (20 mg/kg bw) daily for 28 days in the presence or absence of rutin. The serum contents of MDA, GPx, and SOD were significantly increased after exposure to PFOA. Rutin was able to reduce the oxidative stress induced by PFOA.

Chen et al. (2012) studied the effects of prenatal PFOS exposure on oxidative stress in the lungs of rat offspring. Pregnant female rats were dosed with PFOS (0.1 or 2 mg/kg bw) between GD1 and GD21 daily by gavage. Fresh lung tissue was collected from two male and two female pups from each group for biomarker analysis on PND0 and PND21. Pups from the dams that received the higher dose (2 mg/kg bw) showed significantly increased levels of MDA and significantly decreased GSH level and SOD activity compared with the controls at both time points. The MDA level showed a small but significant increase in the neonatal lungs of pups from dams exposed to PFOS at 0.1 mg/kg.

Crebelli et al. (2019) conducted a subacute experiment by supplying male C56BL/6 mice (age 6–8 weeks) with drinking-water containing PFOA (28, 5.5, or 0.55 mg/L, corresponding to 5, 1, or 0.1 mg/kg bw per day) for 5 weeks. Liver MDA levels were measured using the TBARS method. The study reported no significant changes in liver lipid peroxidation in any of the PFOA-treated groups. However, CCl_4-treated mice (positive control) showed a significant increase in MDA level.

Salimi et al. (2019) studied the potential of PFOA to induce abortion and developmental toxicity in mice. Pregnant female NMRI mice (age 8–9 weeks) were dosed with PFOA at 1, 10, or 20 mg/kg between GD5 and GD9. mt-ROS production, measured using DCFH-DA, was significantly increased in the liver, brain, and heart of the fetuses, but not in the placenta.

Liu et al. (2009) studied the oxidative effects of PFOS in young mouse pups. The pups of Kunming mice were weaned on PND21, then dosed with PFOS at 50 mg/kg bw once by subcutaneous injection on PND7, PND14, PND21, PND28, or PND35. The levels of MDA, SOD, and TAC were determined in liver and brain 24 hours after exposure. No significant changes in MDA levels in the liver or brain were observed in pups of either sex. In the brain, significant decreases in SOD level were only observed in male pups after injections on PND7 and PND21. In the liver, a significantly decreased SOD level was only observed in female pups after injection on PND14. A significant decrease in TAC was only observed in male pup brains after injection on PND21. In the liver, a significant decrease in TAC was observed in female pups after injection on PND21 and in male pups after injection on PND7 and PND14. However, TAC was significantly increased in male pups after injection on PND21.

Wang et al. (2022c) orally dosed male Balb/c mice with PFOS at 0.2, 1, or 5 mg/kg bw daily for 28 days. GSH levels were significantly increased in the livers of mice exposed to PFOS at 5 mg/kg; however, a non-significant decrease was observed in the two lower-dosed groups. The level of GSR in the liver was increased in all the PFOS-exposed mice, but this only reached significance in the group at the highest dose.

Endirlik et al. (2022) examined the effects of PFOA exposure on the livers and brains of male Balb/c mice. The mice were orally dosed with PFOA at 15 or 30 mg/kg for 10 days. Levels of MDA and GSH were measured using the TBARS method and the 5,5′-dithiobis (2-nitrobenzoic acid) method, respectively. GPx, CAT, and Cu-Zn-SOD levels were measured using assay kits. In the liver, no changes in MDA level, but significantly increased GSH and CAT levels and significantly decreased Cu-Zn-SOD and GPx levels, were observed. In the brain, the MDA level significantly increased in mice exposed to PFOA at 30 mg/kg, whereas the GPx level significantly decreased, and no significant changes in the levels of the other biomarkers were observed. It was suggested that PFOA exposure results in a depletion of antioxidative systems and exhibits neurotoxic effects by inducing oxidative stress.

Badr and Birnbaum (2004) studied the oxidative effects of PFOA in male Fischer 344 rats ranging in age from juvenile (age 4 weeks) to post-puberty (age 10 weeks), mature adulthood (age 20 weeks), middle age (age 50 weeks), and senescence (age 100 weeks). The rats were dosed by gavage with PFOA (150 mg/kg bw) or vehicle. The rats were killed between days 0 and 28 post-dosing for the measurement of hepatic peroxisomal β-oxidation and CAT activity in the liver. Hepatic peroxisomal β-oxidation was increased 3–5-fold in all PFOA-dosed groups, with an increased recovery time in older rats. Liver CAT activity was also significantly lower in senescent livers compared with those from all other groups.

Liu et al. (1996) treated adult male Crl:CD BR (CD) rats with APFO (C8) (0.2, 2, 20, or 40 mg/kg per day) for 14 days. β-Oxidation in the liver was measured using [1-^{14}C]palmitoyl-CoA as the substrate, and potassium cyanide was used to inhibit mitochondrial β-oxidation. Hepatic β-oxidation was significantly increased upon PFOA exposure (≥ 2 mg/kg per day) in a dose-dependent manner.

Lv et al. (2018) showed the antioxidative properties of naringin (NAR, 4′,5,7-trihydroxyflavonone-7-rhamnoglucoside), a naturally occurring flavonoid glycoside isolated from citrus fruits, with respect to PFOS-induced oxidative stress. Male mice were exposed to PFOS by oral gavage (10 mg/kg bw) daily for 3 weeks. Levels of MDA, H_2O_2, SOD, and GSH were measured in the livers of the mice in the presence or absence of NAR (100 mg/kg bw). PFOS exposure significantly increased the MDA and H_2O_2 levels and reduced the SOD and GSH levels. Mice co-treated with NAR were protected from PFOS-induced oxidative stress.

[The Working Group noted that the studies described above reported the effects of acute, subacute, and subchronic repeated-dose treatment (up to 28 days) of PFOA or PFOS in vivo. The agents were mostly administered by oral gavage; the i.p. route, administration via drinking-water, or ad libitum feeding were used only in a few studies. Rodent studies dominated, and the dosing regime was typically 5–10 mg/kg per day. It was noted that such doses were much higher than exposures in humans. Despite this, PFOA and PFOS were shown to induce oxidative stress as shown also in studies in exposed humans and in human cells in vitro.]

(ii) Non-human mammalian systems in vitro

A wide range of experiments in vitro in non-human mammalian cells (primary and immortalized cells derived from different organ systems) have been used to explore the oxidative effects of PFOA and PFOS. The studies are briefly described below.

Lindeman et al. (2012) reported no significant increase in oxidatively damaged DNA (Fpg-modified comet assay) in freshly isolated rat testicular cells treated with 100 or 300 μM PFOA for 24 hours. 1,2-Dibromo-3-chloropropane was used as a positive control.

Liver cells of animal origin have been widely used to explore the oxidative stress-inducing

potential of PFOA and PFOS. Xu et al. (2019) isolated primary hepatocytes from young adult male C57BL/6J mice (age, 6 weeks). The liver cells were exposed to PFOA or PFOS (0, 10, 100, 500, or 1000 μM) for 24 hours. PFOA and PFOS significantly increased the ROS (PFOA at ≥ 500 μM; PFOS at 1000 μM), SOD (PFOA and PFOS at ≥ 500 μM), and GSH (PFOA at ≥ 500 μM; PFOS at 100 μM and 1000 μM) levels and significantly reduced CAT activity at all concentrations. [The Working Group noted that PFOA or PFOS induced cell death at a concentration of 1000 μM and that the increases in oxidative end-points are not relevant at this concentration.] Goines and Dick (2022) also reported that PFOS increased ROS production and reduced the GSH level in HepG2 cells that were treated with 100 μM PFOS for 16 hours.

PFOS was shown to induce ROS production in kidney cells. Wen et al. (2021) exposed rat proximal renal tubular cells (NRK-52E) to PFOS (100 μM) and measured ROS production after 1, 3, 6, and 24 hours of exposure. The exposed cells showed significantly increased ROS production after 1 hour, and the ROS levels remained significantly elevated at all the tested time points.

PFOA and PFOS were shown to induce ROS production in other organ systems. For example, Reistad et al. (2013) examined primary cultures of neurons isolated from rat cerebellum (age, 6–8 days; sex not reported). The cultured neurons were exposed to different concentrations (0, 6, 12, 25, 50, or 100 μM) of PFOA or PFOS for 24 hours. PFOA and PFOS significantly increased ROS production in a dose-dependent manner. The estimated half-maximal effective concentration (EC_{50}) values (± SD) were 25 ± 11 μM and 27 ± 9.0 μM for PFOA and PFOS, respectively. Increased ROS production was observed at concentrations close to the serum PFOA and PFOS concentrations found in occupationally exposed workers.

López-Arellano et al. (2019) studied the effects of PFOA on mouse oocytes in vitro. Ovary explants were dissected from 17-day post-coitus mouse CD-1 embryos and cultured for 7 days. Oocytes were then treated with PFOA at 1/4 LC_{50} (28.2 μM) or LC_{50} (112.8 μM) for 24 hours. ROS production was significantly increased in the treated oocytes compared with the controls. A similar study was conducted in porcine oocytes and cumulus cells (Mario et al., 2022). Cumulus cells are a group of granulosa cells that surround the oocytes and play a role in the process of oocyte development and maturation. The cells were treated with PFOA – 1/8 LC_{50} (20 μM) or 1/4 LC_{50} (40 μM) – during in vitro maturation for 44 hours. Exposure to 40 μM PFOA significantly increased ROS production in the cumulus cells. An earlier study (Chen et al., 2021) also showed that PFOS exposure during in vitro maturation (100 μM, 44–48 hours) significantly increased ROS levels in porcine oocytes.

Basini et al. (2022) studied the effects of PFOA on porcine ovarian granulosa cells that were exposed to PFOA (2, 20, or 200 ng/mL equal to 4.83, 48.3, or 483 μM) for 48 hours. RONS, including the O^{2-}, H_2O_2, and NO levels, were assessed, and in contrast to the above findings, these were found to be significantly decreased after treatment with all the tested PFOA concentrations. Non-enzymatic scavenging activity was assessed by the ferric-reducing ability of plasma and was found not to be affected by PFOA exposure at the tested concentrations.

PFOS was suggested to have an adverse effect on cerebellar granule cells by inducing apoptosis via a ROS-mediated alteration in protein kinase C (PKC) signalling. PKC is a pivotal messenger molecule that is involved in neuronal function and development. PFOS (3 or 30 μM) was found to increase ROS production in cerebellar granule cells from Sprague-Dawley rats aged 7 days. NAC (10 mM) pre-treatment 24 hours before PFOS exposure was shown to inhibit ROS production in the cells (Lee et al., 2012). In a study aiming to investigate the potential for PFOS to cause neurotoxicity (Li et al., 2017b),

PFOS (250 µM, 24 hours) significantly increased ROS production in PC12 cells, a cell line derived from a rat pheochromocytoma. When cells were pre-treated with taurine, an antioxidant (80 mM, 30 minutes), PFOS-induced ROS generation was inhibited.

Qian et al. (2010) studied ROS in mouse RAW 264.7 macrophages exposed to PFOS (100 µM, 5 minutes) using electron spin resonance spectroscopic measurement. PFOS significantly induced ROS (by 2.4-fold) in the cells. Electron spin resonance is considered to be a more robust technique for the detection of ROS because of its specificity and sensitivity. The study also showed that the production of ROS plays a role in PFOS-induced actin filament remodelling and the increase in endothelial permeability.

Elumalai et al. (2023) reported that PFOS-induced oxidative stress contributed to the apoptosis of rat insulinoma INS-1 cells. When cells were treated with 50 or 100 µM PFOS for 36 hours, mitochondrial ROS generation increased through NOX2–gp91Phox activation and the inhibition of cAMP-PKA.

Dos Santos et al. (2022) treated the rat β-cell line INS-1E with low concentrations of PFOA (1 nM or 1 µM) for 24 hours. No significant changes in ROS production, measured by fluorescence of DCF-DA, were observed.

Effects of PFOS (75–600 µM, 24 hours) and PFOA (400–1000 µM, 24 hours) on Nrf2$^{-/-}$ knockout and wildtype C57BL/6 mouse astrocytes have also been studied (Alharthy and Hardej, 2021). The study showed that exposure to 600 µM PFOS or 800 µM PFOA significantly increased ROS levels, lipid peroxidation, and apoptosis in both the wildtype and Nrf2$^{-/-}$ astrocytes. However, the effects of PFOA and PFOS were significantly larger in the Nrf2$^{-/-}$ astrocytes than in wildtype cells. The GSH/GSSG ratio was significantly decreased in both wildtype and Nrf2$^{-/-}$ astrocytes treated with PFOA or PFOS. However, the decrease was significantly larger in the Nrf2$^{-/-}$ astrocytes than in the wildtype cells. Pre-treatment with butylated hydroxytoluene before PFOA and PFOS exposure significantly reduced ROS production in wildtype astrocytes but not in Nrf2$^{-/-}$ astrocytes. In summary, the study showed that Nrf2$^{-/-}$ astrocytes were more susceptible to PFOA and PFOS toxicity.

[The Working Group noted that the literature in experimental systems in vitro regarding the potential effects of PFOA and PFOS to induce oxidative stress was quite extensive. Oxidative stress is typically measured by generation of ROS, and this is sometimes coupled with the measurement of antioxidant enzyme activities, including those of SOD, CAT, GSH, and/or GPx. The Working Group identified more studies reporting evidence of oxidative stress induction than those reporting marginal evidence or no evidence.]

Oxidative stress was induced by PFOS in embryonic stem cell-derived cardiomyocytes from 129 mice (Cheng et al., 2013), in rat hepatocytes (Khansari et al., 2017), in rat HAPI microglial cells (Wang et al., 2015c), and in a co-culture of Sertoli cells and gonocytes from male Sprague-Dawley rat pups (Zhang et al., 2013d).

PFOA was shown to induce oxidative stress in MC3T3 osteoblast cells (Choi et al., 2016), in mouse spermatogonial GC-1 cells (Lin et al., 2020a), and in rat brain and liver mitochondria (Mashayekhi et al., 2015). Both PFOA and PFOS induced oxidative stress in rat mesangial cells (Gong et al., 2019).

Studies showing marginal or no effects included a study by Berntsen et al. (2017), in which rat cerebellar granule neurons were exposed to PFOA or PFOS at 75, 150, 300, or 600 µM for 3 hours, and H_2O_2 was used as a positive control. No significant increase in ROS production upon exposure to PFOA or PFOS was observed after 3 hours. PFOA and PFOS exposure for 3 hours resulted in a significant decrease in lipid peroxidation at many of the tested concentrations.

PFOA- or PFOS-induced oxidative stress may also affect neuronal development. Slotkin et al.

(2008) used PC12 cells as an in vitro model to study the effects of PFOA and PFOS exposure on undifferentiated (24 hours) and differentiating (4 days) cells. PFOA (at 10 and 250 μM) and PFOS (at 50, 100, and 250 μM) significantly increased MDA levels in the differentiating cells; 50 μM chlorpyrifos was used as a positive control.

Other evidence that PFOA and PFOS induce oxidative stress in non-human cell lines was provided by several studies. Wang et al. (2015a) used *gpt delta* transgenic mouse embryonic fibroblast cells immortalized by the SV40 T antigen, Oseguera-López et al. (2020) performed a spermatozoa capacitation study using sperm from boars, and Wei et al. (2021) used mouse oocytes, and all three studies showed that PFOS induced oxidative stress in the respective cell types. A study by Suh et al. (2017) provided further evidence that PFOA induces oxidative stress in rat pancreatic β-cell-derived RIN-m5F cells.

In a rabbit renal proximal tubule model (Schnellmann, 1990), PFOS at 10, 20, 50, or 100 μM did not increase MDA levels. However, GSH levels were significantly decreased in cells exposed to 100 μM PFOS for 15 minutes.

Synopsis

[The Working Group noted that there is some evidence that PFOS induces oxidative stress in exposed humans, whereas the evidence for PFOA is mixed. Ten studies examined the effects of PFOS, of which six generated positive results, two marginal, and two negative results. Eleven studies in exposed humans examined the effects of PFOA; of these, only three studies showed a positive association of PFOA with oxidative stress, with one marginal and seven negative results.

In human primary cells in vitro, there is evidence that PFOA and PFOS induce oxidative stress. Similarly, PFOA and PFOS were found to induce ROS production in most in vitro studies using immortalized human cell lines, including HepG2 cells and other human cells, confirming the observations in both in vitro and in vivo studies in humans.

In experimental systems in vivo and in vitro, there is evidence that PFOA and PFOS induce oxidative stress.

The PFOA and PFOS concentrations used in exposure experiments in various non-human mammalian cells were similar to those used in human cell studies. The findings suggested that PFOA and PFOS can induce oxidative stress in vitro at concentrations similar to those used in human cells, in the micromolar concentration range. Some studies reported increased levels of 8-OHdG or 8-oxodG in cells or biological fluids, as measured by LC-MS/MS, which provided evidence for oxidative stress and potential oxidative damage to DNA. The generation of oxidative stress by PFOA and PFOS exposure is further supported by increased ROS production and decreased antioxidant defence end-points. Co-treatment with antioxidants has been shown to reduce oxidative stress, which provides additional evidence that oxidative stress may be involved when experimental systems are exposed to PFOA or PFOS.]

4.2.6 Induces chronic inflammation

(a) Humans

(i) Exposed humans

See Table 4.18.

Changes in inflammatory markers, including C-reactive protein (CRP) cytokines such as the interleukins (ILs) IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, and IL-10, TNF-α, interferon-gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), neutrophil count; lymphocyte count; leptin; and adiponectin; as well as parameters related to eczema and rhino-conjunctivitis, atopic dermatitis, and lung function; were examined as part of the KC "induces chronic inflammation".

In total, the Working Group had access to 18 studies that investigated associations between

Table 4.18 End-points relevant to chronic inflammation in humans exposed to PFOA or PFOS

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
Inflammatory markers in general population exposure							
35 inflammatory proteins	Serum/ plasma	Europe HELIX project; in 1101 mother–child pairs, PFAS measured in blood collected during pregnancy (prenatal exposure) and in children of age 8 years (postnatal exposure) (range, 6–12 years). 35 inflammatory proteins measured in the children's plasma Cross-sectional design.	Maternal PFOS: median, 6.2 ng/mL Child PFOS: median, 1.5 ng/mL *n* = 1101	↓ MIG, MIP1-β (prenatal PFOS) No association with adiponectin, BAFF, C-peptide, CRP, EGF, eotaxin, FGF-2, G-CSF, HGF, IFN-α, IFN-γ, IL-10, IL-12, IL-13, IL-15, IL-17, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IP10/CXCL10, leptin, MCP-1/CCL2, MIP-1α/CCL3, PAI-1, or TNF-α	Maternal age and education, pre-pregnancy BMI, parity, cohort, child ethnicity, age at examination, and sex	Potential divergence of PFAS exposure profiles in childhood from gestational exposure profiles because of factors such as transplacental transfer, breastfeeding, and dietary exposure. Sample size relatively large. Possibility of loss to follow-up and selection bias in longitudinal birth cohorts, children of mean age 8 years. Both mixture-approach statistical analysis and linear regression analysis were performed. All significance levels were set to 0.05 in this study, but it is unclear whether the linear analysis involved multiple correction.	Papadopoulou et al. (2021)
			Maternal PFOA: median, 2.2 ng/mL Child PFOA: median, 1.9 ng/mL *n* = 1101	↑ IL-1β (prenatal PFOA) ↓ IL-8, HPG (postnatal PFOA) No association with adiponectin, BAFF, C-peptide, CRP, EGF, eotaxin, FGF-2, G-CSF, HGF, IFN-α, IFN-γ, IL-10, IL-12, IL-13, IL-15, IL-17, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IP10/CXCL10, leptin, MCP-1/CCL2, MIP-1α/CCL3, PAI-1, or TNF-α			

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
HbA1c, adiponectin, leptin, CRP, SHBG, and IL-6	Plasma	USA Prospective prebirth cohort with 3 years of follow-up. Measurements in mothers during and after pregnancy	PFOS: median, 24.8 ng/mL, *n* = 450–454, depending on the marker, during pregnancy PFOA: median, 5.6 ng/mL, *n* = 450–454, depending on the marker during pregnancy	No association with HbA1c, adiponectin, leptin, CRP, SHBG, or IL-6 No association with HbA1c, adiponectin, leptin, CRP, SHBG, or IL-6	Age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, parity, and breastfeeding history	Loss to follow-up between pregnancy and 3 years postpartum, requiring correction for potential selection bias. Limited power to detect subtle effects, potentially resulting in false negatives; small effect sizes with wide confidence intervals; and no adjustment for multiple testing.	Mitro et al. (2020)
IFN-γ, IL-6, IL-10, TNF-α, CRP	Serum	Atlanta, USA African American Maternal–Child Cohort Longitudinal assessment	PFOS: median, 2.19 ng/mL; *n* = 425 PFOA: median, 0.72 ng/mL; *n* = 425	↑ TNF-α and IFN-γ at both 8–14 wk and 24–30 wk of gestation ↑ IL-6 and CRP at 24–30 wk of gestation No associations with IFN-γ, IL-6, IL-10, TNF-α, or CRP at either 8–14 wk or 24–30 wk of gestation	Models were adjusted for gestational age at sample collection, maternal age, education, prenatal BMI, and parity	PFOS and PFOA were detected in > 98% of samples at 8–14 wk of gestation; inflammatory markers measured at up to two time points (8–14 wk and 24–30 wk of gestation).	Tan et al. (2023)

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
87 inflammatory proteins	Plasma	Sweden Prospective adult population-based cohort Cross-sectional design	PFOS: median, 13.4 ng/mL; *n* = 965 PFOA: median, 3.3 ng/mL; *n* = 965	No association with CRP or other inflammatory markers ↓ CRP	Sex, sample storage time in freezer, smoking, exercise habits, education, energy and alcohol intake, BMI, glomerular filtration rate, glucocorticoid and COX-inhibitor treatment	Sample size was moderate, multiple covariate adjustments and correction for multiple testing were applied. Older individuals, sensitivity analysis included. CRP assessed using ELISA, Older Swedish participants, limiting the generalizability of the findings to other age and ethnic groups.	Salihović et al. (2020b)
CRP, IL-6, IL-1β, adiponectin, and leptin	Serum	Taiwan, China Young adults (age 12–30years) Cross-sectional design	PFOS: median, 8.93 ng/mL; *n* = 287 PFOA: median, 2.39 ng/mL; *n* = 287	No associations No association with CRP, IL-6, IL-1β, adiponectin, or leptin	Adjustment for age, sex, additional lifestyle factors, and measured parameters; a significant association was only identified when it remained consistent across all three models, avoiding model-dependent results	Sample size was relatively small.	Lin et al. (2011)
IL-6, IL-10, and TNF-α	Serum of women with overweight or obesity during pregnancy or afterwards	San Francisco Bay Area, USA Participation in the MAMAS Cross-sectional design	PFOS: median, 2.83 ng/mL; *n* = 103 PFOA: median, 1.4 ng/mL; *n* = 103	↑ IL-6 No association with IL-10 or TNF-α ↑ IL-6 No association with IL-10 or TNF-α	Age, race/ethnicity, time-varying BMI, parity, education, smoking status, number of gestational weeks at baseline, and visit	Sample size was small, which may have limited the ability to detect associations (*n* = 103), lack of data on breastfeeding	Zota et al. (2018)
CRP, absolute neutrophil count, and lymphocyte count	Serum	USA. NHANES 2005–2006. Adults aged ≥ 20 years. Cross-sectional design	PFOS: median, 11.4 ng/mL; *n* = 6652	↓ Neutrophil count ↑ Lymphocyte count No association with CRP	Age, sex, race/ethnicity, education, poverty income ratio, BMI, and serum cotinine	Sample size was relatively large, potential bias from participants taking anti-inflammatory or immunomodulatory medication.	Omoike et al. (2021)

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
			PFOA: median, 3.2 ng/mL; *n* = 6652	↓ Neutrophil count ↑ Lymphocyte count No association with CRP			Omoike et al. (2021) (cont.)
MCP-1, HO-1, and IL-8	Maternal plasma or serum	China Prospective, nested case–control study Cross-sectional assessment	PFOS: median, 1.79 ng/mL *n* = 519 (144 cases and 375 controls) PFOA: median, 0.79 ng/mL; *n* = 519 (144 cases and 375 controls)	↑ MCP-1 No association with IL-8 or HO-1 ↓ IL-8 No association with MCP-1 or HO-1	Crude models were adjusted for gestational age. Full models included covariates that were significantly associated with the levels of the biomarkers or PFOS and PFOA	Case–control study, sample size was small (144 cases and 375 controls). Participants were recruited only during the first and second trimesters (4–22 wk of gestation).	Liu et al. (2020a)
Leptin, adiponectin	Umbilical cord serum	China SMBCS Prospective birth cohort Cross-sectional assessment	PFOS: median, 1.94 µg/L; *n* = 1111 PFOA: median, 3.76 µg/L; *n* = 1111	↑ Adiponectin ↑ Leptin (women) ↑ Adiponectin ↑ Leptin (women)	Maternal factors (age, occupation type, education level, family annual income, pre-pregnancy BMI, gestational weight gain, weekly physical activity, smoking during pregnancy, parity); infant factors (sex and gestational age); and an interaction term for PFAS and sex	Sample size was relatively large, biomarkers not specific for chronic inflammation.	Ding et al. (2023)

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
Inflammatory markers in communities with elevated exposure							
IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, GM-CSF, TNF-α	Serum	USA Population-based cohort study, adults with median age 61 years Cross-sectional design	PFOS: median, 8.2 ng/mL; *n* = 212 PFOA: median, 3.3 ng/mL; *n* = 212	No association with IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, GM-CSF, or TNF-α ↓ IL-1β No association with IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, GM-CSF, or TNF-α	Age, BMI, sex, smoking history, and race/ethnicity	Sample size was small (*n* = 212), may not be generalizable to non-AFFF-exposed populations; the biomarkers were not all measured in the entire sample, which further reduced the power. Limitations of the study included low detection frequency of cytokines and non-fasting samples, suggesting that cytokine concentrations may have been influenced by recent food intake.	Barton et al. (2022)
TNFα, IL-6, IL-8, IFN-γ, adiponectin, and leptin	Serum	Mid-Ohio Valley, USA The C8 Health Study of adults Cross-sectional design	PFOS: mean, 26.9 ng/mL; *n* = 200 PFOA: mean, 94.6 ng/mL; *n* = 200	↑ Adiponectin ↓ TNF-α, IL-8 No association with IL-6, IFN-γ, or leptin ↑ IFN-γ ↓ TNF-α No association with IL-6, IL-8, adiponectin, or leptin	Age, alcohol consumption, BMI, sex, and eGFR	Serum samples were randomly selected from a larger cohort; pilot study with no multiple comparisons.	Bassler et al. (2019)
End-points of disease outcome							
Eczema and rhino-conjunctivitis	Maternal plasma	Hokkaido, Japan Prospective birth cohort with 7 years of follow-up Longitudinal design	PFOS: median, 5.1 ng/mL; *n* = 2689 PFOA: median, 1.9 ng/mL; *n* = 2689	↓ Eczema No association with rhino-conjunctivitis ↓ Eczema No association with rhino-conjunctivitis	Sex parity, maternal age at delivery, maternal smoking during pregnancy, BMI pre-pregnancy, and annual household income during pregnancy	Large sample size, loss to follow-up, self-reported questionnaires, with the possibility of recall bias affecting outcomes, lack of inflammatory biomarker measurement.	Ait Bamai et al. (2020)

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
Lung function, asthma, atopic dermatitis, rhinitis, LRTIs	Serum at age 10 years	Norway ECA prospective birth cohort with 10–16 years of follow up. Longitudinal design	PFOS: median, 5.2 ng/mL; *n* = 378	↑ LRTIs (entire sample and boys aged 10–16 years) No association with reduced lung function, asthma, atopic dermatitis, or rhinitis	BMI at age 16 years, puberty status, maternal education, and physical activity level at age 16 years (frequency of activities leading to breaking sweat and shortness of breath); after 10 years of follow up: physical activity and maternal education	Sample size was small, potential selection bias related to socioeconomic factors and loss to follow-up.	Kvalem et al. (2020)
			PFOA: median, 1.6 ng/mL; *n* = 378	↑ Rhinitis (PFOA) ↑ LRTIs (entire sample and girls aged 10–16 years) No association with lung function, asthma, or atopic dermatitis			

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
Childhood atopic dermatitis	Fetal umbilical cord blood	Shanghai, China Prospective birth cohort with 2 years of follow-up	PFOS: median, 2.5 ng/mL; *n* = 687 PFOA: median, 7.0 ng/mL; *n* = 687	No association with atopic dermatitis ↑ Atopic dermatitis in girls	Infant sex, parity (nulliparous and parous), birth weight, gestational age at delivery, mode of delivery, maternal pre-pregnancy BMI, maternal age, maternal education, maternal ethnicity, paternal age, paternal education, parental history of allergic disorders, paternal smoking during pregnancy, family income, and breastfeeding during the first 6 months; as only a few women consumed alcohol (1.98%) or smoked (0.5%), maternal alcohol consumption and smoking were not included in the fully adjusted model	Diagnosis of atopic dermatitis confirmed by two dermatologists. Adjustment for a large number of potential confounders. Loss to follow-up resulted in a substantial proportion (23%) of participants being excluded from the analysis. Limited sample size for some types of exposure.	Chen et al. (2018a)

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
Lung function, asthma, eczema	Maternal plasma	Spain. Prospective birth cohort with 7 years of follow-up	PFOS: median, 5.8 ng/mL; *n* = 1243	Trends toward associations with lower risks of asthma and eczema; no association with reduced lung function	Maternal age at delivery, parity, previous breastfeeding, pre-pregnancy BMI, region of residence, and country of birth	Large sample size. Lack of correction for multiple comparisons and small effect estimates for the relationships of PFAS with lung function necessitate careful interpretation. The potential for chance findings because of multiple comparisons was acknowledged. Lack of information about *P*-value criteria and statistical significance, reliance on maternal PFAS concentrations as proxies for fetal exposure, and self-reported questionnaires for outcome assessment introduced potential for recall and misclassification bias.	Manzano-Salgado et al. (2019)
			PFOA: median, 2.3 ng/mL; *n* = 1243	Trend toward a positive association with reduced lung function (FVC and FEV1) at age 4 years, no association at age 7 years; no association with asthma or eczema			
Childhood atopic eczema, food allergy, allergic rhinitis, and asthma	Maternal plasma	Norway MoBA study, prospective population-based pregnancy sub-cohort with 7 years of follow-up	PFOS: median, 12.9 ng/mL; *n* = 1943	↓ Common cold, ear infection, urinary tract infection ↑ Bronchitis/ pneumonia No association with atopic eczema, food allergy, allergic rhinitis, asthma, throat infection, pseudocroup, or diarrhoea/gastric flu	Maternal age, maternal BMI, maternal education, parity, and smoking during the pregnancy; correction for multiple testing using false discovery rate	7 years of follow-up, a relatively large number of participants, loss to follow-up and reliance on questionnaire-based outcomes. All samples had PFOS and PFOA concentrations above the LOQ.	Impinen et al. (2019)

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
Childhood atopic eczema, food allergy, allergic rhinitis, and asthma (cont.)			PFOA: median, 2.5 ng/mL; n = 1943	↓ Common cold, urinary tract infection ↑ Bronchitis/ pneumonia, pseudocroup No association with atopic eczema, food allergy, allergic rhinitis, asthma, throat infection, ear infection, or diarrhoea/gastric flu			Impinen et al. (2019) (cont.)
Reduced lung function at birth, asthma, allergic rhinitis, atopic dermatitis	Fetal umbilical cord blood	Norway ECA prospective birth cohort with 2–10 years of follow-up	PFOS: median, 5.2 ng/mL; n = 641 PFOA: median, 1.6 ng/mL PFOA: median, 1.6 ng/mL; n = 641	No association with reduced lung function at birth, asthma, allergic rhinitis, or atopic dermatitis No association with reduced lung function at birth, asthma, allergic rhinitis, or atopic dermatitis	Confounders examined: sex; birth weight; birth month; breastfeeding at 6 and 12 months; maternal smoking during pregnancy; household smoking at birth, at preschool age and at school age; parental asthma; atopic dermatitis and allergic rhinitis; parental education; and household income Regression models were adjusted for sex only and correction for multiple testing was performed using Bonferroni	High follow-up rate after 10 years and representativeness of the study sample. Potential recall bias, especially in questionnaires and interviews, lack of data on important confounding factors, such as parity and previous breastfeeding.	Impinen et al. (2018)

Table 4.18 (continued)

End-point	Biosample type	Location, setting, study design	Exposure level No. of study participants	Response [a]	Covariates adjusted for	Comments	Reference
Lung function	Umbilical cord blood plasma and serum from children	Taiwan, China Prospective birth cohort with 7 years of follow-up	Cord blood PFOS: median, 6.4 ng/mL Children's serum PFOS: median, 5.9 ng/mL; *n* = 165 Cord blood PFOA: median, 2.4 ng/mL Children's serum PFOA: median, 2.7 ng/mL; *n* = 165	Positive association with reduced lung function in children Positive association with reduced lung function in children	Sex, child height, child BMI, birth weight, maternal education, eating habits, prenatal smoking history, history of environmental tobacco smoke exposure, maternal cotinine concentration, gestational age, family income, use of pesticide or incense at home	Sample size was small, lack of multiple testing correction, differences in demographic characteristics of the participants at follow-up compared with the original cohort might have introduced selection bias, reliance on cord blood and blood for the assessment of associations with PFAS exposure, which may not have fully captured the changing concentrations of these compounds over time, particularly during early life and childhood.	Kung et al. (2021)

AFFF, aqueous film-forming foam; BMI, body mass index; COX, cyclooxygenase; CRP, C-reactive protein; ECA, Environment and Childhood Asthma study; eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; FEV1, forced expiratory volume in 1 second: FVC, forced vital capacity; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; HbA1c, glycated haemoglobin; HELIX, Human Early Life Exposome; HO-1, haem oxygenase 1; IFN-γ, interferon-gamma; IL, interleukin; LOQ, limit of quantification; LRTIs, lower respiratory tract infections; MAMAS, Maternal Adiposity, Metabolism, and Stress Study; MCP-1, monocyte chemoattractant protein-1; MoBA, Norwegian Mother, Father and Child Cohort Study; NHANES, National Health and Nutrition Examination Survey; PFAS, perfluoroalkyl and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; SHGB, sex hormone-binding globulin; SMBCS, Sheyang Mini Birth Cohort Study; TNF, tumour necrosis factor; USA, United States of America; wk, week(s).

[a] ↑, increase; ↓, decrease.

human exposure to PFOA and PFOS and end-points relevant to chronic inflammation. Of these, 11 studies examined the relations between PFOS and/or PFOA concentration and circulating markers of inflammation. Furthermore, seven of the studies explored the associations between the concentrations of PFOS and/or PFOA in maternal or umbilical cord blood and health outcomes (atopic dermatitis, eczema, rhinitis, asthma, and lung function) in children.

Measurement of inflammatory end-points

Altogether, 11 studies addressed associations of PFOA and PFOS concentrations with inflammatory markers, including CRP, MCP-1, IL-8, IL-6, neutrophil count, lymphocyte count, IL-10, TNF-α, leptin, and adiponectin. Of these, nine assessed associations either in cross-sectional (n = 7) or prospective (n = 2) settings, involving both children and adults from the general population (study size range, n = 103–6652). In these settings, the primary focus was on examining associations with inflammatory proteins, considering background exposure as the predominant source. Of the 11 studies, two cross-sectional studies examined potential associations in individuals exposed to elevated levels of PFAS: Bassler et al. (2019) focused on participants with elevated exposure due to PFOA-contaminated drinking-water in the C8 Health Study; and Barton et al. (2022) examined a population exposed to drinking-water contaminated with aqueous film-forming foam (AFFF).

Studies of exposure in the general population

The nine studies have been organized in descending order of informativeness, taking into account factors such as study size, prospective versus cross-sectional design, and the specificity of the assessed markers of inflammation for chronic inflammation. Prospective cohort studies are more informative because they enable longitudinal assessments.

In a study of the effects of prenatal (during pregnancy) and childhood (in children aged 8 years) exposure to PFOA and PFOS on cardiometabolic factors and inflammatory status in children, the authors assessed 1101 mother–child pairs from the Human Early Life Exposome project (the children had an average age of 8 years) (Papadopoulou et al., 2021). The covariates included maternal age and education, pre-pregnancy BMI, parity, cohort, child ethnicity, age, and sex. The study reported that prenatal PFOA concentration was positively correlated with that of the pro-inflammatory cytokine IL-1β. Postnatal PFOA concentration was negatively correlated with those of IL-8 and HGF. Prenatal PFOS concentration was negatively associated with those of MIG/CXCL9 and MIP-1β/CCL4. No association was observed between PFOA and/or PFOS concentrations and several end-points, including adiponectin, BAFF, C-peptide, CRP, EGF, eotaxin, FGF-2, G-CSF, IFN-α, IFN-γ, IL-10, IL-12, IL-13, IL-15, IL-17, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IP10/CXCL10, leptin, MCP-1/CCL2, MIP-1α/CCL3, PAI-1, or TNF-α (Papadopoulou et al., 2021). [The Working Group noted that the strengths of this study included the assessment of both prenatal and postnatal exposure to PFOA and PFOS and multiple markers of relevance for chronic inflammation in a large sample of participants from six European cohorts.]

Mitro et al. (2020) conducted a study involving 450 pregnant women recruited between 1999 and 2002 in Massachusetts, USA, to investigate the potential links between PFOA and PFOS plasma concentrations and the markers CRP, IL-6, HbA1c, adiponectin, leptin, and sex hormone-binding globulin (SHBG) during pregnancy and in plasma samples collected 3 years postpartum. The covariates included age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, parity, and breastfeeding history. No significant associations between PFOA or PFOS exposure and CRP, IL-6, HbA1c, adiponectin,

leptin, or SHBG were reported (Mitro et al., 2020).

In the Atlanta African American Maternal–Child Cohort (n = 425), associations between serum concentrations of a PFAS mixture (including PFOA and PFOS) and serum concentrations of IFN-γ, IL-6, IL-10, TNF-α, and CRP at two different time points (8–14 weeks and 24–30 weeks of gestation) were examined. Exposure to PFOS was considered to be the main driver of the effect of the mixture, according to the four different statistical methods used for analysis. When examining associations of the PFOA and PFOS concentrations with various markers, distinctions were observed between the two. PFOS concentration displayed positive associations with IFN-γ and TNF-α during both 8–14 weeks and 24–30 weeks of gestation and were positively associated with IL-6 and CRP only during the 24–30-week period. In contrast, there were no significant associations between PFOA concentration and CRP, IL-6, IFN-γ, IL-10, and TNF-α during both gestation periods (Tan et al., 2023). [The Working Group noted that the findings from this longitudinal assessment indicate disparities in the associations of PFOA and PFOS concentrations with circulating markers of inflammation, suggesting differences between the two agents' induction of circulating markers of chronic inflammation.]

In a cross-sectional study of 965 elderly individuals (age, 70 years; 50% women) from Sweden, associations of PFOA and PFOS with 86 inflammatory protein markers, including multiple cytokines, such as IL-6 and TNF-α, measured using a proximity extension assay, were assessed (Salihović et al., 2020b). Examination of the associations of the PFOA and PFOS concentrations with CRP, measured using a different assay, was included as a sensitivity analysis. The concentration of PFOA was consistently inversely associated with circulating CRP after multiple adjustments (see Table 4.18) and correction for multiple testing (Salihović et al., 2020b). [The Working Group observed that the cross-sectional findings suggest that PFOA concentrations were mainly associated with decreased levels of circulating markers of chronic inflammation, including CRP.]

In a cross-sectional study involving 287 participants (age, 12–30 years) from Taiwan, China, no significant associations of PFOA or PFOS exposure with CRP, IL-6, IL-1β, adiponectin, or leptin were reported (Lin et al., 2011).

In a cross-sectional study of pregnant women with overweight or obesity from the San Francisco Bay area, USA, the associations of prenatal exposure to PFOA and PFOS with levels of inflammatory markers, such as IL-6, IL-10 and TNF-α, in pregnant and postpartum women were assessed (Zota et al., 2018). The study reported a positive association between PFOS and IL-6 ($P < 0.05$). Additionally, the PFOA concentration showed a positive association with the pro-inflammatory cytokine IL-6 ($P < 0.05$). These findings suggest that exposure to PFOA and PFOS is cross-sectionally associated with elevated levels of IL-6 in pregnant and postpartum women.

Using cross-sectional data ($n = 6652$) from the NHANES 2005–2012, PFOA and PFOS concentrations were found to be significantly associated with decreased neutrophil count and increased lymphocyte count in linear models. However, when stratified by quintiles, the associations of PFOA concentration with the neutrophil and lymphocyte counts diminished. No significant associations between PFOA or PFOS and CRP were found (Omoike et al., 2021). The study could not account for potential bias introduced by participants taking anti-inflammatory or immune-modulatory medication. [The Working Group noted that the percentage changes in markers of chronic inflammation were of small magnitude and that the finding of decreased neutrophil count versus increased leukocyte count makes the interpretation of the findings challenging.]

In a nested case–control study comprising 144 women experiencing spontaneous preterm birth and 375 control participants (total, $n = 519$), the associations of serum PFOA and PFOS concentrations (both as continuous variables and after division into quartiles) with inflammatory markers, specifically MCP-1 and IL-8, were examined during weeks 4–22 of gestation. The study reported that in the full cohort, the PFOS concentration was positively associated with that of MCP-1, whereas the concentration of PFOA was inversely associated with that of IL-8, after adjustment for major confounders, including maternal age at delivery, parity, pre-pregnancy BMI, occupation, spontaneous abortion history, medication use, folic acid use, fasting status, pre-pregnancy passive smoking status, the gestational week of sampling (≤ 12 weeks or 12–27 weeks), and the sex of the child (Liu et al., 2020a). [The Working Group observed that the assessed markers may lack specificity for chronic inflammation.]

In 1111 mother–infant pairs from the Sheyang Mini Birth Cohort Study (SMBCS), associations of prenatal exposure to PFOA and PFOS with the leptin and adiponectin concentrations in umbilical cord serum were assessed, and sex-stratification was performed in each model to assess potential sexually dimorphic effects. The PFOA concentration was positively associated with leptin concentration in women only ($P < 0.05$) and positively associated with adiponectin concentration ($P < 0.05$) both in the entire sample and in men. The PFOS concentration was found to be positively associated with that of adiponectin ($P < 0.05$), both in the entire sample and in men, and positively associated with the leptin concentration in women (Ding et al., 2023). [The Working Group noted that the evaluated markers are not specific for chronic inflammation and that use of the findings of this study to draw conclusions regarding chronic inflammation may require additional context and complementary markers to be assessed, in order to provide a comprehensive assessment of the inflammatory state.]

Studies in communities with elevated exposure

Two studies investigated associations between elevated exposure to PFOA and PFOS in contaminated drinking-water and inflammation end-points (see Table 4.18).

Barton et al. (2022) evaluated associations of PFAS exposure via AFFF-contaminated drinking-water with IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, granulocyte–macrophage colony-stimulating factor (GM-CSF), and TNF-α in the PFAS Assessment of Water and Resident Exposure (PFAS-AWARE) study ($n = 212$). All the models were adjusted for age, race/ethnicity, smoking history, sex, and BMI. The authors reported an inverse association between serum PFOA concentration and the probability of detecting IL-1β. No significant associations were observed with IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, GM-CSF, or TNF-α. The authors recognized certain constraints, such as the infrequent detection of specific cytokines, including IL-1β, and the possibility that cytokine levels could have been affected by recent food consumption, because of non-fasted samples (Barton et al., 2022).

Bassler et al. (2019) included 200 adult C8 Health Study participants with an elevated exposure to PFOA (PFOA mean concentration, 94.6 ng/mL). Associations of PFOA and PFOS concentrations with the inflammatory markers TNF-α, IL-6, IL-8, IFN-γ, adiponectin, and leptin were assessed. The PFOA concentration was positively associated with that of IFN-γ and inversely associated with that of TNF-α. The PFOS concentration was positively associated with that of adiponectin and inversely associated with those of TNF-α and IL-8. No significant associations were observed between the PFOS concentration and those of IL-6, IFN-γ, and leptin (Bassler et al., 2019). [The Working Group

noted that in the context of elevated community PFOA exposure, both elevated and decreased concentrations of chronic inflammatory markers were reported.]

Measurement of end-points of disease outcome

Studies of exposure in the general population

In a prospective cohort study including 2689 children in Japan in which maternal enrolment occurred during the first trimester of pregnancy, with follow-up questionnaires administered when the children reached age 7 years, higher maternal PFOS concentrations were associated with a decreased risk of eczema. No significant association was reported with rhino-conjunctivitis. Similarly, PFOA concentration was also inversely associated with eczema, and no significant association was found for rhino-conjunctivitis (Ait Bamai et al., 2020).

The Environment and Childhood Asthma (ECA) Study was a prospective birth cohort including 378 participants with PFOA and PFOS measurements made at age 10 years and follow-up data collected at age 10 years (cross-sectional data) and age 16 years (longitudinal data). The cross-sectional data collected at age 10 years showed associations of the PFOA and PFOS concentrations with asthma. The longitudinal data showed no associations of the PFOA or PFOS concentration with atopic dermatitis. No associations were observed between PFOA or PFOS and lung function. Notably, PFOA concentration was positively associated with rhinitis in all participants at age 16 years. For airway infections, the longitudinal data showed that PFOA and PFOS concentrations were positively associated with lower respiratory tract infections (LRTIs) between ages 10 and 16 years (Kvalem et al., 2020). [The Working Group noted that the study's longitudinal approach minimized selection bias and the chance of misclassifying health outcomes. The asthma diagnosis was based on medication use and clinical diagnosis, further enhancing its accuracy and reducing misclassifications.]

The association of prenatal exposure to PFOA and PFOS with childhood atopic dermatitis was investigated in a prospective birth cohort study including 687 children who had completed a 2-year follow-up. Only in female children (n = 328), a log-unit increase in PFOA concentration was found to be associated with an increase of 2.1-fold in atopic dermatitis risk (adjusted odds ratio, 2.07; 95% CI, 1.13–3.80) after adjusting for potential confounders (Chen et al., 2018a).

Four studies reported no significant associations of PFOS and/or PFOA concentrations with atopic dermatitis/eczema, food allergy, rhinitis, asthma, and/or lung function (Impinen et al., 2018, 2019; Manzano-Salgado et al., 2019; Kung et al., 2021). More specifically, in a prospective cohort study conducted in Spain involving 1243 mother–child pairs, the associations of the prenatal concentrations of PFOA and PFOS with asthma, eczema, and lung function were investigated (see Section 1.6.2 for details). Although the findings were not significant, the study showed that the PFOA and PFOS concentrations were associated with asthma and eczema during childhood (Manzano-Salgado et al., 2019). [The Working Group noted that the interpretation of the findings was hampered since the study presented trends in associations rather than statistically significant comparisons, requiring cautious interpretation.]

In a prospective cohort study conducted in Norway, the associations of prenatal exposure to PFOA and PFOS with the development of asthma, allergies, and common infectious diseases in early childhood was assessed. The study involved a prospective cohort of 1943 mother–child pairs and followed participants up to age 7 years. Health outcomes were assessed at age 7 years, including asthma, allergies, and common infections. Questionnaires were completed at ages 3 and 7 years. PFOS was inversely associated with

the common cold, ear infection, and urinary tract infection between the age of 0–3 years (n = 1207). In addition, PFOS was positively associated with bronchitis/pneumonia, whereas no significant associations were observed with atopic eczema, food allergy, allergic rhinitis, asthma, throat infections, pseudocroup, or diarrhoea/gastric flu at ages 0–3 years and 6–7 years. The PFOA concentration was inversely associated with the common cold and urinary tract infection at age 0–3 years. PFOA was also positively associated with bronchitis/pneumonia and pseudocroup. No associations with atopic eczema, food allergy, allergic rhinitis, asthma, throat infections, ear infections, or diarrhoea/gastric flu were identified at ages 0–3 years and 6–7 years (Impinen et al., 2019).

In a study of the 0–2- and 2–10-year intervals obtained from the 2- and 10-year follow-up investigations of a prospective general population birth cohort, the ECA study, performed in Norway, associations of PFOA and PFOS with physician-diagnosed asthma, wheeze, obstructive airway disease severity, reduced lung function, atopic dermatitis, rhinitis, rhino-conjunctivitis, allergic sensitization, common cold episodes, and LRTIs were assessed. Adjustment for maternal age, maternal BMI, maternal education, parity, and smoking during pregnancy; and correction for multiple testing using the FDR were performed. No associations between PFOA or PFOS and lung function at birth, asthma, allergic rhinitis, or atopic dermatitis were observed (Impinen et al., 2018).

Kung et al. (2021) conducted a study examining the potential link between prenatal or childhood exposure to PFOA and PFOS and the development of lung function during childhood. This investigation included 165 children from Taiwan, China. In the entire cohort, the PFOA and PFOS levels in cord blood were inversely associated with lung function. In particular, intrauterine PFOS exposure was inversely correlated with mean FEV1 (forced expiratory volume in the first second), FVC (forced vital capacity), and PEF (peak expiratory flow) in childhood, especially in subgroups with lower birth weight and allergic rhinitis (Kung et al., 2021).

(ii) Human cells in vitro

See Table 4.19.

Human primary cells

Overall, five studies were available: four studies evaluated the effects of PFOA and PFOS exposure on cytokines (IL-6, IL-8, TNF-α, IL-4, IL-10, and IFN-γ) in peripheral blood leukocytes (Brieger et al., 2011; Corsini et al., 2011, 2012; Maddalon et al., 2023a) and the fifth focused on the IL-6 and IL-1β responses to PFOS exposure in human primary decidual stromal cells during early pregnancy (Yang et al., 2016).

Brieger et al. (2011) reported a significant association between PFOS exposure and decreased TNF-α release by human peripheral blood mononuclear cells in response to lipopolysaccharide (LPS)-stimulation at both the 4-hour and 24-hour time points. Notably, there was no significant correlation of IL-6 with either PFOA or TNF-α.

Corsini et al. (2011) measured the release of pro-inflammatory cytokines (IL-6, IL-8, TNF-α, IL-4, IL-10, and IFN-γ) in LPS-stimulated human peripheral blood leukocytes and in the human promyelocytic cell line THP-1. The study showed that both PFOA and PFOS reduced the release of TNF-α in peripheral blood leukocytes and in THP-1 cells, but only PFOS reduced the release of IL-6 in peripheral blood leukocytes. PFOA reduced the release of IL-8 at 100 µg/mL, and PFOS reduced IL-8 release at 1–100 µg/mL in a dose-dependent manner in THP-1 cells. Both PFOA and PFOS reduced the release of T-cell derived cytokines (IL-4 and IL-10), whereas IFN-γ release was reduced only by PFOS in peripheral blood leukocytes (Corsini et al., 2011).

Table 4.19 End-points relevant to chronic inflammation in human cells in vitro exposed to PFOA or PFOS

End-point	Assay	Tissue, cell line	Result[a]	Concentration (LEC or HIC) or range	Comments	Reference
Primary cells						
IL-6 and TNF-α	ELISA	PBMCs	↓ TNF-α after 4 and 24 h No change in IL-6 No change in IL-6 or TNF-α	PFOS, 100 μg/mL (LEC) PFOS, 1–100 μg/mL PFOA, 1–100 μg/mL		Brieger et al. (2011)
IL-6, IL-8, TNF-α, IL-4, IL-10, IFN-γ	ELISA	Peripheral blood leukocytes	↓ IL-6, TNF-α, IL-4, IL-10, IFN-γ No change in IL-8 ↓ TNF-α (LEC, 1 μg/mL), IL-4, IL-10 (LEC, 10 μg/mL) No change in IL-6, IFN-γ, or IL-8	PFOS, 0.1 μg/mL (LEC) PFOS, 0.1–10 μg/mL PFOA, 10 μg/mL (LEC) PFOA, 0.1–10 μg/mL	Relevance to human exposure and concentration effects	Corsini et al. (2011)
IL-6, TNF-α, IL-10, IFN-γ	ELISA	Peripheral blood leukocytes	↓ IL-6, IL-10, IFN-γ after PFOS treatment in blood leukocytes obtained from a female donor ↓ TNF-α after PFOS treatment of female and male donor or after PFOA in female donor	PFOS, 10 μg/mL (LEC) PFOS, 0.1 μg/mL (LEC) PFOA, 1 μg/mL (LEC)	Sex of the donor considered	Corsini et al. (2012)
IL-8 and TNF-α	ELISA	PBMCs from male and female donor	↓ IL-8 and TNF-α (male donor only)	PFOS, 0.2 μM	Sex of the donor considered	Maddalon et al. (2023a)
IL-6 and IL-1β	qPCR	Primary human decidual stromal cells of early pregnancy	No change	PFOS, 0.1 μM		Yang et al. (2016)
Cell lines						
IL-8, TNF-α	ELISA	Promyelocytic cell line THP-1	↓ IL-8, TNF-α ↓ TNF-α ↓ IL-8	PFOS, 1 μg/mL (LEC) PFOA, 10 μg/mL (LEC) PFOA, 100 μg/mL (LEC)	Relevance to human exposure and concentration effects	Corsini et al. (2011)
TNF-α	ELISA	Promyelocytic cell line THP-1	↓ TNF-α	PFOS, 0.1 μg/mL PFOA, 10 μg/mL (LECs)		Corsini et al. (2012)

Table 4.19 (continued)

End-point	Assay	Tissue, cell line	Result[a]	Concentration (LEC or HIC) or range	Comments	Reference
IL-4, GATA-3, IFN-γ (Th1 and Th2 responses)	ELISA, western blot, qPCR	Human Jurkat cells	↓ IFN-γ, ↑ IL-4 ↓ IFN-γ, ↑ IL-4	PFOS, 1 μM (LEC) PFOA, 1 μM (LEC)		Yang et al. (2021)
IL-6, IL-8	qPCR, flow cytometry	HCEpiC, HCEC, and RPE cells	↑ IL-6, IL-8	PFOA, 400 ppm for 16 h	Only PFOA was detected in all the indoor carpet samples, and had the highest (37 458 ng/g) concentrations of all the perfluorinated compounds	Tien et al. (2020)
IL-6, TNF-α, and IL-10; mRNA expression of *IL6*, *TNFA*, and *IL10*	ELISA and mRNA by PCR	Human placental trophoblast (HTR-8/Svneo) cells	↓ IL-6, ↑ TNF-α ↑ IL-10 ↓ mRNA expression of *IL10* at low concentrations ↑ *IL6* mRNA expression at high concentrations	PFOS, 0.01 mg/L (LEC) PFOS, 0.1 mg/L (LEC) PFOS, 0.01 mg/L (LEC) PFOS, 0.1 mg/L (LEC)		Zhu et al. (2020)
IL-6	ELISA	CCD-18Co myofibroblasts	↓ IL-1β-induced IL-6 production ↓ IL-1β-induced IL-6 production	PFOS, 0.6 μM (300 ng/mL) every 24 h to 96 h PFOA, 0.36 μM (0.15 ng/mL) every 24 h to 96 h	PFOS showed greater cytotoxicity than PFOA in CCD-18Co myofibroblasts	Giménez-Bastida et al. (2015)
CXCL-8, CXCL-10, IL-1α, IL-1β, IL-6	ELISA	Bronchial epithelial cells (HBEC3-TK)	↑ IL-1α, ↑ IL-1β at a PFOS concentration of 10 μM with, or ≥ 30 μM without, immune stimulation via TLR3 No change in IL-6, ↓ CXCL-10 and CXCL-8 (with TLR3 stimulation)	PFOS concentrations ranged from 0.13 to 60 μM and exposure lasted for 48 h		Sørli et al. (2020)

Table 4.19 (continued)

End-point	Assay	Tissue, cell line	Result[a]	Concentration (LEC or HIC) or range	Comments	Reference
			No significant changes in cytokine release were observed at non-cytotoxic PFOA concentrations in unstimulated cells; ↑ IL-1β in TLR3-stimulated cells	PFOA concentrations ranged from 0.13 to 10 μM and exposure lasted for 48 h		Sørli et al. (2020) (cont.)
mRNA expression of *IL6*, *IL8*, *TNFA*, *NFKB1*, *MAPK8*	qPCR	Human pancreatic cell line (PANC-1)	No changes in *IL8*, *TNFA*, *NFKB1*, *MAPK8*. ↓ IL-6 at 100 μM	PFOA, 10, 50, or 100 μM	LD_{50} was 195.74 μM	Abudayyak et al. (2021a)
IL-6, IL-8	ELISA	Human hepatocarcinoma cell line (HepG2)	↑ IL-6, ↓ IL-8 (at both 25 and 50 μM)	PFOA, 10, 25, or 50 μM		Abudayyak et al. (2021b)
Tachykinin	LC-MS/MS	Astroglial SVG p12 cells	↑ tachykinin at 80 μM	PFOA, 10 or 80 μM for 48 h	No positive control	Osemwegie et al. (2021)
Mast cell-mediated inflammatory response, eicosanoids		Human mast cells (HMC-1)	↑ eicosanoids (PFOS)	PFOS or PFOA, 30 μM		Park et al. (2021)
IL6, *TNFA*	qPCR	BV2 microglial cells	↑ *IL6* at all time points ↑ *TNFA* after 1 and 3 h ↑ *IL6* at 1 or 10 μM, after 6 h ↑ *TNFA* at 10 μM after 6 h	PFOS, 1 μM for 1, 3, or 6 h PFOS: 0.1, 1.0, or 10 μM for 6 h		Zhu et al. (2015)
IL-6, and TNF-α	ELISA		↑ IL-6 and TNF-α at all time points ↑ IL-6 ↑ TNF-α	PFOS: 1 μM for 6, 12, or 24 h 0.1 μM (LEC) for 24 h 1 μM (LEC) for 24 h		

Table 4.19 (continued)

End-point	Assay	Tissue, cell line	Result[a]	Concentration (LEC or HIC) or range	Comments	Reference
IL6, *IL1B*, and *TNFA*	qPCR	Human placental cell lines (HTR-8/SVneo and JEG-3 cells)	↑ *IL6*, *IL1B*, and *TNFA*	PFOS, 0–100 μM 50 μM (LEC) in HTR-8/SVneo 20 μM (LEC) in JEG-3 cells	Primarily focused on the immediate effects of PFOS exposure; the study did not include an extensive exploration of concentration–response relationships for the observed outcomes	Li et al. (2021a)
IL-8, TNF-α	ELISA	Human monocytic cell line THP-1	↓ IL-8 at 20 μM ↓TNF-α at all time points	PFOS, 0.2, 2, or 20 μM 0.2 μM (LEC)	LPS 10 ng/mL was added for 24 h	Masi et al. (2022)
TNFA, *IL1B*, *IL6*, *IL8*, *COX2*	RT-PCR and qPCR	Human mast cells (HMC-1)	↑ *TNFA* and *IL8* ↑ *IL1B* and *IL6* ↑ *COX2* ↑ histamine ≥ 25 μM	PFOA, 50–400 μM for 12 h 200 μM (LEC) 50 μM (LEC) 100 μM (LEC) PFOA, 25–100 μM for 24 h 25 μM (LEC)		Singh et al. (2012)

CXCL, CXC motif chemokine ligand; ELISA, enzyme-linked immunosorbent assay; h, hour(s); HCEC, human corneal endothelial cells; HCEpiC, human corneal epithelial cells; HIC, highest ineffective concentration; HMC, human mast cells; IFN, interferon; IL, interleukin; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LD_{50}, median lethal dose; LEC, lowest effective concentration; LPS, lipopolysaccharide; mRNA, messenger RNA; PCR, polymerase chain reaction; PBMC, peripheral blood mononuclear cell; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; ppm, parts per million; qPCR, quantitative polymerase chain reaction; RPE, retinal pigment epithelial; RT, reverse transcription; Th, T-helper; TLR, toll-like receptor; TNF, tumour necrosis factor.

[a] ↑, increase; ↓, decrease.

In another study, similar results were obtained using human leukocytes and the human promyelocytic cell line THP-1, and the authors further showed that PFOA and PFOS affect nuclear factor kappa B (NF-κB) activation, thereby suppressing cytokine secretion by immune cells (Corsini et al., 2012).

More recently, Maddalon et al. (2023a) investigated male and female peripheral blood mononuclear cells that were treated with PFOS at 0.2 μM. The authors observed that PFOS reduced both IL-8 and TNF-α release for male donors only, and in case of TNF-α, a statistically significant difference between male and female donors was observed. [The Working Group noted that the results of these studies suggested that PFOA and PFOS exposure was associated with an attenuated inflammatory response in peripheral blood leukocytes.]

Yang et al. (2016) investigated the impact of PFOS exposure on decidual stromal cell function. PFOS alone had no effects on the pro-inflammatory cytokines IL-6 or IL-1β (Yang et al., 2016).

Human cell lines

One study conducted in vitro experiments using Jurkat cells to investigate the mechanisms of PFOA- and PFOS-mediated T-helper (Th) Th1 and Th2 responses. The results showed that both PFOA and PFOS enhanced the Th2 response (IL-4) in Jurkat cells at 1.0–50 μM via STAT6 activation, but inhibited Th1-related gene expression (IFN-γ). The effects of PFOS on IL-4 and IFN-γ were reversed in Jurkat cells that did not express STAT6, suggesting that PFOA and PFOS exposure aggravate inflammation (Yang et al., 2021).

Tien et al. (2020) investigated whether PFOA in indoor particulate matter induced inflammation in corneal and retinal cells. Exposure to PFOA was found to promote the secretion of IL-6 and IL-8. The authors suggested that exposure to PFOA in particulate matter may increase the risk of age-related macular degeneration by inducing oxidative stress and inflammation in the retina. Only PFOA was detected in all the indoor dust samples and it was present at the highest concentrations, of the substances investigated (Tien et al., 2020). [The Working Group noted that the observed response might be attributed to both dust particles and the presence of PFOA in the dust.]

Zhu et al. (2020) investigated the effects of PFOS on the secretion of IL-6, TNF-α, and IL-10, as well as the mRNA expression of *IL6*, *TNFA*, and *IL10*, in human placental trophoblast (HTR-8/Svneo) cells. The cells were exposed to PFOS at 0, 0.01, 0.1, or 1.0 mg/L for 24 hours, and the survival rates, secretion levels, and mRNA expression were measured. PFOS exposure reduced the survival rate of the cells at higher concentrations and induced changes in the secretion of inflammatory cytokines. Specifically, PFOS reduced IL-6 secretion at lower concentrations but increased TNF-α secretion and *IL6* mRNA expression at higher concentrations. In addition, PFOS increased IL-10 secretion at higher concentrations but reduced *IL10* mRNA expression at lower concentrations (Zhu et al., 2020).

Giménez-Bastida et al. (2015) selected the CCD-18Co myofibroblast cell model to investigate the ability of PFOA and PFOS to modulate inflammatory mechanisms. IL-1β, an essential pro-inflammatory cytokine, was added to the cells to induce an inflammatory response. The study showed that PFOA and PFOS individually possess the ability to impair cell viability, induce cell proliferation, and reduce cell inflammation by reducing IL-1β-induced IL-6 production by subepithelial myofibroblasts of the colonic CCD-18Co line at non-cytotoxic concentrations (Giménez-Bastida et al., 2015) (see also Section 4.2.10).

Another study assessed the effects of PFOA and PFOS on the respiratory system using bronchial epithelial cells (HBEC3-TK) (Sørli et al., 2020). PFOA and PFOS inhibited lung

surfactant function at 0.5 mM in an acellular test and induced a pro-inflammatory response at micromolar concentrations. PFOA and PFOS were not associated with reduced cell viability at 10 µM. PFOS increased the release of the pro-inflammatory cytokine IL-1α, and also increased that of IL-1β at ≥ 30 µM without immunostimulation by the toll-like receptor 3 (TLR3) and at 10 µM with TLR3 stimulation. PFOS reduced the release of the chemokine CXCL8 at 3.3 µM and reduced that of CXCL10 at 10 µM when cells were immunostimulated. In contrast, PFOA at 10 µM increased only the release of IL-1β in HBEC3-TK that were immunostimulated by TLR3. The authors acknowledged that the concentrations of PFOA and PFOS tested in the assay (0.5 mM) were considerably higher than what would be deposited in the alveoli of a person inhaling contaminated air, which may affect the study's relevance to human exposure (Sørli et al., 2020).

The role of inflammation in PFOA-induced pancreatic toxicity was investigated by measuring the mRNA expression levels of *IL6*, *IL8*, *TNFA*, *NFKB1*, and *MAPK8* in a human pancreatic cell line (PANC-1). PFOA was reported to induce dose-dependent cytotoxicity (IC_{50}, 195.74 µM), with apoptosis being the main cell-death pathway induced. PFOA did not significantly increase the mRNA expression of the analysed immune response-related biomarkers (Abudayyak et al., 2021a).

Abudayyak et al. (2021b) also investigated the effects of PFOA on human hepatocarcinoma (HepG2) cells and found that PFOA significantly increased IL-6 levels (≤ 1.8-fold; $P \leq 0.05$) but reduced IL-8 levels at 25 µmol/L (40% decrease) and 50 µmol/L (35% decrease) (Abudayyak et al., 2021b).

Osemwegie et al. (2021) investigated the effects of the in vitro exposure to PFOA of the astroglial SVG p12 cell line. The study measured the gene expression of the inflammatory marker tachykinin, which was increased by 80 µM PFOA treatment for 48 hours (Osemwegie et al., 2021).

The effects of PFOA and PFOS on human mast cells have also been examined. The results showed that PFOS had the greatest impact, increasing degranulation and the production of inflammatory eicosanoids in mast cells (Park et al., 2021).

PFOS had effects on BV2 microglia, and its potential contribution to inflammation in the central nervous system was assessed (Zhu et al., 2015). The results showed that PFOS increased BV2 cell activation after 12 hours of treatment at 0.1–10 µM. Treatment with PFOS at 1 µM for 1–6 hours significantly increased *IL6* mRNA expression and increased *TNFA* mRNA expression at 1 µM after treatments of 1 and 3 hours, but not longer. In addition, cells treated with a higher concentration of PFOS (10 µM) increased the expression of both *IL6* and *TNFA* after 6 hours of treatment (Zhu et al., 2015).

A study by Li and colleagues aimed to examine the potential impact of PFOS on the development of the human placental cell lines HTR-8/SVneo and JEG-3 cells, mediated through the PPARγ pathway. The mRNA expression of *IL6*, *IL1B*, and *TNFA* were significantly increased by PFOS exposure in HTR-8/SVneo cells at 50 and 100 µM. In JEG-3 cells, PFOS increased the mRNA expression of *IL6* when present at 10 µM to 30 µM, and of *IL1B* and *TNFA* at 20 and 30 µM (Li et al., 2021a).

Masi et al. (2022) investigated the effect of exposure to PFOS on the expression of receptor for activated C kinase 1 (RACK1) receptor and immune cell activation. The results showed that PFOS exposure downregulated RACK1 expression when treated with low doses (0.2–20 µM) for 18 or 24 hours, and reduced LPS-induced IL-8 and TNF-α production at 20 µM and 0.2 µM, respectively.

In another study, the role of PFOA in human mast cell (HMC-1)-mediated allergic inflammation and the underlying mechanisms was investigated (Singh et al., 2012). The HMC-1 cells were treated with PFOA (25–400 µM) for 12 or

24 hours. PFOA exposure was found to increase histamine release after 24 hours at concentrations of ≥ 25 μM and to increase the gene expression of *TNFA*, *IL1B*, *IL6*, *IL8*, and *COX2* in the mast cells after 12 hours (Singh et al., 2012).

(b) Experimental systems

(i) Non-human mammalian systems in vivo

Pro-inflammatory effects

See Table 4.20.

Quist et al. (2015) examined adverse hepatic changes at both the microscopic and ultrastructural levels in CD-1 mice exposed prenatally to low doses of PFOA that were within the higher bound of the reference interval for human exposure. Pregnant CD-1 mice were exposed orally by gavage to PFOA at doses ranging from 0.01 to 1 mg/kg on GD1 to GD17, and histopathological changes in the livers of the offspring were observed on PND21 and PND91. Prenatal exposure to low doses of PFOA induced histopathological changes in the liver of offspring on PND21 and PND91, including chronic active periportal inflammation, which primarily involved lymphocytes and macrophages, with fewer plasma cells and occasional neutrophils. These effects were dose-dependent (Quist et al., 2015).

In a 2-year feeding study in rats exposed to PFOA (NTP, 2020; see also Section 3.1.2), the incidence of chronic active inflammation in the glandular stomach and of focal inflammation in the liver of male rats was increased in post-weaning exposure groups at 150 or 300 ppm at age 16 weeks, compared with controls. The study in male rats was stopped at the interim time point, and a second study was started at lower exposures (up to 80 ppm). Glandular stomach chronic inflammation in female rats was not increased at the 16-week time point, but it was at the 2-year time point (NTP, 2020).

Filgo et al. (2015) (see also Section 3.1.1) investigated the severity of chronic liver inflammation in three mouse strains – CD-1, 129/SV wildtype (WT), and 129/SV knockout (KO) – that were exposed to PFOA from GD1 to GD17. Chronic inflammation increased in severity only in CD-1 mice and, notably, severity was increased at the two highest doses, but incidence was not increased. Indeed, no increase in the incidence of chronic inflammation was observed in any of the three strains. [The Working Group noted that the study investigated only female mice, which are recognized as being less responsive to PFOA than are males. Among the three strains, the evidence of chronic inflammation in two 129/SV strains (WT and KO) was inconclusive; only CD-1 exhibited a positive but not significant response.]

In a study by Kamendulis et al. (2022), mice (age 8 weeks) were exposed to drinking-water containing PFOA at 5 ppm for 4 or 7 months (see Sections 3.1.2, 4.2.5). The chronic inflammation score was investigated at age 6 and 9 months, and was based on histopathological staining of sections of the pancreas. The study reported an increased inflammation score at 9 months compared with controls. [The Working Group noted that the evidence supporting a connection between PFOA and chronic pancreatic inflammation in the experimental model was inconclusive.]

Son et al. (2009) investigated the effect of PFOA exposure on immune organs (the spleen and thymus) of male ICR mice. The ICR mice were exposed to drinking-water containing PFOA at various doses for 21 days. The study showed that PFOA increased the expression of TNF-α, IL-1β, and IL-6 in the spleen, which also showed enlargement, with hyperplasia of the white pulp in the groups at 250 ppm. No changes were observed in the thymus (Son et al., 2009).

Guo et al. (2021b) also investigated the potential of PFOA to induce splenic atrophy in male mice. Mice were exposed to different

Table 4.20 End-points relevant to chronic inflammation effects in non-human mammalian systems in vivo exposed to PFOA or PFOS

End-point, assay	Species, strain, sex	Tissue	Result[a]	Dose (LED or HID) or range	Route, duration, dosing regimen	Comments	Reference
Pro-inflammatory effects							
Chronic active periportal inflammation, by histology	Pregnant mouse, CD-1, female	Livers of offspring	↑ in offspring at PND91 and PND21, dose-related	PFOA, 0.01, 0.1, 0.3, or 1 mg/kg	Oral gavage from GD1 to GD17, daily	PFOA ammonium salt	Quist et al. (2015)
Chronic inflammation (active or focal), by histology	Rat, Sprague-Dawley, both sexes	Glandular stomach	+ active inflammation in males after 16 weeks	PFOA, 0/150, 0/300 ppm (only postweaning exposure) in study 1	Feed, 16-week interim time point of 2-year feeding study (study 1)	PFOA	NTP (2020)
		Liver	+ focal inflammation in males after 2 years	PFOA, 0/40, 0/80, 300/80 ppm in study 2	Feed, 2-year study (study 2)		
		Forestomach	+ active inflammation in females after 2 years	PFOA, 0/1000 ppm (only postweaning) or 300/1000 ppm (both exposures) in study 1			
Chronic inflammation, by histology	Pregnant mouse, CD-1, 129/SV WT, and 129/SV KO, female	Livers of offspring	+ only in CD-1 mice at 1 and 5 mg/kg bw	PFOA, 0.01–5 mg/kg per day	Oral gavage daily from GD1 to GD17	PFOA ammonium salt; dose-related increases in severity scores in PFOA-exposed livers	Filgo et. al. (2015)
Chronic inflammation, by histology	Mouse, Pdx-1 (KC), both sexes	Pancreas	(↑) at 9-month time point	PFOA, 5 ppm	Drinking-water, from age 8 weeks until 6 or 9 months (time points of analysis)	Inconclusive evidence	Kamendulis et al. (2022)

Table 4.20 (continued)

End-point, assay	Species, strain, sex	Tissue	Result[a]	Dose (LED or HID) or range	Route, duration, dosing regimen	Comments	Reference
Tnf, *Il1b*, *Il6*, by RT-PCR	Mouse, ICR, male	Spleen Thymus	↑ *Tnf*, *Il6* at 250 ppm ↑ *Il1b* at 50 and 250 ppm No changes	PFOA, 0, 2, 10, 50, or 250 ppm	Oral (drinking-water) for 21 days	PFOA ammonium salt; spleen hyperplasia	Son et al. (2009)
TNF-α, IL-1β, IL-6, IRF5, IL12p70, macrophages, by ELISA	Mouse, BALB/c, male	Spleen and serum	↑ TNF-α, IL-1β, IL-6, and IRF5 at 2 and 10 mg/kg per day (spleen and serum) ↑ IL-1β, IL-6, and IL12p70 at ≥ 0.4 mg/kg per day (serum) ↑ TNF-α at ≥ 2 mg/kg per day (serum) ↑ IL12p70 at 10 mg/kg per day (serum) ↑ macrophage ratio at all doses with dose dependence	PFOA, 0, 0.4, 2, or 10 mg/kg per day	Oral (gavage) for 28 days, daily		Guo et al. (2021b)
IL-6, TNF-α, IFN-γ, IL-4, by ELISA	Mouse, C57BL/6, male	Liver	↓ TNF-α, IFN-γ, IL-4, IL-6	PFOA, 0.002% w/w, 3 ± 0.7 mg/kg per day	Oral (dietary exposure), 10 days		Qazi et al. (2013)
IL-5, IL-13, IL-4, IL-12, IL-2, IFN-γ, IL-17α, IL-1α, IL-6, and TNF-α, by Luminex	Mouse, $B6C3F_1$, female	Serum	↓ IL-5, IL-13, IL-12 at high dose ↓ IL-17α, IL-1α at both doses ↑ TNF-α at high dose No significant effects on IL-4, IL-2, IFN-γ, or IL-6	PFOA, 1.88 (low) or 7.5 (high) mg/kg per day	Oral (in drinking-water), for 4 weeks. 5 days prior the end of the exposure, one single intraperitoneal injection (300 mg/mouse in 0.5 mL) of keyhole limpet hemocyanin	Effects of PFOA alone not investigated	De Guise and Levin (2021)
IL-6, COX-2, CRP, by ELISA Inflammatory cells, by histology	Mouse, Kunming, male	Liver	↑ IL-6, COX-2, and CRP at 10 mg/kg per day ↓ IL-6, COX-2, CRP at 2.5 and 5 mg/kg per day ↑ infiltration of inflammatory cells (and hypertrophy), dose-dependent	PFOA, 2.5, 5, or 10 mg/kg per day	Oral for 14 consecutive days		Yang et al. (2014)

Table 4.20 (continued)

End-point, assay	Species, strain, sex	Tissue	Result[a]	Dose (LED or HID) or range	Route, duration, dosing regimen	Comments	Reference
Liver inflammation, steatosis, IL-1β, by histology, immunohistochemistry, ELISA	Mouse, C57BL/6J, male	Liver and serum	↑ steatosis after both diets ↑ liver inflammation in HFD-fed mice ↑ IL-1β (serum) after both diets	PFOS, 5 mg/kg per day	Oral for 4 weeks; HFD or chow diet ± PFOS		Qin et al. (2022b)
Tnf, *Il1b*, *Cox2*, *Il10*, by qPCR	Mouse, C57BL/6J, male	Colon, serum, cerebral cortex, hippocampus	↑ *Tnf* and *Cox2* at 3 mg/kg ↑ *Il1* at 1 and 3 mg/kg ↓ *Il10* at 1 and 3 mg/kg in colon ↓ *Il10* and ↑ *Tnf* in serum ↑ *Tnf* at 1 mg/kg and *Cox2* at 1 and 3 mg/kg in cortex and at 3 mg/kg in hippocampus ↓ *Il10* in hippocampus at 3 mg/kg	PFOA, 0.5, 1, or 3 mg/kg per day	Oral for 5 weeks		Shi et al. (2020)
TNF-α, IL-1β, IL-6, macrophages ($CD11b^+$ cells), by ELISA, RT-PCR (cytokines); flow cytometry (CD11b)	Mouse, C57BL/6, male	Peritoneal cavity, spleen	↑ TNF-α and IL-6 at ≥ 25 mg/kg, ↑ IL-1β at ≥ 5 mg/kg ↑ % macrophages in peritoneal cavity at ≥ 1 mg/kg ↑ TNF-α and IL-6 at ≥ 125 mg/kg, ↑ IL-1β at ≥ 50 mg/kg ↑ macrophages at ≥ 50 mg/kg in spleen	PFOS, cumulative doses of 0.5, 1, 5, 25, or 50 mg/kg; max 2.08 mg/kg per day	Oral for 60 days	PFOS concentrations were measured in the serum PFOS was the potassium salt	Dong et al. (2012a)
TNF-α and IL-6, by ELISA	Mouse, $B6C3F_1$, female	Serum, peritoneal lavage fluid	Serum cytokines: ↓ TNF-α at 1 mg/kg (↑ at 300 mg/kg), ↑ IL-6 at 1 mg/kg (↓ at 3 mg/kg) Peritoneal macrophage cytokines: ↑ TNF-α at 300 mg/kg, no change in IL-6 Peritoneal lavage fluid: ↓ TNF-α and IL-6	PFOS, 1, 3, or 300 mg/kg total administered dose, corresponding to 0, 0.0331, 0.0993, or 9.93 mg/kg per day	Oral for 28 days	PFOS potassium salt	Mollenhauer et al. (2011)

Table 4.20 (continued)

End-point, assay	Species, strain, sex	Tissue	Result[a]	Dose (LED or HID) or range	Route, duration, dosing regimen	Comments	Reference
Il1b, *Tnf*, *Nfkb1*, *Creb1*, AP-1(mRNA expression), by qPCR	Rat, Sprague-Dawley, pregnant female	Hippocampus, cortex	Hippocampus of offspring: ↑ *Il1b*, ↑ *Tnf* on PND0 and PN21 ↑ AP-1 at all doses on PND0 and at 0.6 and 2 mg/kg per day on PND21 ↑ *Nfkb1* at 0.6 and 2 mg/kg per day on PND0 and at 2 mg/kg on PND21 ↑ *Creb1* at 0.6 and 2 mg/kg per day on PND0 and PND21 Cortex: ↑ Il1b at 0.6 mg/kg per day on PND0 and PND21, at 2 mg/kg per day on PND21 ↑ *Tnf*, AP-1, *Nfkb1* at 2 mg/kg per day on PND0 and PND21 ↑ CREB at 0.6 and 2 mg/kg per day on PND0 and PND21	PFOS: 0.1, 0.6, or 2.0 mg/kg per day	Oral, from GD2 to GD21	PFOS potassium salt	Zeng et al. (2011)
TNF-α and TGFβ3, by ELISA, western blot	Mouse, BALB/c, male	Testicular homogenates	↑ TNF-α, TGFβ3	PFOA, 0–20 mg/kg per day	28 consecutive days		Lu et al. (2016c)
IL-6, IFN-γ, TNF-α, HGF, by ELISA	Mouse, C57BL/6J, male	Serum	↑ IL-6, IFN-γ, TNF-α, HGF	PFOA, 1, 5, 10, or 20 mg/kg per day	Oral gavage up to 28 days		Soltani et al. (2023)
IL-1β, IL-6, TNF-α, by ELISA	Rat, Sprague-Dawley, female	Serum	↑ IL-6, TNF-α No change in IL-1β	PFOS, 0.3 mg/kg per day	Oral daily gavage of pregnant (GD1 to birth) and lactating (PND1 to PND21) mothers		Liu et al. (2023b)
Tnf, *Il6*, *Nfkb1*, and *Mapk8* gene expression, by qRT-PCR	Rat, Wistar, male	Liver	↑ *Tnf*, *Il6*, *Nfkb1*, and *Mapk8* gene expression	PFOA, 10 mg/kg per day	Oral daily gavage for 4 weeks		Naderi et al. (2023)

Table 4.20 (continued)

End-point, assay	Species, strain, sex	Tissue	Result[a]	Dose (LED or HID) or range	Route, duration, dosing regimen	Comments	Reference
Crp, TNF-α, IL-6, and *Nfkb1* gene expression, by RT-PCR, histopathology, ELISA	Rat, albino, male	Jejenum	↑ CRP, TNF-α, IL-6, *Nfkb1*	PFOS, 5 mg/kg per day	Oral daily gavage for 28 days		Shalaby et al. (2023)
CRP, IL-6, COX-2, inflammatory cell infiltration, oedema, by ELISA, histopathology	Mouse, Kunming, male	Liver	↑ CRP, IL-6, COX-2, inflammatory cell infiltration, oedema	PFOA, 10 mg/kg per day	Intragastrically administered for 14 days		Zou et al. (2015)
Anti-inflammatory effects							
Oedema, dorsal-to-ventral paw thickness measured with micro-callipers	Rat, Sprague-Dawley, male	Paw	↓ oedema	PFOA, 20, 50, or 100 mg/kg	Injection into paw 30 min before induction of inflammation	Carrageenan-induced oedema; dose–response relation	Taylor et al. (2002)
Leukocyte activation, PGE2 synthesis, by ELISA	Rat, Sprague-Dawley, female	Serum	↓ leukocytes and PGE2	PFOA, 150 mg/kg	SC	Acute pancreatitis induced by cerulein	Griesbacher et al. (2008)
Inflammatory oedema formation, by histology		Pancreas	No change				

AP, activator protein; bw, body weight; COX, cyclooxygenase; CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; GD, gestational day; HFD, high-fat diet; HID, highest effective dose; IFN, interferon; IL, interleukin; IRF, interferon regulatory factor; KO, knock-out; LED, lowest effective dose; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PG, prostaglandin; PND, postnatal day; ppm, parts per million; qRT-PCR,quantitative reverse transcription-polymerase chain reaction; SC, subcutaneous; TGF, transforming growth factor; TNF, tumour necrosis factor; WT, wildtype; w/w, weight per weight.

[a] ↑, increase; ↓, decrease; (↑), decrease in a study with limitations; +, occurrence.

concentrations of PFOA (0, 0.4, 2, or 10 mg/kg per day) for 28 days. PFOA induced splenic atrophy and increased levels of TNF-α, IL-1β, IL-6, and IRF5 in the spleen at the two highest doses (2 and 10 mg/kg). In the serum, levels of the cytokines IL-1β, IL-6, and IL12p70 were increased by 0.4 mg/kg at higher doses, whereas TNF-α was significantly increased at doses ≥ 2 mg/kg. In addition, dose-dependent increases in activated macrophages were found in the spleen (Guo et al., 2021b).

The effects of exposure to PFOA on immune-mediated liver damage in mice was assessed by Qazi et al. (2013). PFOA exposure at 3 ± 0.7 mg/kg per day for 10 days was associated with decreased hepatic levels of IL-6, TNF-α, IFN-γ, and IL-4, and PFOA caused marked hypertrophy of hepatocytes.

In the study conducted by De Guise and Levin (2021), an investigation of levels of pro-inflammatory markers (IL-5, IL-13, IL-4, IL-12, IL-2, IFN-γ, IL-17α, IL-1α, IL-6, and TNF-α) was performed in mice exposed to low (1.88 mg/kg per day) or high (7.5 mg/kg per day) doses of PFOA in drinking-water for 4 weeks. The results showed a significant reduction in the serum concentrations of the Th2 cytokines IL-5 and IL-13, the Th1 cytokine IL-12, a non-significant dose-dependent increase in IL-2, and an increase in IFN-γ at the highest PFOA exposure. Both doses of PFOA reduced IL-17α and IL-1α, whereas TNF-α was increased at the highest dose of PFOA (De Guise and Levin, 2021).

The hepatic effects of PFOA exposure were also assessed in a study of mice orally dosed with different concentrations of PFOA for 14 days (Yang et al., 2014). The results showed that only high doses of PFOA (10 mg/kg per day) were associated with higher levels of IL-6, COX-2, and CRP (Yang et al., 2014). In addition, hepatic hypertrophy was induced, as indicated by a significant increase in relative liver weight; in addition, a significant increase in inflammatory cells was observed on histological examination.

Qin et al. (2022b) investigated the effects of chronic PFOS exposure (5 mg/kg per day) on liver disease progression by exploring the initiation of NLRP3 inflammasome activation and its potential role in liver inflammation. The study assessed several end-points, including the activation of liver inflammation and steatosis, as well as the release of the pro-inflammatory cytokine IL-1β under a high-fat diet (HFD) or chow diet. The study reported significant findings, including the activation of the NLRP3 inflammasome, an increase in IL-1β secretion, and hepatocyte steatosis in both chow- and HFD-fed animals exposed to PFOS, but exacerbation of liver inflammation around the portal vein and steatosis was also observed in HFD-fed mice exposed to PFOS (Qin et al., 2022b).

Shi et al. (2020) investigated the potential effects of PFOA exposure on the gut and brain in male C57BL/6J mice. Mice were exposed to different concentrations of PFOA (0, 0.5, 1, or 3 mg/kg bw per day) for 35 days. PFOA exposure resulted in increased TNF-α, IL-1 β, and COX-2 and decreased IL-10 levels in the colon. Different results were observed in serum and the brain (see Table 4.20).

Dong et al. (2012a) investigated the effect of 60-day oral exposure to the potassium salt of PFOS on the inflammatory response in peritoneal macrophages, splenic macrophages, and serum of adult male C57BL/6 mice. The mice were dosed daily by oral gavage with PFOS at various doses, ranging from 0.0083 to 2.0833 mg/kg per day, to achieve total administered doses (TADs) of 0.5 to 125 mg/kg over the treatment period. The study reported that PFOS exposure significantly increased the percentage of peritoneal macrophages ($CD11b^+$ cells) at concentrations of ≥ 1 mg/kg TAD, and PFOS exposure elevated the ex vivo production of IL-1β in the peritoneal cavity at concentrations of ≥ 5 mg/kg TAD. Furthermore, PFOS exposure markedly enhanced the ex vivo production of TNF-α, IL-1β, and IL-6 by peritoneal and splenic

macrophages when stimulated with LPS. In addition, the serum levels of TNF-α, IL-1β, and IL-6 were also significantly elevated in response to LPS stimulation (Dong et al., 2012a).

Mollenhauer et al. (2011) investigated the effects of PFOS exposure on the inflammatory markers TNF-α and IL-6 in both serum and peritoneal lavage fluid in adult female B6C3F$_1$ mice. The mice were orally exposed to PFOS at 0, 1, 3, or 300 mg/kg TAD, corresponding to daily doses of 0, 0.0331, 0.0993, or 9.93 mg/kg per day, for 28 days. The study reported that the serum TNF-α levels were significantly decreased by PFOS at 1 mg/kg TAD compared with controls, whereas the serum IL-6 levels were increased. The IL-6 concentrations in peritoneal lavage fluid decreased with increasing PFOS dose. Additionally, the number of splenocytes expressing intracellular IL-6 was significantly decreased in the 3 mg/kg treatment group compared with controls (Mollenhauer et al., 2011).

The pro-inflammatory effects of PFOS on glial activation in the hippocampus and cortex were investigated in a study of rat offspring. The dams received different doses of PFOS via gavage (0.1, 0.6, or 2.0 mg/kg bw) from GD2 to GD21. The study reported increased levels of IL-1β and TNF-α, and elevated mRNA levels of *Jun*, *Nfkb1*, and *Creb1* in the hippocampus at lower doses than in the cortex at PND0 or PND21 (Zeng et al., 2011).

Lu et al. (2016c) investigated the effect of PFOA exposure (0–20 mg/kg per day) on the cytokines TNF-α and TGFβ3 in BALB/c male mice. The authors observed that PFOA increased the TNF-α and TGFβ3-based inflammatory response in testes.

Soltani et al. (2023) investigated the effects of PFOA in C57BL/6 J male mice exposed at 1, 5, 10, or 20 mg/kg. The study reported that PFOA increased the circulating levels of cytokines such as IL-6, IFN-γ, TNF-α, and HGF.

Liu et al. (2023b) investigated the effects of PFOS exposure during pregnancy and lactation in rats. The study reported that PFOS increased the levels of circulating TNF-α and IL-6 on PND14 in the pups.

Naderi et al. (2023) investigated the effects of PFOA exposure on the expression of *Tnfa*, *Il6*, *Nfkb1*, and JNK genes in Wistar male rats exposed to 10 mg/kg. The study reported that PFOA increased the expression of *Il6*, *Tnfa*, *Nfkb1*, and JNK genes in the liver.

Shalaby et al. (2023) investigated *Crp*, *Tnfa*, *Il6*, and *Nfkb1* gene expression in response to PFOS exposure at a dose of 5 mg/kg per day. The study showed increased expression of *Crp*, *Tnfa*, *Il6*, and *Nfkb1* genes.

Zou et al. (2015) investigated the potential of quercetin to offset the adverse effects of PFOA in mice treated intragastrically with PFOA at a dose of 10 mg/kg per day alone or in combination with quercetin at a dose of 75 mg/kg per day for 14 consecutive days. The study showed that PFOA changes the liver morphology by disrupting liver architecture and inducing marked oedema, vacuolar degeneration, hepatocellular necrosis, and inflammatory cell infiltration. In addition, PFOA significantly increased levels of CRP, IL-6, and COX-2, but supplementation with quercetin reduced these levels (Zou et al., 2015).

Overall, when assessing data from experimental animal models, the aggregated findings from the evaluated studies displayed a mixture of outcomes. In a broader context, there is a notable tendency towards a positive correlation of PFAS exposure with chronic inflammation, as indicated by the histological data, and this connection is particularly robust for PFOA, in contrast to PFOS.

Anti-inflammatory effects

See Table 4.20.

Taylor et al. (2002) investigated the effects of PFOA on the inflammation and hyperalgesia induced by intraplantar injection of carrageenan in male Sprague-Dawley rats, with the aim of better understanding the contribution

Table 4.21 End-points relevant to chronic inflammation in non-human mammalian cells in vitro exposed to PFOA or PFOS

End-point	Assay	Tissue, cell line	Result[a]	Concentrations, range	Reference
TNF-α, IL-1β, IL-6, and IL-8	ELISA, western blot	Rat RBL-2H3 mast cells	↑ IL-1β, IL-6, and IL-8 at 100 μM ↑ TNF-α, IL-1β, IL-6, and IL-8 ≥ 10 μM in IgE-stimulated cells	PFOA, 10, 50, 100, or 500 μM	Lee et al. (2017)
TNF-α, IL-6	ELISA	Rat Kupffer cells and primary hepatocytes	↑ TNF-α, IL-6 in Kupffer cells at all time points No change in hepatocytes	PFOS, 100 μM for 0 or 48 h	Han et al. (2018b)
TNF-α	ELISA	Rat C6 glioma cells	↑ TNF-α	PFOS, 0, 0.1, 1, 5, 10, 20, 50, or 100 nM for 12 h or 20 nM for 0, 1, 3, 6, 12, 24, or 48 h	Chen et al. (2018b)
TNF-α	ELISA	Rat HAPI microglial cells	↑ TNF-α > 1 nM and following 3 h of exposure	PFOS, 0–200 nM for 6 h or 20 nM for 0, 1, 3, 6, 8, 12 or 24 h. 0.1% DMSO as the control	Yang et al. (2015)

DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; h, hour(s); IgE, immunoglobulin E; IL, interleukin; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; TNF, tumour necrosis factor.
[a] ↑, increase.

of PPARs to the inflammatory process. The oedema (swelling) and thermal hypersensitivity were assessed by measuring the dorsal-to-ventral paw thickness. The results showed that PFOA inhibited carrageenan-induced oedema and thermal hypersensitivity. The authors concluded that PFOA exhibits anti-inflammatory and/or anti-hyperalgesic effects in vivo by interfering with the initiation of inflammation (Taylor et al., 2002). In another study, the potential anti-inflammatory effects of PFOA in cerulein-induced acute pancreatitis in female rats was investigated (Griesbacher et al., 2008). The study showed that PFOA significantly reduced leukocyte accumulation and prostanoid synthesis, and thereby displayed a potential for anti-inflammatory effects in cerulein-induced acute pancreatitis (Griesbacher et al., 2008).

(ii) Non-human mammalian systems in vitro

See Table 4.21.

The immunological effects of PFOA and PFOS exposure on macrophages via sirtuin regulation was assessed using the murine macrophage cell line RAW 264.7 (Park et al., 2019). The results showed that both PFOA and PFOS had effects on sirtuins. More specifically, PFOA reduced *Sirt7* and *Sirt4* expression at 0.5 μM, but increased *Sirt1*, *Sirt3*, *Sirt4*, *Sirt5*, and *Sirt6* gene expression at 5 μM. In contrast, PFOS increased *Sirt2*, *Sirt3*, *Sirt5*, and *Sirt6* gene expression (Park et al., 2019).

Lee et al. (2017) investigated the correlation between PFOA exposure and allergic inflammation by examining its effect on RBL-2H3 mast cell degranulation and allergic symptoms. The results showed that PFOA activated NF-κB, leading to enhanced expression of TNF-α, IL-1β, IL-6, and IL-8 in IgE-stimulated mast cells (Lee et al., 2017).

Han et al. (2018b) investigated the effects of PFOS exposure on the inflammatory responses of Kupffer cells (liver macrophages). Rat Kupffer cells and primary hepatocytes were exposed to 100 μM PFOS for various time periods (0–48 hours). The study demonstrated that PFOS

induced the production of TNF-α and IL-6 in Kupffer cells (Han et al., 2018b).

Chen et al. (2018b) investigated the effects of PFOS exposure on the TNF-α inflammatory response in rat C6 glioma cells. The C6 cells were exposed to different concentrations (0–100 nM) of PFOS for 12 h or 20 nM PFOS for different periods of time (0, 1, 3, 6, 12, 24, or 48 hours). The study reported that PFOS increased the TNF-α inflammatory response (Chen et al., 2018b).

Yang et al. (2015) investigated the effect of PFOS exposure on the TNF-α inflammatory response in rat HAPI microglia. The cells were treated with PFOS at different concentrations for 6 hours (0–200 nM) or at 20 nM for 0, 1, 3, 6, 8, 12, or 24 hours. The study reported that PFOS increased the TNF-α inflammatory response at ≥ 5 nM for 6 hours and at 20 nM after 3 hours or longer (Yang et al., 2015). Overall, exposure to PFOA and PFOS consistently increased chronic inflammatory responses, as demonstrated by increased IL-6 and TNF-α, in various cell types in vitro.

Synopsis

[Overall, the Working Group noted that the findings of studies of human populations, both cross-sectional and longitudinal, that examined inflammatory markers (cytokines and proteins), neutrophil and leukocyte counts, and inflammatory disease outcomes (eczema, atopic dermatitis, asthma, and allergic rhinitis) in relation to PFOA and PFOS exposure yielded mixed results. Moreover, the associations varied by the biomarker investigated, sex, and exposure source, highlighting the complexity of these relationships. The evidence is not informative enough to conclude that PFOA and PFOS induce chronic inflammation in exposed humans.

In human primary cell studies, a trend emerged for decreases in the levels of chronic inflammatory markers in response to PFOA and PFOS treatment, although the outcomes varied depending on the specific model and assay used. In experimental systems in vivo, the findings were also mixed, but in a broader context, there was a discernible inclination towards a positive association with chronic inflammation, which was more pronounced for PFOA than for PFOS. While the evidence from the in vitro studies is clearer, the results from human and experimental systems in vivo collectively support a nuanced relationship of PFOA and PFOS exposure with chronic inflammation.]

4.2.7 Is immunosuppressive

See Table 4.22.

(a) Humans

(i) Exposed humans

The evaluation of the evidence on whether PFOA and PFOS exhibit the key characteristic of carcinogens "is immunosuppressive" was prompted by observations of clinical end-points related to immunosuppression (e.g. by infections), the effects on related immune end-points (vaccination, immune cell subpopulation monitoring, performance of functional assays on immune cells collected from exposed humans), or the measurement of biomarkers in fluids from exposed humans (cytokines or other mediators of inflammation). The most informative and relevant studies are reported in Table 4.22.

The Working Group first described the clinical outcomes observed in humans, mostly children exposed to the two agents. The strengths of the studies were: serum levels representing combined exposure from all sources and routes, including contributions from metabolites of precursors, the monitoring of diverse types of infections (respiratory tract and digestive tract), and/or responses to diverse vaccine antigens (tetanus, diphtheria, influenza, COVID-19, etc.), and the combination of longitudinal and cross-sectional studies including a large number of individuals. Confounding factors, such as smoking, BMI, maternal education, sex of the

Table 4.22 End-points relevant to immunosuppression in humans exposed to PFOA or PFOS

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Infectious diseases	Maternal plasma	Hokkaido, Japan Study on the environment and children's health to age 4 years Cross-sectional study	PFOS, 4.92 ng/mL; PFOA, 2.01 ng/mL (median) 1558 mother–child pairs	+ (increased OR of total infectious diseases for PFOS) Q4 versus Q1, OR, 1.61; 95% CI, 1.18–2.21; *P* for trend, 0.008	Maternal age, number of older siblings, maternal smoking during pregnancy, maternal education, infant sex, breastfeeding period	Cross-sectional study with a large number of mother–child pairs recruited. Limitations were: infectious disease incidences were based on maternal reports that were not corroborated by medical records. No studies on the validity of self-reported physician-diagnosed infections. These could have resulted in some level of outcome misclassification.	Goudarzi et al. (2017a)
Infectious diseases	Maternal plasma	Hokkaido, Japan Cohort for the study of the environment and children's health Prospective birth cohort with 7 years of follow-up	PFOS, 5.12 ng/mL; PFOA, 1.94 ng/mL (median) 2689 mother–child pairs; finally analysed 2206 children	+ PFOA, increased risk of pneumonia: OR, 1.17 (95% CI, 1.01–1.37); *P* = 0.043 for the children as a whole (*n* = 2689) RSV infection: OR, 1.58 (95% CI, 1.13–2.22); *P* = 0.008, *P* for trend = 0.038, for children with no siblings (*n* = 379) PFOS was inversely associated with increased risks of pneumonia and RSV infection	Sex, parity, maternal age at delivery, maternal smoking during pregnancy, BMI pre-pregnancy, annual household income during pregnancy	Longitudinal birth cohort design with a large sample size. Limitations were: loss to follow-up, reliance on questionnaire-based outcomes, possibility of recall bias affecting, health outcomes recorded using parental self-reported questionnaires.	Ait-Bamai et al. (2020)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Respiratory tract infection	Fetal umbilical cord blood	Oslo, Norway Environment and Childhood Asthma study Prospective birth cohort with 2–10 years of follow-up	PFOS, 5.2 ng/mL; PFOA, 1.6 ng/mL (median) *n* = 641	+ positive associations of the cord blood concentrations of PFOS and PFOA with airway infections; significant association of lower respiratory tract infection in infants aged 0–10 years with PFOS (β = 0.50; 95% CI, 0.42–0.57) and PFOA (β = 0.28; 95% CI, 0.22–0.35)	Confounders examined were sex; birth weight; birth month; breastfeeding at age 6 and 12 months; maternal smoking during pregnancy; household smoking at birth, at preschool age, and at school age; parental asthma, atopic dermatitis and allergic rhinitis; parental education; and household income	Strengths of the study: high follow-up rate at 10 years; highly representative study sample; prospective longitudinal design, extensive characterization of the children from birth to age 2 years, as well as at 10 years; use of questionnaires, structured interviews, and clinical examinations of the children. Limitations: potential recall bias, especially in questionnaires and interviews, lack of data on important confounding factors, such as parity and previous breastfeeding.	Impinen et al. (2018)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Infectious diseases	Maternal plasma	Norway, MoBA nationwide cohort. Prospective population-based pregnancy sub-cohort with 7 years of follow-up	PFOS, 12.9 ng/mL, PFOA, 2.5 ng/mL (medians) *n* = 1943 *n* = 1270 (questionnaire after 3 years) *n* = 972 (questionnaire after 7 years)	+ positive associations between bronchitis and/or pneumonia with PFOS (RR, 1.20;95% CI, 1.07–1.34; *P* = 0.001) and PFOA (RR, 1.27, 95% CI, 1.12–1.43; *P* < 0.001) (age 0–3 years) Positive association between viral pseudocroup and PFOA (RR, 1.22; 95% CI, 1.07–1.38; *P* = 0.002) (age 0–3 years) Positive association between gastric flu/ diarrhoea and PFOA (RR, 1.48; 95% CI, 1.31–1.67; *P* < 0.001) (age 6 –7 years)	Maternal age, maternal BMI, maternal education, parity, and smoking during the pregnancy Correction for multiple testing using false discovery rate	Longitudinal prospective cohort design with 7 years of follow-up, sample size was relatively large. All samples had PFOS and PFOA concentrations above the LOQ. Loss to follow-up at 7 years and reliance on questionnaire-based outcomes. Possible that a single illness episode could be reported with more than one of the symptoms of infection, making the number of episodes more uncertain.	Impinen et al. (2019)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Infectious diseases	Maternal plasma	Odense, Denmark Odense Child Cohort; data collected 2010–2012 Prospective cohort	PFOS, 7.52 ng/mL; PFOA, 1.68 ng/mL (medians) 1503 mother–child pairs	+ PFOS doubling in mothers associated (adjusted HR, 1.23) with a 23% increase in the risk of hospitalization because of any infection + PFOA and PFOS doubling increased the risk of LRTI by 27% (adjusted HR, 1.27; 95% CI, 1.01–1.59) and 54% (adjusted HR, 1.54; 95% CI, 1.11–2.15), respectively	Maternal educational, maternal, parity, BMI, smoking during pregnancy	Prospective cohort study in 1503 mother–child pairs. Estimation of the number of severe infections may be imprecise. Analyses of some specific types of infections based on a small number of events (gastrointestinal tract, 40 events).	Dalsager et al. (2021)
Response to vaccination (tetanus and diphtheria)	Maternal and infant plasma	Faroe Islands, Denmark National Hospital Faroe Island Birth cohort Prospective study	Maternal PFOS GM, 27.3 ng/mL; age 5 years children PFOS GM, 16.7 ng/mL Maternal PFOA GM, 3.20 ng/mL; age 5 years children PFOS GM, 4.06 ng/mL 656 recruited 1997–2000; *n* = 587, follow-up to 2008	+ Prenatal exposure to PFOS and PFOA negatively associated with anti-diphtheria antibody concentrations, at age 5 and 7 years for PFOS and 7 years for PFOA – (negative) association between anti-tetanus titre and PFOA at age 7 years	Pairwise; possible effect of PCB exposure, birth weight, maternal smoking during pregnancy, duration of breastfeeding	Prospective cohort study addressing the response to vaccination as the end-point. Average sample size. Closed population studied (fishing community in the Faroe Islands).	Grandjean et al. (2012)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Response to vaccination (tetanus, diphtheria)	Infant blood collected at birth and at ages 18 months and 5 years (clinical examinations)	Faroe Islands, Denmark Birth Cohort 5, born in 2007–2009 Prospective birth cohort	Age 18 months, PFOS GM, 7.1 ng/mL; age 5 years, PFOS GM, 4.7 ng/mL Age 18 months, PFOA GM, 2.8 ng/mL; age 5 years, PFOA GM, 2.2 ng/mL Total, *n* = 490; age 18 months, *n* = 275; and age 5 years, *n* = 349	+ (at age 5 years, 152 (44%) children had antibody concentrations lower than the protective level of 0.1 IU/mL for diphtheria and 126 (36%) for tetanus)	Breastfeeding duration, maternal serum PCB concentrations, caesarean section; others not mentioned	Clear vaccination schema, prospective study with follow-up.	Grandjean et al. (2017)
Response to vaccination (*Haemophilus influenzae*, diphtheria, tetanus) and IFN-γ production by PBMCs	Infant blood collected at age 1 year	Berlin, Germany Cross-sectional study	Data expressed as means Formula-fed children, PFOS, 6.8 ± 3.4 ng/mL; PFOA, 3.8 ± 1.1 ng/mL Breastfed children, PFOS, 15.2 ± 6.9 ng/mL; PFOA, 16.8 ± 6.6 ng/mL 101 healthy children age 1 year (21 formula-fed, 80 breastfed for at least 4 months)	+ PFOA associations of adjusted PFOA level with antibody levels for Hib, tetanus IgG1, and diphtheria, $P < 0.05$) No significant associations of PFOS with Hib, or tetanus or diphtheria antibodies + association of PFOA level with a lower production of IFN-γ by PBMCs stimulated with tetanus and diphtheria toxoid found in 55 children) No effect of PFOA or PFOS on infections during the first year of life	Children (age 341–369 days) of German parents, either breastfed for a maximum of 2 weeks ("formula-fed" children) or breastfed (exclusively breastfed for ≥ 4 months) Healthy infants with no acute or chronic diseases, including atopic eczema, no medication, vaccinated	Strengths: children breastfed for a long time with relatively high levels of internal exposure to persistent organic pollutants. High stability of the associations identified using different methods of evaluation, broad spectrum of other contaminants also measured as possible confounders. Limitation: small sample size: 101 children (51 boys, 50 girls) examined; 21 formula-fed, and 80 breastfed for at least 4 months.	Abraham et al. (2020)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Response to vaccination (rubella)	Blood from participants	US NHANES for years 1999–2000 and 2003–2004 Cross-sectional study	Mean PFOA concentration 6 ng/mL in men, 4.3 ng/mL in women, 4.8 ng/mL in youth Mean PFOS concentrations were 28.1 ng/mL, 22.1 ng/mL, and 25.1 ng/mL, respectively; 581 women, 621 men, and 1012 young participants (aged 12–18 years)	+ associations of both PFOA ($P = 0.0016$) and PFOS ($P = 0.0295$) quartiles with rubella titres after adjusting for covariates	Age, race/ethnicity, educational level (high school), BMI, parity, live births	Limitations: cross-sectional study, temporal nature of the associations between PFAS and humoral immune response could not be determined. No young children in the cohort. No information on vaccination status or recent rubella infection. No information on age when vaccination was performed, other infections, or variation in vaccination rates and schedules.	Pilkerton et al. (2018)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Response to vaccination (mumps, rubella, and measles antibodies)	Blood from participants	US NHANES 1999–2000 and 2003–2004 Cross-sectional study	GM PFOS, 20.8 ng/mL; PFOA, 4.13 ng/mL; 1191 children	+ (doubling of PFOS associated with a 7.4% decrease in mumps antibodies; doubling of PFOS and PFOA associated with 5.9% and 6.6% decreases in mumps antibodies if analysis restricted to seropositive children) + (doubling of PFOS was associated with a 13.3% decrease in rubella antibodies; for PFOA decrease not significant) No associations between PFOS or PFOA with measles antibody level	No information on vaccination status in NHANES; however, in early 1990s, most US states have required a measles/mumps/ rubella booster before starting school; parity	Limitations: possible exposure misclassification, although less risk than in a typical cross-sectional study, because these compounds have long serum half-lives. Lack of information on receipt and/or timing of measles/mumps/rubella vaccination.	Stein et al. (2016)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Response to vaccination (rubella, measles, and mumps antibodies)	Blood from participants	US NHANES 2003–2004 and 2009–2010 cycles Cross-sectional study	GM PFOA in entire sample, 3.33 ng/mL; 3.42 ng/mL in those with lower folate; 3.15 ng/mL in those with higher folate PFOS in entire sample, 12.44 ng/mL; 12.88 ng/mL in those with lower folate; 11.57 ng/mL for those with higher folate 819 adolescents aged 12–19 years	+ inverse associations of serum PFOS ($P = 0.22$) and PFOA ($P = 0.03$) with rubella antibodies, and between PFOA ($P = 0.01$) and mumps antibodies, only in adolescents with blood folate concentrations < 66th percentile (lower folate group) No associations in adolescents with higher RBC folate levels (higher folate group)	Demographic characteristics, including age (continuous), sex (dichotomous), race, household income, using self-reported questionnaires; income-to-poverty ratio, weight, height, dietary information	NHANES study collected high quality data, good sample size. Seropositivity for rubella and measles was used as a proxy for rubella/measles/mumps vaccination, because of lack of information on vaccination or boosting for the study sample.	Zhang et al. (2023d)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Response to vaccination (influenza virus, A/H3N2 strain)	Blood from participants	Mid-Ohio Valley region of Ohio and West Virginia, USA Cross-sectional study (pre-post measurements)	PFOA: first quartile: 0.25–13.7 ng/mL; second quartile: 13.8–31.5 ng/mL; third quartile: 31.6–90 ng/mL; fourth quartile: 90.4–2140 ng/mL. PFOS: first quartile: 0.1–5.8 ng/mL; second quartile: 5.9–9.2 ng/mL; third quartile:9.3–14.5ng/mL; fourth quartile: 14.7–42.3 ng/mL *n* = 411	+ PFOA (serum concentrations between 13.7 and 90 ng/mL) associated with a reduced response to A/H3N2 influenza virus + PFOA associated with an increased risk of not attaining the antibody threshold considered to offer long-term protection) – (no evidence that PFOS serum concentration was associated with reduced vaccine responses; no associations of self-reported colds or influenza with PFAA concentrations)	Age, medical comorbidities, and medications	Serum samples collected pre-vaccination and 21 ± 3 days post-vaccination in 2010. Number of participants was only 411. Analysis revealed a strong effect of previous influenza vaccination on the immune response, based on self-reported information.	Looker et al. (2014)

Table 4.22 (continued)

End-point	Biosample type	Location Setting, study design	Exposure level No. of study participants	Response[a]	Covariates adjusted for	Comments	Reference
Response to vaccination against COVID-19	Blood from participants	Ronneby, Sweden Adults (age 20–60 years) from the Biomarker Cohort and a background-exposure group from Karlshamn Observational study	Ronneby group, median PFOS serum level, 47 ng/mL (adults, *n* = 309) Background group, median exposure PFOS serum level, 4 ng/mL (*n* = 47) 2 doses of mRNA vaccine Spikevax	– (PFOA and PFOS levels were not associated with antibody levels after 5 weeks and 6 months)	Sex, age, smoking Exclusion of individuals with previous SARS-CoV-2-infection Others mentioned in the questionnaire, but analysis not provided	PFAS levels were measured before vaccination and 5 weeks (*n* = 350) and 6 months (*n* = 329) after the second vaccine dose. Prospective design with measurement of antibody responses at defined time points after vaccination. Number of participants used to assess background exposure was small. Possibility that there was an effect of PFAS within the background exposure levels cannot be excluded.	Andersson et al. (2023)
Response to vaccination against COVID-19	Blood from participants	Factories in Alabama and Wisconsin, USA Employees and retirees from two facilities (Spring 2021) Observational study	415 participants (757 observations, repeated measures analyses)	+ fully adjusted IgG concentration was −3.45% (95% CI, −7.03 to 0.26%) per 14.5 ng/mL IQR increase in PFOS + For PFOA, −1.95% (95% CI, −4.35 to 0.51) per 3.59 ng/mL IQR increase in PFOA	Age, sex, race/ethnicity, site, BMI, smoking, immunocompromising conditions Antigenic stimulus group and time since last antigenic stimulus used as precision variables	SARS-CoV-2 neutralizing antibodies were measured to assess the polyclonal response to SARS-CoV-2. Wide distribution of serum concentrations of PFOS and PFOA (increased statistical power to detect an association). Potential longer-term associations with PFAS not assessed.	Porter et al. (2022)

β, adjusted Poisson regression estimate; BMI, body mass index; CI, confidence interval; COVID-19, disease caused by SARS-CoV-2-infection; GM, geometric mean; Hib, *Haemophilus influenzae* type b; HR, hazard ratio; IFN-γ, interferon-gamma; IgG, immunoglobulin G; IQR, interquartile range; IU, international unit; LOQ, limit of quantification; LRTI, lower respiratory tract infection; MoBA, Norwegian Mother, Father and Child Cohort Study; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; PBMCs, peripheral blood mononuclear cells; PCB, polychlorinated biphenyl; PFAA, perfluoroalkyl acids; PFAS, perfluoroalkyl and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; Q, quartile; RR, Poisson regression rate ratio; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; US, United States; USA, United States of America.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study).

infant, breastfeeding period, and others were considered in the analyses. The weaknesses of the studies were mainly restricted to the less detailed self-reported questionnaires used in some of the studies.

Increased risk of infections

The Hokkaido Study on the relation between environment and children's health recruited 20 787 pregnant women from February 2003 to March 2012. Using this cohort, Goudarzi et al. (2017a) examined the relation between prenatal exposure to various perfluoroalkyl acids (PFAA), including PFOS, and the prevalence of infectious diseases in children aged up to 4 years (Goudarzi et al., 2017a). A total of 1558 mother–child pairs were included in this data analysis. PFAA concentrations were measured in maternal plasma samples collected at 28–32 weeks of gestation. Medical history of common infectious diseases, based on physicians' diagnoses, was extracted from the questionnaires completed by the mothers. The median serum concentrations were 4.92 ng/mL for PFOS and 2.01 ng/mL for PFOA, and 67.1% of the children (n = 1046) were diagnosed with at least one of the diseases defined as infectious. After adjusting for appropriate confounders (maternal age, number of older siblings, maternal smoking during pregnancy, maternal education, infant sex, and breastfeeding period), the highest quartile of PFOS concentration was associated with an increased OR for infectious diseases as a whole in all the children. No associations between infectious diseases and the other PFAAs examined, including PFOA, were found (Goudarzi et al., 2017a).

[The Working Group noted that prenatal exposure to PFOS seemed to be associated with infectious disease occurrence in early life. As in other studies, the classification of infectious diseases was based on maternal reports that were not corroborated by medical records. No studies on the validity of self-reported physician-diagnosed infections were conducted.]

In a subsequent study conducted on the same Hokkaido cohort, Ait Bamai et al. (2020) investigated the effects of prenatal exposure to PFAS on the prevalence of infectious diseases in children aged up to 7 years (Ait Bamai et al., 2020). Of the children diagnosed with infectious diseases between ages 2 and 4 years, the analysis was limited to 2206 children (out of a total 2689) for whom data on PFAS measurements and questionnaires, administered to mothers during the first trimester of pregnancy and when children were 7 years old, were available. Median concentrations in the blood were 1.94 ng/mL for PFOA and 5.12 ng/mL for PFOS. Prenatal exposure to PFOA was associated with increased risk of infections in all the children (n = 2689) and with increased risk of respiratory syncytial virus (RSV) infection among children without any siblings (n = 379), and PFOS was inversely associated with increased risks of pneumonia and RSV infection.

In the study by Impinen et al. (2018), the objective was to determine whether prenatal exposure to PFAS was associated with asthma or other allergic diseases, or respiratory tract infections, in childhood. PFAS were measured in cord blood from 641 infants in the ECA prospective birth cohort study cohort in Oslo, Norway. The results showed that reported airway infections were significantly associated with cord blood concentrations of PFOA and PFOS (median concentrations of PFOA and PFOS were 1.6 and 5.2 ng/mL, respectively) and, specifically, that LRTIs in infants aged 0–10 years were associated with PFOA ($\beta = 0.28$; 95% CI, 0.22–0.35) and PFOS ($\beta = 0.50$; 95% CI, 0.42–0.57). Associations were also found for perfluorooctanesulfonamide, perfluorononanoic acid (PFNA), and perfluoroundecanoic acid. [The Working Group considered that, although it is not known how cord blood concentration is linked to overall future exposure, it represents a good marker of gestational exposure. These observations suggested that PFOA and PFOS were associated with an increased number of

respiratory tract infections in the first 10 years of life, suggesting immunosuppressive effects of these two molecules. The common cold, asthma, allergic rhinitis, atopic dermatitis, and allergic sensitization were not significantly associated with PFOA or PFOS.]

The potential association between PFAS plasma level and the risk of infectious diseases was also investigated over a 7-year follow-up in mothers and children recruited in Norway between 1999 and 2008 in the Norwegian Mother and Child Cohort Study (MoBa) (Impinen et al., 2019). Blood samples were obtained from the mothers during mid-pregnancy (n = 1943). The median blood concentrations were 12.9 ng/mL for PFOS and 2.5 ng/mL for PFOA. From age 0 to 3 years, positive associations were found between bronchitis and/or pneumonia with both PFOA and PFOS, and between pseudocroup and PFOA only. A positive association between PFOA and gastric flu/diarrhoea from age 6 to 7 years was also found.

Dalsager et al. (2021) investigated the association between the maternal serum concentrations of PFAS during pregnancy and the rate of hospitalization of the children due to common infectious diseases between birth and age 4 years (1503 mother–child pairs were included). Serum samples collected from first-trimester pregnant women from the Odense Child Cohort (OCC) in 2010–2012 were analysed for concentrations of PFOS, PFOA, and other PFAS. Hazard ratios (HRs) expressing the relative change in the instantaneous risk of hospitalization associated with a doubling in maternal PFAS concentration were used. A doubling in maternal PFOS concentration was associated with a 23% increase in the risk of hospitalization due to any infection (HR, 1.23; 95% CI, 1.05–1.44). Every doubling of PFOA or PFOS increased the risk of LRTI by 27% (HR, 1.27; 95% CI, 1.01–1.59) or 54% (HR, 1.54; 95% CI, 1.11–2.15), respectively.

[The Working Group considered that these studies thoroughly addressed the effects of exposure during pregnancy or early life on infectious diseases in children. An association was found between PFOS and the overall risk of infectious disease, and of PFOA and PFOS exposure with the risk of LRTIs, including RSV.]

The objective of the study conducted by Granum et al. (2013) was to investigate the effect of prenatal exposure to PFAS on the responses to paediatric vaccines and immune-related health outcomes in children up to age 3 years (Granum et al., 2013). In the prospective BraMat birth cohort (n = 99), a subcohort of MoBa, pregnant women from Oslo and Akershus were recruited during 2007–2008. Maternal PFAS levels in the blood were measured at delivery (median concentrations for PFOA and PFOS were 1.1 and 5.5 ng/mL, respectively), and the children had their levels measured at age 3 years (not reported by the authors). There was a positive association between the maternal concentration of PFOA and the number of episodes of common cold in the children, and between PFOA and the number of episodes of gastroenteritis.

Zhang et al. (2022b) studied data from the NHANES, including for 517 children aged 3–11 years (2013–2014 cycle) and 2732 adolescents aged 12–19 years (2003–2016 cycles) (Zhang et al., 2022b). PFAS concentrations were measured in serum samples from random subsamples (one third of the total population in that age group of the cycle) for children (age 3–11 years) and adolescents (age 12–19 years). The common cold was self-reported by the participant or parent on the basis of having had a head cold or chest cold during the last month. In children, serum PFOA concentrations were related to the common cold, although the confidence intervals were wide (OR, 1.32; 95% CI, 0.67–2.62). No association was seen for PFOS and the common cold among the children. For adolescents, serum PFOS concentration showed a positive relationship with the study outcome (OR, 1.13; 95% CI, 0.96–1.32), although

with limited precision. No positive relation was found for PFOA. The Bayesian kernel machine regression model was used to study the effect of the mixture. The results showed a clear trend for common cold estimates to increase across quantiles of the total PFAS concentration in the children, whereas no clear pattern was found for the adolescents. [The Working Group considered the above two studies to be of low informativeness, because they were based on a small number of people and because the self-reported questionnaire completed by the participant or parent only described having a head cold or chest cold during the last month.]

The aim of the prospective cohort study conducted by Okada et al. (2012) was to evaluate possible associations between maternal PFOA and PFOS levels in pregnant women and infectious diseases in their infants during the first 18 months of life. Pregnant women were recruited between 2002 and 2005 from the Sapporo region of Japan. Maternal serum PFOA and PFOS levels in blood taken from a peripheral vein after the second trimester, or in certain cases immediately after delivery, were measured and related to the IgE levels in cord blood, reflecting the prenatal exposure of the fetus (n = 231), and to infectious diseases (n = 343) during the following 18 months. The median PFOA and PFOS concentrations were 1.3 ng/mL and 5.2 ng/mL, respectively. The development of infectious diseases at age 18 months was determined from questionnaires to mothers. The results showed no association between maternal PFOA and PFOS levels and infant infectious diseases at age 18 months. [The Working Group noted that this study had some limitations, with a relatively small sample size that was probably insufficient for the identification of significant relationships of PFOA and PFOS exposure with infectious diseases.]

Wang et al. (2022d) investigated the associations of prenatal exposure to PFAS with acute infectious diseases, including the common cold, bronchitis/pneumonia, and diarrhoea, during early childhood. A prospective cohort in Shandong, China, of 235 mother–infant pairs recruited between September 2010 and 2013 from the Laizhou Wan (Bay) birth cohort (LWBC) was studied. Ten selected PFAS were measured in maternal serum, including PFOA and PFOS. The geometric mean concentrations of PFOA and PFOS were and 44.88 ng/mL and 4.39 ng/mL, respectively. Questionnaires completed by parents were used to collect detailed information on acute infectious diseases after 1 year of follow-up. The questionnaire data were confirmed by the medical records. The OR for diarrhoea was 4.99 (95% CI, 1.86–13.39) per log-unit increase in PFOA. When stratified by breastfeeding duration (at least 4 months, or not), the relation between PFAS exposure and diarrhoea was more pronounced among the breastfed infants. However, there were no associations between prenatal PFAS exposure and the common cold or bronchitis/pneumonia (Wang et al., 2022d). [The Working Group noted that the sample size of the study was small, and the results should be interpreted with caution.]

Effects on responses to vaccination

In the following paragraphs, the potential effects of PFOA and PFOS on the response to vaccination are evaluated. The immune response to vaccination is normally dependent on the adaptive immune system, which involves antigen presenting cells and T and B lymphocytes. The response to common vaccines represents a relevant end-point to address the consequences for the immune system of the exposure to chemicals. Indeed, the concentrations of antigen-specific antibodies in serum represent a useful and clinically relevant indicator of immune function in humans. The studies are reported in descending order of informativeness.

Grandjean et al. (2012) studied the relationship between PFAS exposure and vaccination with tetanus and diphtheria toxoids in infants using the serum antibody concentrations at ages

5 and 7 years as a marker. ELISA was used for tetanus antibody and Vero cell-based neutralization assay was used for diphtheria antibody. The study was a prospective study of a birth cohort from the National Hospital in the Faroe Islands, Denmark, with a total of 656 consecutive single-child births recruited during 1997–2000, 587 of which had follow-up until 2008. Maternal serum PFAS concentrations were measured during the third trimester. Maternal geometric mean serum concentrations were 3.20 and 27.3 ng/mL for PFOA and PFOS, respectively. Geometric mean serum concentrations at age 5 years were 4.06 and 16.7 ng/mL for PFOA and PFOS, respectively. Prenatal exposure to both PFOA and PFOS, as indicated by the maternal serum concentrations, was negatively associated with the anti-diphtheria antibody concentrations. The strongest negative correlations were for the association between maternal PFOS and pre-booster diphtheria antibody concentration at age 5 years, with a two-fold higher exposure being associated with a difference of –39% (95% CI, –55% to –17%) in the diphtheria antibody concentration. For the antibody concentrations at age 7 years, an increase of two-fold in PFOA exposure associated with differences of –36% (95% CI, –52% to –14%) and –25% (95% CI, –43% to –2%) for tetanus and diphtheria, respectively. PFOS exposure was associated with a difference in diphtheria antibody of –28% (95% CI, –46% to –3%) and a non-significant difference of –23.8% (95% CI, –44.3% to 4.2%) for tetanus. These results showed that elevated exposures to PFAS were associated with reduced humoral immune responses to routine childhood immunizations in children aged 5 and 7 years. [The Working Group noted the following strengths: the serum levels represented the combined exposure to all exposure sources and routes, including metabolites of precursors, over a relatively long period of time, due to the long half-lives of PFOA and PFOS. In addition, the study considered co-exposure to smoking and polychlorinated biphenyls (PCBs).]

In a subsequent study, Grandjean et al. (2017) attempted to link the PFAS-associated decreases in antibody concentrations to past exposure during infancy using a new prospective birth cohort in the Faroe Islands (Cohort 5, born in 2007–2009). A total of 381 children participated in the examinations at age 5 years, and 370 of these had also participated at age 18 months. To increase the statistical power of the study, joint statistical analyses were conducted with a previous birth cohort in the Faroe Islands (Grandjean et al., 2012), in which the PFAS exposure was higher. The geometric mean PFOS concentration at age 5 years was 16.7 ng/mL in the 2012 study versus 4.7 ng/mL in the 2017 study. The geometric mean PFOA concentration at age 5 years was 4.06 ng/mL in the 2012 study versus 2.2 ng/mL in the 2017 study. The serum concentrations of antibodies against the tetanus and diphtheria vaccines were determined at age 5 years. This study confirmed the inverse associations of prenatal exposure with the antibodies against both the tetanus and diphtheria toxoids. A doubling of the exposure to PFOA led to a decrease of about 30% in the antibody concentration for tetanus at age 5 years, whereas the association with diphtheria was weaker. Using the same cohorts of people recruited in the Faroe Islands, Mogensen et al. (2015b) estimated the combined (analysis of multiple exposures) change in antibody concentrations for tetanus and diphtheria. In this study, it was not possible to attribute causality to any single PFAS compound. Hence, the three 7-year concentrations, i.e. the concentrations of PFOA, PFOS, and perfluorohexanesulfonic acid (PFHxS), were combined, and the study showed that an increase of two-fold in PFAS was associated with a decrease of 54.4% (95% CI, 22.0–73.3%) in the antibody concentration after vaccination (Mogensen et al., 2015b).

A cross-sectional study in 101 healthy children (aged 1 year) from Berlin, Germany (21 formula-fed, and 80 breastfed for at least 4 months), which included measurements of

internal levels of POPs and a broad panel of biological parameters, was performed at the end of the 1990s (Abraham et al., 2020). The plasma levels (mean ± SD) of PFOA and PFOS were 3.8 ± 1.1 and 6.8 ± 3.4 μg/L in the 21 formula-fed children, and 16.8 ± 6.6 and 15.2 ± 6.9 μg/L in the 80 children who were exclusively breastfed for at least 4 months, respectively. This study showed significant correlations of the level of PFOA, but not of PFOS, with the adjusted levels of vaccine antibodies against *Haemophilus influenza* type b (Hib; $r = 0.32$), tetanus ($r = 0.25$), and diphtheria ($r = 0.23$). These results showed an association between exposure to PFOA and lower levels of antibodies. Moreover, significant associations of PFOA level with lower production of IFN-γ after the stimulation of peripheral blood lymphocytes with tetanus ($r = -0.33$; $P = 0.01$) or diphtheria ($r = -0.24$; $P = 0.08$) toxoid were found in 55 children, suggesting alterations in the cell-mediated immune system. However, no relevant associations were observed between PFOA or PFOS and lymphocyte proliferation after specific or nonspecific stimulation with vaccine components (Abraham et al., 2020). [The Working Group noted that the strengths of the study were that studies on children in their first year are especially relevant because infants have a relatively high exposure if breastfed and may have a higher susceptibility, because their immune system is developing; and the use of a sensitive analytical method to measure PFOA, PFOS, and PFHxS in almost all samples. The plasma concentrations represented the combined exposure from all exposure sources and routes, including metabolites of precursors, and although measured at a single time point, represent exposure over a relatively long period of time, because of the long half-lives of PFOA and PFOS. Also, co-exposures to other substances, including several carcinogens, were considered in the analysis.]

To examine whether serum PFOA and PFOS were associated with a reduced immunity-response to rubella immunization, and whether interactions with sex or ethnicity warranted data stratification, Pilkerton et al. (2018) analysed a nationally representative sample of individuals aged 12 years in NHANES, USA, for the years 1999–2000 and 2003–2004. In total, 581 adult women, 621 adult men (age, 19–49 years), and 1012 young participants (age, 12–18 years) were included. The average serum PFOA concentrations were 6 ± 0.3 ng/mL in men, 4.3 ng/mL in women, and 4.8 ng/mL in the young participants. The average serum PFOS concentrations were 28.1 ng/mL in men, 22.1 ng/mL in women, and 25.1 ng/mL in young participants. Whole-group linear regression analyses of the young participants showed no significant associations of the rubella titre with either PFOA or PFOS, after adjusting for covariates (sex, age, race/ethnicity, educational level, and BMI), nor were there interactions of these PFAS with sex or ethnicity. In adults, there were significant associations of both PFOA ($P = 0.0016$) and PFOS ($P = 0.0295$) quartiles with the rubella titre after adjusting for covariates. In adults, when the effect size analyses were stratified by sex, a significant negative association was observed only for PFOA in men; the association for PFOS was borderline.

Stein et al. (2016), in a cross-sectional study, examined the relations between PFAS serum concentration and measles, mumps, and rubella antibody concentrations in the NHANES cohorts of 1999–2000 and 2003–2004 ($n = 1191$). The study included 1191 children. The geometric means for serum concentrations were 20.8 ng/mL (95% CI, 19.1–22.7 ng/mL) for PFOS and 4.13 ng/mL for PFOA (95% CI, 3.76–4.53 ng/mL). A doubling of PFOS was associated with a 7.4% (95% CI, –12.8% to –1.7%) decrease in mumps antibodies. When restricted to seropositive children, a 6.6% (95% CI, –11.7% to –1.5%) decrease in mumps antibodies was associated with a doubling of PFOA and a 5.9% decrease with a doubling of PFOS.

The largest decrease in antibody concentration (13%) was observed for the association of rubella antibodies with a doubling of PFOS, although only in the seropositive subsets.

Crawford et al. (2023) with financial support by the industry, conducted a systematic review, using PROSPERO to determine the closeness of the association between a doubling in serum PFAS concentration and the difference in $\log_e$[antibody concentration] after vaccination (Crawford et al., 2023). The literature retrieved from PubMed and Web of Science searches was evaluated, and five PFAS, including PFOA and PFOS, were assessed across 14 reports deemed eligible and published between 2012 and 2022. The evaluation of the responses to diphtheria, rubella, and tetanus infections, including a meta-analysis, were supportive of an association with PFAS, with stronger associations identified for PFOA, PFOS, and PFHxS than for PFNA or perfluorodecanoic acid.

In a study conducted in 819 adolescents aged 12–19 years in the NHANES 2003–2004 and 2009–2010 cycles who had detectable serum levels of rubella and measles antibody, Zhang et al. (2023d) found inverse associations of the serum concentrations of PFOA ($P = 0.03$), and to a lesser extent PFOS ($P = 0.22$), with rubella antibodies. An inverse correlation was also found between the serum concentrations of PFOA ($P = 0.01$) and mumps antibodies. However, these associations were present only in adolescents with lower folate levels (measured as a ratio between whole-blood and serum folate levels) and not in adolescents with higher levels. [The Working Group noted that the study did not investigate the potential mechanism or the effect of folate.]

An investigation of the relation between the antibody response after vaccination with an inactivated trivalent influenza vaccine and the circulating levels of PFOA and PFOS was performed in the USA (Looker et al., 2014). The study participants comprised 411 adults who lived for at least 1 year between 1950 and 2004 in one of six water districts in the Mid-Ohio Valley region of the USA, where the inhabitants had elevated PFOA exposure from contaminated drinking-water. To assess the response to influenza vaccination, haemagglutination-inhibition tests were conducted on serum samples collected pre-vaccination and 21 ± 3 days post-vaccination in 2010. Serum samples were also analysed for PFOA and PFOS concentrations. The concentrations of PFOA were as follows: first quartile: 0.25–13.7 ng/mL; second quartile: 13.8–31.5 ng/mL; third quartile: 31.6–90 ng/mL; and fourth quartile: 90.4–2140 ng/mL. The concentrations of PFOS were as follows: first quartile: 0.1–5.8 ng/mL; second quartile: 5.9–9.2 ng/mL; third quartile: 9.3–14.5 ng/mL; and fourth quartile: 14.7–42.3 ng/mL. Questionnaires were completed regarding the occurrence and frequency of recent (during the last 12 months) respiratory infections. The results showed that PFOA at serum concentrations between 13.7 and 90 ng/mL was associated with a reduced response to influenza virus A/H3N2. The authors also found an increased risk of not attaining the antibody threshold considered to offer long-term protection. However, there was no evidence that the PFOS serum concentration was associated with the vaccine response. The authors also found no evidence of an association between self-reported colds or influenza and PFOA or PFOS concentration, suggesting that the extent of suppression of the vaccine response and the associations with disease outcomes are difficult to assess, particularly in a small study sample (Looker et al., 2014).

Andersson et al. (2023) performed an observational study with prospective design of 309 adults from the Ronneby Biomarker Cohort in Ronneby, Sweden, who had a median PFOS serum concentration of 47 ng/mL, and a group from another town with background exposure ($n = 47$) who had a median PFOS serum concentration of 4 ng/mL. These groups received two doses of the Spikevax mRNA vaccine against

COVID-19 (SARS-CoV-2). The serum levels of seven PFAS were measured before vaccination. IgG against the SARS-CoV-2 spike antigen was measured before vaccination and 5 weeks (n = 350) and 6 months (n = 329) after the second vaccine dose. PFAS exposure, regardless of how it was estimated, was not negatively associated with antibody levels after COVID-19 vaccination.

In a study by Porter et al. (2022), a total of 415 participants were included in repeated measures analyses (757 observations) of antibodies against SARS-CoV-2. The participants were current and retired workers from two manufacturing facilities with historical use of PFAS in Alabama and Wisconsin, USA; the study was sponsored by the company. The log-transformed concentrations of anti-spike IgG and neutralizing antibodies were modelled in relation to the concentrations of PFAS at enrolment, after adjusting for antigenic stimulus. The median concentration of PFOS was 121.50 ng/mL and that of PFOA was 31.7 ng/mL. The fully adjusted IgG concentration was 3.45% lower (95% CI, –7.03% to 0.26%) for each 14.5 ng/mL (interquartile range) increase in PFOS. For PFOA, the results were comparable to those for PFOS: the concentration was 1.95% lower (95% CI, –4.35% to 0.51%) for each 3.59 ng/mL (IQR) increase in PFOA.

One of the objectives of the study conducted by Granum et al. (2013) was to investigate the effect of prenatal exposure to PFAS on the responses to paediatric vaccines. To this end, the antibody levels specific for four vaccines in the Norwegian Childhood Vaccination Program were measured: measles, rubella, tetanus, and Hib (Granum et al., 2013). Pregnant women in the prospective BraMat birth cohort (n = 99), a subcohort of MoBa, were recruited during 2007–2008 in Oslo and Arkershus, Norway. Blood samples were collected from the mothers at the time of delivery and from the children at age 3 years. In multivariate models, increased concentrations of all four PFAS, including PFOA (median, 1.1 ng/mL; interquartile range, 0.2–2.7 ng/mL) and PFOS (median, 5.5 ng/mL; interquartile range, 1.4–11.0 ng/mL), in maternal blood (50 samples tested) were significantly associated with reduced levels of anti-rubella antibodies in the children at age 3 years (PFOA bivariate $\beta = -0.40$; 95% CI, −0.64 to −0.17; $P = 0.001$; and PFOS bivariate $\beta = -0.08$; 95% CI, 0.14 to −0.02; $P = 0.007$). However, no significant associations were found between the concentrations of PFAS, including PFOA and PFOS, and antibody levels developed in response to the other vaccines, including tetanus toxoid (Granum et al., 2013). [The Working Group noted that a major limitation of the study was the very small study sample.]

In a randomized controlled trial conducted in rural regions close to Bissau, Guinea-Bissau, where exposure to PFAS is generally low compared with that in all other parts of the world (237 infants; median serum PFOS concentration, 0.77 ng/mL; median serum PFOA concentration, 0.68 ng/mL), a doubling of serum PFOS concentration in children vaccinated at age 4–7 months was associated with a 21% lower concentration of measles antibodies (95% CI, –37% to –2%) measured at age 9 months for each 1 ng/mL increment in blood concentration (Timmermann et al., 2020).

Kaur et al. (2023) investigated the relation between maternal plasma PFAS concentration (n = 72) and SARS-CoV-2 anti-spike IgG antibody protein levels in a New York City, USA-based pregnancy cohort, the Gen C cohort, established in April 2020. Of all the congeners tested, the nine congeners that were measurable in the maternal plasma negatively correlated with the SARS-CoV-2 anti-spike IgG antibody level ($P < 0.05$) in multivariable analyses. There was a significant association for PFOA ($\beta = -0.62$; 95% CI, −1.11 to −0.12; $P = 0.017$), but not for PFOS ($\beta = -0.33$; 95% CI, −0.85 to 0.20; $P = 0.209$). The mean maternal serum levels were 1.10 ng/mL for PFOA and 1.84 ng/mL for PFOS.

In Greenlandic children who participated in the INUENDO cohort and the IVAAQ cohort,

Timmermann et al. (2022) examined associations between exposure to PFAS, PCBs, and mercury in all forms and blood concentrations of diphtheria and tetanus vaccine antibodies after vaccination. The study included cross-sectional data for children aged 7–12 years, collected in Greenland during 2012–2015. A total of 338 children were eligible for the study and had blood samples available, and 175 of these had available vaccination records. In the children, the median concentration of PFOS was 8.68 ng/mL and that of PFOA was 2.28 ng/mL. The authors found that higher childhood exposure to environmental chemicals was associated with lower antibody concentrations after vaccination and with higher odds of not having a sufficient antibody concentration to be protected against diphtheria. The ORs for not being protected against diphtheria were 1.14 (95% CI, 1.04–1.26) for PFOS and 1.41 (95% CI, 0.91–2.19) for PFOA (Timmermann et al., 2022). [The Working Group noted that the concentrations of the specific diphtheria antibodies were quite low, probably due to the time interval since the most recent vaccination or booster.]

End-points of immunity

Associations between neonatal serum concentrations of PFOA and PFOS and immunoglobulin (Ig) isotype profiles in dried blood spots from newborns were assessed in a prospective cohort of infants (3175 infants in the Upstate KIDS prospective birth cohort study, 2008–2010) (Jones et al., 2022). After correction for multiple comparisons, higher PFOA levels in the neonates' blood samples were associated with higher IgA, IgM, and IgG2 levels, and lower levels of IgE, in single-pollutant models, with P values ranging from 0.002 to < 0.00001. However, PFOS showed no significant association with the Ig isotype profile. When PFOA and PFOS were examined as a mixture, with estimates adjusted for infant sex, maternal BMI, race, parity, age, and infertility treatment, small (but significant) to marginal joint effects of the mixture on the outcome isotypes, consistent with single-pollutant PFAS models, were found (Jones et al., 2022). [The Working Group considered that the clinical relevance of these observations remains to be established.]

Zhu et al. (2016) assessed the polarization of T-helper lymphocytes in 231 asthmatic children and 225 non-asthmatic children (controls) from the Genetic and Biomarkers study for Childhood Asthma (GBCA) in northern Taiwan, China. The levels of Th1 (IFN-γ and IL-2) and Th2 (IL-4 and IL-5) cytokines were measured using ELISA. The Th2/Th1 cytokine concentration ratio and the IgE levels were in accordance with the asthmatic status of the patients. The results showed that asthmatic children had significantly higher serum PFAA concentrations, but not higher concentrations of specific PFAS, compared with the healthy controls. In male asthmatic children, significant positive associations were found between the IL-4/IFN-γ ratio and PFOS and PFOA (correlation coefficients, Spearman $\rho = 0.210$; $P = 0.008$; and Spearman $\rho = 0.290$; $P < 0.001$, respectively), and between the IL-5/IFN-γ ratio and PFOS and PFOA (Spearman $\rho = 0.198$; $P = 0.017$; and Spearman $\rho = 0.189$; $P = 0.017$, respectively). In female asthmatic children, significant associations were found only of the IL-4/IFN-γ ratio with PFOS (Spearman $\rho = 0.291$; $P = 0.013$), and of the IL-5/IFN-γ ratio with PFOS (Spearman $\rho = 0.245$; $P = 0.037$). [The Working Group considered that the limitation of these analyses should be noted, with only single serum samples being collected for cytokine measurement.]

Lopez-Espinosa et al. (2021) studied the associations of PFHxS, PFOA, PFOS, and PFNA with leukocyte counts (neutrophils, monocytes, eosinophils, lymphocytes, and basophils). Serum PFAS concentrations were measured, and leukocyte types were counted in 42 782 (between 2005 and 2006) and 526 (during 2010) adults living in an area with PFOA contamination of

drinking-water in the Mid-Ohio Valley, USA. The circulating numbers of immune cells (CD3+ T-cells, CD3+ CD4+ T-helper cells, CD3+ CD8+ T-cytotoxic cells, CD3+ CD4+ CD8+ double-positive T-cells, CD16+ CD56+ natural killer (NK) cells, and CD19+ B cells) were measured only in the 2010 adults. Data on the study participants were collected through questionnaires, and the covariates sex, race/ethnicity, age, education, BMI (available for the 2005–2006 survey), tobacco consumption, alcohol intake, a present or past diagnosis of an immune disease and/or cancer, and the use of anti-inflammatory medication (available for the 2010 survey) and other medication were considered. A weak consistent association was shown for both PFOA and PFOS with the total lymphocyte count, but no clear associations with the various lymphocyte subtypes. No clear associations were found between the total leukocyte count and PFAS level, with statistical significance only being reached for PFHxS and PFOS in the 2005–2006 survey (Lopez-Espinosa et al., 2021). [The Working Group noted that the small differences in the overall lymphocyte count observed in the study cannot be considered clinically meaningful.]

(ii) Human cells in vitro

Mechanisms of PFOA- and PFOS-induced immunosuppression were evaluated in vitro using human primary immune cells (lymphocytes and monocytes) and human immune cell lines. In a recent study, Kasten-Jolly and Lawrence (2022) showed a significant reduction in the proliferation of human T-cells (from peripheral blood mononuclear cells (PBMCs) isolated from healthy staff of the New York State Department of Health) in response to phytohaemagglutinin (PHA; polyclonal activation) or influenza antigen (memory T-cell activation) in the presence of 100 μM PFOA or PFOS (cells were exposed to increasing concentrations of the two chemicals at 1, 10, and 100 μM). Moreover, both PFOA and PFOS at the concentration of 100 μM significantly ($P < 0.05$) suppressed the release of IFN-γ, a cytokine known to be important for the immune response to viruses. The production of other cytokines and chemokines by human PBMCs was also affected by PFOA (MIP3α/CCL20, ITAC, IL-10, IL1-β, IL-6, and IL-7) and PFOS (same as for PFOA, plus GM-CSF, fractalkine, IL-17, IL-13, IL-21, and TNF-α). PFOS was more potent than PFOA. However, for both molecules, IL-2 production was significantly augmented, despite an inhibition of proliferation. These effects were significant with 100 μM of PFOA or PFOS, except for CCL20, the production of which was increased by 10 μM of either chemical.

The cytokines released by T lymphocytes were evaluated using a whole-blood assay and peripheral blood from healthy donors after stimulation with the polyclonal activator PHA. Both PFOA and PFOS reduced PHA-induced IL-4 and IL-10 release, whereas IFN-γ release was affected only by PFOS (Corsini et al., 2011). In all the tests performed, PFOS was a more potent inhibitor of cytokine production than PFOA. At the concentration of 0.1 μg/mL (equivalent to 241 nM PFOA and 200 nM PFOS), only PFOS was able to reduce the secretion of TNF-α, IL-4, IL-6, IL-10, and IFN-γ. The same assay was used to assess the release of pro-inflammatory cytokines after LPS stimulation. Both PFOA and PFOS induced a dose-related decrease in TNF-α production, IL-8 release was unaffected, and the release of IL-6 was reduced by PFOS, but not by PFOA (Corsini et al., 2011). Similar findings were reported in the human promyelocytic cell line THP-1, with PFOS causing significant decreases in the LPS-induced release of TNF-α and IL-8, starting from a concentration of 1 μg/mL. In these models, the suppression of cytokine production by PFOA was dependent upon PPARα activation, whereas the effects of PFOS were independent of PPARα activation. On the other hand, Midgett et al. (2015) demonstrated that PFOA did not have an impact on IL-2 production, but PFOS

at a concentration of 100 μg/mL suppressed IL-2 production in both the Jurkat T-cell line and human primary CD4+ T cells isolated from leukocytes of healthy donors (n = 11).

Using human primary lymphocytes (6 donors) as a model, together with integrative omics analyses, including of the transcriptome and lipidome, and bioinformatics analysis, Li et al. (2020c) observed that PFOS moderately altered the production of interleukins (IL-1, IL-4, IL-6, and IL-8) in human lymphocytes and dysregulated clusters of genes and lipids that play important roles in immune function, such as in lymphocyte differentiation, the inflammatory response, and the immune response. The same group performed a metabolomic analysis on human lymphocytes and highlighted alterations in organic acids, including taurine and NG-dimethyl-l-arginine; organooxygen molecules, such as d-ribulose 5-phosphate; and lipid compounds, such as carnitines; in lymphocytes treated with PFOA (Li et al., 2020d).

Corsini et al. (2012), using a human whole-blood assay and LPS stimulation, confirmed the inhibitory effect of PFOS, and to a lesser extent PFOA, on TNF-α production. The concentrations used were 0.1, 1, and 10 μg/mL. These results were confirmed using the human promyelocytic cell line THP-1.

In another study that used the monocytic THP-1 cell line under LPS stimulation, Masi et al. (2022) also observed that PFOS (0.2–20 μM) induced a dose-dependent downregulation of RACK-1 receptor promoter transcriptional activity, mRNA expression, and protein levels, which was mirrored by reductions in IL-8 and TNF-α production and CD86 expression.

Houck et al. (2023) tested 147 PFAS using a phenotypic screening platform of human primary cell co-culture systems, the BioMAP Diversity PLUS panel. This platform models tissues and the disease biology of organs (vasculature, immune system, skin, and lung). The platform includes 12 assays that use 12 different human primary cell systems and includes 148 mechanistic end-points, of which several are modulators and effectors of vascular and immune biology. The authors included four known immunosuppressants (azathioprine, methotrexate, dexamethasone, and cyclosporine A), covering different mechanisms of immunosuppressive action, in the test set, along with the 147 PFAS (Houck et al., 2023). PFOA and PFOS reduced the level of IL-10 in the co-culture system, and their mechanisms of action were different from those of other known immunosuppressants.

Human NK-cell activity has recently been shown to be slightly inhibited after 24 hours of exposure of human primary PBMCs from male donors to 0.2 μM PFOS (Maddalon et al., 2023b).

[The Working Group noted that PFOS and, to a lesser extent, PFOA reduced the production of some cytokines, with the possible involvement of PPARα and lipid metabolism, and also altered lymphocyte proliferation and NK-cell activity. The latter effect was only in cells isolated from male donors.]

(b) Experimental systems

(i) PFOA in non-human mammalian systems in vivo

Effects on immune end-points

PFOA was evaluated using standard assays of immune function (Yang et al., 2002a; De Witt et al., 2008; McDonough et al., 2020; De Guise and Levin, 2021).

In the study by De Witt et al. (2008), sheep red blood cell (SRBC) IgM antibody titres were suppressed in C57BL/6J and C57BL/6N mice in a dose-dependent manner at a PFOA exposure of ≥ 3.75 mg/kg per day (ammonium salt; purity, ≥ 98%) for 10–15 days given via the drinking-water. However, the IgG titres were elevated at PFOA exposure levels of 3.75 and 7.5 mg/kg per day (De Witt et al., 2008).

Using a diet supplemented with PFOA at 0.02% (w/w), Yang et al. (2002a) showed a clear decrease in the production of antibodies of both IgM and IgG isotypes against horse erythrocytes injected intravenously ($5–10 \times 10^7$ cells in 200 mL of Earle balanced solution) into C57BL/6 mice.

A significant decrease in the IgM antibody response to the T-cell-dependent antigen keyhole limpet haemocyanin (KLH) was observed with PFOA at a dose of 1.88 mg/kg per day given to $B6C3F_1$ mice in their drinking-water for 4 weeks (De Guise and Levin, 2021).

Altered T-lymphocyte populations were observed in male ICR mice exposed to 0, 2, 10, 50, or 250 ppm (0, 0.49 ± 0.04, 2.64 ± 0.15, 12.63 ± 1.15, or 47.21 ± 3.57 mg/kg per day) PFOA (free acid; purity, 98%) in drinking-water for 21 days. In the spleen, all the doses of PFOA reduced CD8+ lymphocytes, and CD4+ lymphocytes were increased by PFOA at 50 and 250 ppm. Exposure to PFOA at 250 ppm increased CD8+ lymphocytes in the thymus. However, PFOA induced a significant decrease in body weight from the initial value at the two highest doses (Son et al., 2009) (see also Section 4.2.7(c) and Section 4.2.6(b)(i)).

Iwai and Yamashita (2006) treated male Crj:CD(SD)IGS rats orally for 14 days with PFOA (ammonium salt, 10% aqueous solution). The doses of PFOA were 0, 0.5, 5, and 50 mg/kg per day. Blood parameters (haematocrit, erythrocyte count, and haemoglobin) were reduced by PFOA at 50 mg/kg, but the reticulocyte count was not affected. No influence on lymphocyte subsets in blood (T-cells and NK cells) was observed at any of the tested doses. [The Working Group noted that the circulating levels of PFOA were not measured in this study.]

Loveless et al. (2008) conducted a study with the objective of comparing the effects of PFOA (ammonium salt, 19.5% aqueous solution) in male CD rats and CD-1 mice. Both species were dosed by oral gavage with *n*-PFOA at 0.3, 1, 10, or 30 mg/kg per day for 29 days. In the rats, the production of anti-IgM SRBC antibodies was not affected; however, systemic toxicity (reduced body-weight gain and increased corticosterone levels) was observed at the 10 and 30 mg/kg doses. Systemic toxicity was also observed in mice at the 10 and 30 mg/kg doses. In mice, reduced IgM antibody production after 10 mg/kg was observed (20% suppression), along with reduced spleen and thymus weights and cell numbers, as well as microscopic depletion/atrophy of lymphoid tissue (thymus and spleen). [The Working Group noted that non-specific alteration of the immune response to SRBCs (a T-cell-dependent antigen) because of systemic toxicity of PFOA in mice cannot be excluded.]

The proliferation in response to both T-cell (concanavalin A, ConA) and B-cell (LPS) activators of ex vivo spleen cells derived from male C57BL/6 mice fed for 10 days with a diet containing 0.02% (w/w) PFOA was attenuated by the PFOA treatment (Yang et al., 2002a).

Because of concerns regarding skin exposure, the immunotoxicity of PFOA (free acid; purity, 96%) after dermal exposure (0.5–2% w/v, or 12.5–50 mg/kg per dose administered topically on the dorsal portion of the ear) has been also evaluated in mice. The IgM antibody response to SRBCs was significantly reduced in the spleen after 4 days of dermal exposure in $B6C3F_1$ mice (Shane et al., 2020). In addition, PFOA exposure caused a significant decrease in thymus and spleen weights after 4 and 14 days of exposure. Immune-cell phenotyping identified a reduction in the frequency of splenic B-cells (Shane et al., 2020).

Effects on the developing immune system in mice

Experiments have also been conducted in mice to study the effects of PFOA and PFOS on the developing immune system (Keil et al., 2008; Hu et al., 2010, 2012; Zhong et al., 2016; Torres et al., 2021). The mean SRBC-specific IgM antibody titres in the female offspring (PND48) of C57BL/6 mouse dams exposed to

PFOA (ammonium salt; purity, ≥ 98%) from GD6 to GD17 were not affected by doses of up to 1 mg/kg bw given in the drinking-water (Hu et al., 2010). The adult offspring of dams exposed to PFOA at 2 mg/kg during gestation and lactation showed a reduction in the percentage of splenic CD4+CD25+Foxp3+ T-cells and in IL-10 production, suggesting alterations to regulatory T cells (Hu et al., 2012).

Effects in models of infection in mice

One study showed that PFOA (purity, 96%) can accumulate in the lungs, making this organ more susceptible to viral infections (Ahmad et al., 2021). Ahmad et al. (2021) observed in CD-1 mice orally exposed to 5 or 20 mg/kg per day PFOA for 10 days that it accumulated in the lungs, with mean concentrations of 14.14 ± 2.95 and 36.41 ± 15.09 µg/g, respectively, measured in the lung tissue. Changes in mRNA expression of the DNA methylation regulator genes encoding DNA methyltransferases (*Dnmts*) and tet methylcytosine dioxygenases (*Tets*) were observed, along with the genes encoding the membrane proteins angiotensin converting enzyme 2 (*Ace2*) and transmembrane serine protease 2 (*Tmprss2*), which are involved in SARS-CoV-2 virus infection (see also Section 4.2.4).

(ii) PFOS in non-human mammalian systems in vivo

Effects on immune end-points

Experimental data derived mainly from in vivo experiments in rodents (rats or mice) exposed for 7–60 days using the oral route (gavage or food intake). In vivo antibody production was evaluated after immunization with a specific antigen, and ex vivo experiments measured lymphocyte proliferation, cytokine production, and NK cell activity. Doses of PFOS above 20 mg/kg (total administered dose, TAD) were found to be toxic (reduced body weight) and were considered not to be relevant in general.

In vivo daily exposure of $B6C3F_1$ mice for 28 days to PFOS (potassium salt; stated purity, > 98%) at 0, 0.166, 1.66, 3.31, 16.6, 33.1, or 166 µg/kg bw per day, given by gavage, significantly reduced the T-cell-dependent production of antibodies against SRBCs in cells isolated from the spleen, assessed using a plaque-forming cell assay. The lowest observed effect level was 0.05 mg/kg TAD over 28 days in male mice and 0.5 mg/kg TAD in female mice, corresponding to 1.66 and 3.31 µg/kg per day, respectively (Peden-Adams et al., 2008). The measured PFOS serum concentrations at these doses were 91.5 ± 22.2 ng/mL and 666 ± 108 ng/mL (mean ± SD), respectively. NK-cell activity was significantly increased in male mice at the doses of 0.5, 1, and 5 mg/kg TAD, but not in females. The serum trinitrophenyl (TNP)-specific IgM titres were also reduced by PFOS after TNP-LPS (TNP conjugated with LPS) challenge, suggesting that the humoral immune effects may be attributed to B-cells rather than T cells, because both T-dependent (SRBC) and T-independent (TI) (TNP-LPS) antigen administration resulted in suppressed IgM production (Peden-Adams et al., 2008).

Dong et al. (2009) also observed a significant reduction in anti-SRBC IgM antibodies in male C57BL/6 mice daily exposed for 60 days by gavage to PFOS (potassium salt; purity, > 98%) at 0, 8.33, 83.33, 416.67, 833.33, or 2083.33 µg/kg bw per day, with a no observed adverse effect level (NOAEL) of 8.33 µg/kg per day (0.5 mg/kg TAD) and a lowest observed adverse effect level of 83.33 µg/kg per day (5 mg/kg TAD). The measured PFOS serum concentration for 0.5 mg/kg TAD was 0.674 ± 0.166 mg/L.

In a follow-up study, Dong et al. (2011) used a similar protocol but slightly different doses. The NOAEL for a decrease in IgM was 16.7 µg/kg per day and the lowest observed adverse effect level was 83.3 µg/kg per day (TAD, 5 mg/kg per day).

Antibodies to SRBC were also found to decrease after 7 days' exposure to PFOS (potassium salt; purity, > 98%) by gavage at 5 mg/kg per

day, and the PFOS level was 110.46 ± 6.18 mg/L (Zheng et al., 2009).

Delayed-type hypersensitivity (DTH), a marker of cell-mediated immunity, in adult male C57BL/6 mice treated with PFOS orally for 60 days was not altered by doses up to 50 mg/kg, corresponding to PFOS at 833.3 µg/kg bw per day and a serum level of 51.71 ± 3.81 mg/L (Dong et al., 2011).

In a study by McDonough et al. (2020), a formulation containing several PFAS (a commercial brand of electrochemically fluorinated AFFF) was administered for 10 days via gavage to female and male C57BL/6 mice (PFOS + PFOA, at 0, 1.88, 3.75, 7.5, or 10 mg/kg bw). The results showed that antigen-specific antibody production was suppressed, on average, by 13% in male mice and by 12.4% in female mice across all the doses (McDonough et al., 2020). [The Working Group considered that these results obtained in vivo in mice might suggest that T-cell-dependent antibody production was the main target of PFOS.]

Splenic T-cell immunophenotypes (expressing CD4 and CD8 markers or not) were minimally altered in B6C3F_1 female mice (age 7–8 weeks), but all the T-cell subpopulations were slightly but significantly modulated in male mice by PFOS at 0.1–0.5 mg/kg TAD for 28 days by the oral route. The PFOS doses corresponded to 131 ± 15.2 ng/mL for male mice and 123 ± 18.7 ng/mL for female mice (Peden-Adams et al., 2008).

Also, the absolute numbers of splenic cells expressing CD19/CD21 or major histocompatibility complex (MHC) II markers in adult female B6C3F_1 mice treated orally with PFOS for 28 days were not altered at doses up to 5 mg/kg TAD (Fair et al., 2011). The splenic and thymic cellularity of adult male C57BL/6 mice was significantly reduced at oral doses of PFOS from 25 mg/kg for 60 days. At this same dose, splenic and thymic CD4+ lymphocytes were altered in adult male C57BL/6 mice. The NOAEL was 5 mg/kg TAD. Splenic CD8+ and B-cell numbers were also affected at 50 mg/kg and above (Dong et al., 2009). Splenic and thymic cellularity, including CD4+, CD8+, and B-cells, were also decreased in adult male C57BL/6 mice after oral exposure to PFOS for 7 days at doses from 20 mg/kg (Zheng et al., 2009). [The Working Group considered that PFOS mostly altered lymphocyte function, but also induced a decrease in the number of cells at doses of 25 mg/kg and above.]

Other immune parameters (lymphoproliferation, NK cell activity, and cytokines) have been measured ex vivo in mice using splenocytes. NK cells are an important component of the immune surveillance for cancer and provoke the lysis of cells not expressing MHC class I molecules, such as cancer and virally infected cells.

NK cell activity, measured with a chromium assay and expressed in lytic units, was increased in male B6C3F_1 mice at 0.5, 1, and 5 mg/kg TAD compared with controls, but was not altered in females after 28 days' exposure to PFOS by gavage (Peden-Adams et al., 2008). An augmentation of splenic NK cell activity, determined by lactate dehydrogenase release, was also observed in adult male C57BL/6 mice after oral exposure to PFOS at 5 mg/kg TAD for 60 days (Dong et al., 2009).

T-lymphocyte and B-lymphocyte proliferation, measured after polyclonal activation using mitogens (ConA or LPS), was not affected in B6C3F_1 mice exposed by gavage to PFOS for 28 days at doses up to 5 mg/kg TAD, and in male C57BL/6 mice after oral exposure to PFOS for 60 days at doses up to 25 mg/kg TAD (Peden-Adams et al., 2008; Dong et al., 2009).

T-cell proliferation in response to ConA stimulation in PFOA-exposed Balb/c mice was inhibited after PFOS (potassium salt; purity, > 98%) exposure, and the T-cell receptor signalling, calcium signalling, and p38 MAPK signalling pathways, as measured by microarray data analysis, and calcium ion influx were augmented in mouse splenocytes (Lv et al., 2015). However, splenic T-lymphocyte proliferation was slightly

decreased in male C57BL/6 mice after oral exposure to PFOS for 7 days at 5 mg/kg per day (Zheng et al., 2009).

Ex vivo IL-6 production by B-cells was significantly increased by in vitro stimulation with either anti-CD40 or LPS in female $B6C3F_1$ mice exposed orally for 28 days to PFOS (potassium salt; stated purity, > 98%) at 1 mg/kg TAD (Fair et al., 2011). IL-4 secretion was increased by exposure to PFOS at ≥ 5 mg/kg TAD administered to male C57BL/6 mice daily via gavage for 60 days (Dong et al., 2011) and also after oral exposure to PFOS for 7 days at 5 mg/kg per day (Zheng et al., 2011).

Pierpont et al. (2023) measured immune parameters after chronic exposure of C57BL/6 mice to PFOS (potassium salt; purity, 88.9%). Mice were exposed to PFOS at 0.15, 1.5, 15, or 50 µg/kg for 28 days, after which B cells, T cells, and granulocytes from the bone marrow, liver, spleen, lymph nodes, and thymus were assessed. No effects of PFOS on the major T- or B-cell populations, macrophages, dendritic cells, basophils, mast cells, eosinophils, neutrophils, serum antibodies, or selected serum cytokines were observed. All the experimental groups had elevated serum PFOS levels, with the highest levels obtained at 50 µg/kg per day (females, 2792.0 ± 295.3 ng/mL, and males, 2159.2 ± 164.6 ng/mL).

Interestingly, when male $B6C3F_1$ mice were exposed to a PFOS tetraethylammonium salt at a TAD of 7 mg/kg bw (equivalent to a TAD for PFOS anion of 5.55 mg/kg) in the diet for 28 days, a serum concentration of 11 µg/mL was yielded. However, no effects were found on the cellular compositions of the thymus and spleen, the number of splenic cells secreting IgM antibodies against SRBCs or TNP-LPS (Qazi et al., 2010). [The Working Group noted that only a single dose administered via the diet was used in the study.]

Lefebvre et al. (2008) also addressed the effects of PFOS on immune system function (potassium salt; stated purity, ≥ 98%) using exposure via the diet, but in Sprague-Dawley rats. The rats were exposed for 28 days to PFOS at 2–100 mg/kg diet (corresponding to approximately 0.14–7.58 mg/kg per day). The body-weight reductions induced were significant in male and female rats exposed to PFOS at 50 or 100 mg/kg diet. There were no effects on the DTH response to KLH, KLH-specific IgG in the serum, or splenic T- and B-cell proliferation in response to a mitogen ex vivo (Lefebvre et al., 2008).

Effects on the developing immune system in mice

In the $B6C3F_1$ pups of dams exposed orally to PFOS (potassium salt; purity, 91%) during GD1–GD17, NK cell function and IgM production were significantly decreased at age 8 weeks. The NOAEL was 0.1 mg/kg per day (Keil et al., 2008). C57BL/6 mouse pups were evaluated for developmental immunotoxicity after maternal oral exposure to PFOS (potassium salt; purity, > 98%) of 0.1, 1.0, or 5.0 mg/kg per day during GD1–GD17 (Zhong et al., 2016). The results showed alterations in splenic and thymic cellularity, in T- and B-cell proliferation measured ex vivo after 5 mg/kg exposure, in splenic NK-cell activity at age 8 weeks, and in the antibody response at age 4 but not 8 weeks.

Effects in models of infection in mice

Experimental models of infection are often used to address the consequences of immunosuppression on host resistance.

A 21-day exposure to PFOS (potassium salt) at 25 µg/kg per day, corresponding to PFOS at 670 ± 47 ng/mL in the serum, resulted in a significant increase in emaciation and mortality in response to influenza A virus in $B6C3F_1$ mice (Guruge et al., 2009).

In another study in C57BL/6 mice exposed to PFOS (potassium salt; purity, 88.9%) at 1.5 µg/kg per day for 4 weeks or to 3 µg/kg per day for 14 days, no effects were found on influenza virus clearance or antibody or T-cell-specific antiviral responses, indicating that for these doses

and durations, PFOS did not suppress the antigen-specific immune response. The PFOS serum levels were 99.6 ± 4.4 and 116 ng/mL ± 2.8 ng/mL for 1.5 and 3 µg/kg per day, respectively (Torres et al., 2021).

In a mouse model of *Citrobacter rodentium* infection, persistent treatment with PFOS at 2 mg/kg (potassium salt; purity, > 98%) increased the bacterial count, and this was accompanied by increases in inflammatory cytokine concentrations and dysbiosis, suggesting an alteration in intestinal immunity (Suo et al., 2017).

(c) Mechanism of action of PFOA and PFOS on the immune system

Taylor et al. (2023) confirmed in C57BL6 mice treated for 15 consecutive days the significant decrease of IgM antibodies directed against SRBCs. They also observed changes in the numbers of B-cell subsets as well as mitochondrial markers after PFOA (free acid) exposure, indicating that the B-cell developmental trajectory could be altered through effects on B-cell differentiation or proliferation, leading to suppression of the T-cell-dependent antibody response (TDAR).

PFOA and PFOS have been described to have pro-inflammatory effects, mediated through cytokine production (as also reported in Section 4.2.6), which could indirectly modulate the immune response. Son et al. (2009) found that levels of TNF-α, IL-1β, and IL-6 were augmented in the spleen in male mice exposed to PFOA (ammonium salt; purity, 98%) in drinking-water for 21 days. However, most in vitro studies conducted with human or rodent immune cells showed a decrease in pro-inflammatory cytokine production. PFOA and PFOS reduced the number of macrophages (CD11b+ cells) in the bone marrow, but not in the spleen or peritoneal cavity, in C57BL/6 mice receiving 0.02% (w/w) PFOS (tetraethylammonium salt; purity, 98%) or PFOA (free acid; purity, 96%) in the diet for 10 days (Qazi et al., 2009). This high-dose, short-term exposure also augmented the inflammatory responses to LPS. Dietary treatment of male C57BL/6 mice with 0.002% (w/w) PFOA or 0.005% (w/w) PFOS for 10 days attenuated the hepatic levels of TNF-α, IFN-γ, and IL-4 (Qazi et al., 2010).

[The Working Group noted that the mechanisms of action of PFOA and PFOS on the immune system are still a matter of debate.]

A role of PPARα in the PFOA-induced reduction in thymus weight and cellularity has been evoked in mice (Yang et al., 2000, 2001). In PPARα-null mice, reductions in spleen weight and in the number of splenocytes after PFOA treatment were absent, and the decrease in the number of thymocytes was significantly less marked (Yang et al., 2002b). The response of splenocytes isolated from the spleens of PFOA-treated PPARα-null mice to appropriate T- or B-cell activators in vitro was not altered compared with wildtype mice (Yang et al., 2002b). However, De Witt et al. (2016) found that exposure to PFOA at 30 mg/kg suppressed the TDAR in both WT and PPARα KO C57BL/6 mice, suggesting that the suppression of TDAR might be independent of PPARα involvement.

To determine whether the immunotoxicity of PFOA (free acid; purity, > 96%) is associated with lipid metabolism, male BALB/c mice were fed either a regular diet or an HFD, and exposed to PFOA at doses of 0, 5, 10, or 20 mg/kg per day for 14 days (Wang et al., 2014a). The results suggested that an excess of dietary lipids did not prevent the PFOA-induced immune suppression caused by peroxisome proliferators. Moreover, immunomodulation by PFOA was via the PPAR pathway and involved the induction of mitochondrial damage and the lymphocyte apoptosis pathway.

Ten-day treatment of male 129/Sv PPARα-null mice with different dietary doses (0.001%–1% w/w) of PFOS showed that the thymic changes were partially dependent on PPARα (Qazi et al., 2009). It has been suggested that PFOS (free acid;

purity, > 98%) may indirectly affect the immune organs by interfering with lipid metabolism, leading to co-senescence of the thymus and spleen (Wang et al., 2011).

Corsini et al. (2011) conducted in vitro experiments showing that PFOA and PFOS (free acids) suppressed LPS-induced TNF-α production in human primary cultures and THP-1 cells, whereas IL-8 was suppressed only in THP-1 cells, and IL-6 release was reduced only by PFOS. Both PFOA and PFOS reduced PHA-induced IL-4 and IL-10 release from T-cells, whereas IFN-γ release was affected only by PFOS. In all instances, PFOS was more potent than PFOA. Using siRNA, a role for PPARα in PFOA-induced immunotoxicity could be shown, whereas an inhibitory effect on LPS-induced I-κB degradation was identified that could explain the immunomodulatory effect of PFOS, suggesting different mechanisms of action (Corsini et al., 2011).

Maddalon et al. (2023b) showed that PFOS (free acid) reduced RACK-1 expression in a recent report. RACK-1 is a kinase involved in immune function and cytokine expression.

Recently, Zhang et al. (2023e) used a systemic evidence map (SEM) approach and found 1155 studies showing that PFAS were involved in either immune effects or chronic inflammation, of which 321 qualified for inclusion in their data set. The SEM showed decreased B-cell activation and altered levels of T-cell subtypes and immunoglobulins, confirming PFAS-induced immunosuppression (Zhang et al., 2023e).

Cell death is often observed after immunosuppressant treatment, explaining the observations of lymphoid organ atrophy. Adult male C57BL/6 mice treated with PFOS (potassium salt; purity, > 98%) at 0, 1, 5, or 10 mg/kg per day by gavage daily for 7 days had more apoptotic cells than in control mice. The PFOS-induced production of ROS and alteration in mitochondrial membrane potential could lead to the apoptosis of splenocytes and thymocytes (Zhang et al., 2013d).

In adult C57BL/6 mice dosed daily by oral gavage with PFOS (potassium salt; purity, > 98%) at 0, 0.0167, 0.0833, or 0.8333 mg/kg per day, yielding target PFOS TADs of 0, 1, 5, or 50 mg/kg, respectively, for 60 days, PFOS induced p53-dependent apoptosis through Bcl-xl downregulation, without changing Bcl-2 or Bax expression (Dong et al., 2012b). The release of cytochrome c and activation of caspase-3 confirmed the involvement of the mitochondria.

Synopsis

[The Working Group noted that in exposed humans, PFOA and PFOS have been found to be associated with the augmentation of LRTIs and diarrhoea and reduced vaccination efficacy in children in several environmental studies conducted in different countries. An association between PFOA and a reduced response to influenza vaccination has recently been identified in adults, and also for SARS-CoV-2 vaccination in a maternal cohort. The limitations of environmental studies are mainly associated with the use of self-reported data in questionnaires and the existence of confounding factors. Importantly, these observations were supported by evidence that PFOA and PFOS affect the response of human primary immune cells (proliferation and cytokine production). Moreover, evidence of immunosuppression after exposure to PFOA and PFOS, which mainly affected the TDAR (T-cell-dependent antibody response), has also been reported in several animal experiments.]

4.2.8 Modulates receptor-mediated effects

See Tables S4.23–S4.26 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636), and Table 4.27.

With regard to the modulation of receptor-dependent pathways in humans, most epidemiological data have come from studies on the receptor

ligands (e.g. changes in the serum concentrations of ligands that activate receptor-dependent pathways) or hormones that regulate the expression of receptor ligands (e.g. thyroid-stimulating hormone TSH and luteinizing hormone LH). The epidemiological studies considered here can be found in Table S4.23. Human in vitro studies have investigated the activation/antagonism of multiple nuclear receptors, with a focus on the activation of PPARα. However, rodent studies have made it clear that PFOA and PFOS target more than just PPARα (Rosen et al., 2010; Attema et al., 2022; Su et al., 2022a). Table S4.24 summarizes the available studies that investigated the ability of PFOA and PFOS to bind and/or activate nuclear receptors in human systems in vitro. Evidence was gathered from transcriptional and biological assays performed in human primary hepatocytes and human liver cell models, as well as binding and reporter assays.

(a) Humans

(i) Thyroid hormone pathway

Exposed humans

See Table S4.23 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Child–adult exposure

Several studies have investigated the associations of PFOA and PFOS with markers of thyroid function and disease in children and adults. [The Working Group selected the most informative studies, based on a prospective study design, a high level of exposure (including occupational and contaminated community exposure), and large study size.]

Two prospective cohort studies of female and male adults (USA) and children (Republic of Korea) have investigated the associations of serum PFOA and PFOS levels with TSH, free triiodothyronine (FT3), total triiodothyronine (TT3), free thyroxine (FT4), and/or total thyroxine (TT4) (Blake et al., 2018; Kim et al., 2020).

In the study conducted in adults, serum collected at the initial enrolment examination and at subsequent follow-up examinations during the years 1991–2008 was analysed for PFOA, PFOS, TSH, and TT4 levels. An IQR increase in serum PFOS, but not PFOA, was associated with a 9.75% (95% CI, 1.72%–18.4%) increase in TSH, but neither PFOA nor PFOS was associated with TT4 (Blake et al., 2018). [The Working Group noted, however, that due to a change in protocol, fewer repeated TT4 measurements than TSH measurements were available. This was also a highly exposed community.]

In children, TSH levels were measured at age 2, 4, and 6 years. The relation of serum PFOA and PFOS concentrations with TSH levels at the three time points was assessed by repeated-measures analysis using linear mixed models. Serum levels of FT4 and T3 were measured once (at age 6 years). PFOA was associated with a low TSH level only in male participants, and no association was detected for PFOS (Kim et al., 2020). The PFOA level at age 6 years was not associated with T3, but it was associated with high FT4 at age 6 years, primarily in male participants (Kim et al., 2020). The PFOS level at age 6 years was associated with high levels of T3 at the same age, primarily in male participants, but was not associated with FT4 (Kim et al., 2020). Age 6 years was the one time point at which a significantly higher PFOS concentration was reported in male than female participants (Kim et al., 2020).

There have been several cross-sectional studies conducted in highly exposed populations. A cross-sectional study of highly exposed adult male workers in a fluorochemical plant, conducted by the industry, showed that their serum PFOA levels were associated with a decrease in FT4 within the normal reference range, without associations with TSH, T3, or TT4 (Olsen and Zobel, 2007). A cross-sectional study

of highly exposed adult female and male workers exposed to fluorochemicals from two manufacturing facilities showed that the serum PFOS levels in men from all the locations combined were associated with an increase in T3, but no associations were seen with TSH, FT4, or TT4. No associations of the serum PFOS levels with TSH, FT3, FT4, or TT4 were found in women in another industry-conducted study (Olsen et al., 2003b). Large cross-sectional studies (n > 10 000) have reported few associations of serum PFOA or PFOS levels with TSH or thyroid hormones. A cross-sectional study of males and females aged 14–39 years in the Veneto region of Italy found no associations of serum PFOA or PFOS levels with TSH (Gallo et al., 2022). [The Working Group noted that this was an analysis of a highly exposed community.]

In a large study of children and adolescents from a community contaminated by PFOA from a fluorochemical-production plant (the C8 study cohort in the Mid-Ohio Valley, USA), serum PFOS, but not PFOA, was associated with a small (1.1%) IQR increase in TT4 (Lopez-Espinosa et al., 2012). Neither the PFOA nor the PFOS serum level was associated with TSH (Lopez-Espinosa et al., 2012).

Similarly, moderately sized cross-sectional studies (n = 1000–2000) have reported associations between serum PFOA or PFOS levels and TSH and thyroid hormones. In a study of cross-sectional data from male and female adolescents, adults, and older adults in the USA (NHANES 2007–2008), serum PFOA level was associated with increased TSH and TT3 levels, but not with free iodothyronine (FT3), FT4, or TT4 (Jain, 2013). No associations of serum PFOS level with TSH, FT3, TT3, FT4, or TT4 were found (Jain, 2013). However, when low iodine and high thyroid peroxidase antibody (TPOAb) levels were taken into account in a subset of the same study sample, serum PFOA and PFOS levels were associated with increases in TSH, FT3, and TT3, and PFOS was negatively associated with FT4. The authors observed that in the T0I0 group, with normal TPOAb and iodine concentrations, serum PFOA was associated with increased FT3 levels (1.2%; 95% CI, 0.1–2.4%) (Webster et al., 2016).

A study of NHANES data from the 2007–2008 and 2009–2010 cycles stratified by age and sex reported that whereas PFOA was associated with an increase in FT3 in both sexes, it was associated with an increase in TT3 only in women (Wen et al., 2013). PFOA was not found to be associated with TSH or FT4/TT4, and the PFOS level was not found to be associated with TSH or thyroid hormones in this study (Wen et al., 2013).

A similar analysis of NHANES data from the 2011–2012 participants showed that the PFOA level was not associated with TSH, FT3, TT3, FT4, or TT4, and that the PFOS level was only associated with an increase in FT4 (van Gerwen et al., 2020). However, when analysed by sex and age, the PFOA level in the same cohort was associated with reduced TSH (in females aged 12–19 years), increased TT3 and FT3 (in women aged 60–80 years), and increased FT4 (in women aged 20–39 years). PFOA was not associated with TSH or thyroid hormones in male participants in any age group (Lewis et al., 2015). The PFOS levels in the 2011–2012 NHANES cohort were reported to be associated with increased TSH (in male participants aged 12–19 years) and increased FT4 (in women aged 20–39 years). PFOS was not associated with TT3, FT3, or TT4 in male or female participants, nor with FT4 in male participants (Lewis et al., 2015).

Lastly, in a cross-sectional study of older (aged 63.5 ± 13.6 years, mean ± SD) women and men, the PFOA serum level was associated with lower TSH concentrations and higher FT4 concentrations, but was not associated with FT3 (Li et al., 2022d). The PFOS serum level in this study was associated with lower TSH and FT3, but higher FT4 (Li et al., 2022c). In contrast, in a study incorporating cross-sectional data from adolescents, adults, and older adults, no associations of PFOA

or PFOS with TSH or FT4 levels were observed (Ji et al., 2012).

Several smaller studies (n = 31–633) have been conducted to investigate associations of the PFOA and PFOS serum levels with TSH and thyroid hormones in children and adults. [The Working Group considered these studies to be moderately informative, because of the smaller number of participants and/or the lower PFOA or PFOS serum levels.]

No association between the PFOA serum level and TSH was found in the majority of the small studies (Bloom et al., 2010; Ji et al., 2012; Raymer et al., 2012; Lin et al., 2013; Shrestha et al., 2015; Crawford et al., 2017; Li et al., 2017c; Kang et al., 2018; Khalil et al., 2018; Caron-Beaudoin et al., 2019; Li et al., 2021b, women only).

Two studies reported that the PFOA level in serum was associated with increased TSH level (Byrne et al., 2018; Liu et al., 2022b). One study reported that the PFOA level in adolescent male participants was associated with decreased TSH level (Li et al., 2021b).

No association between the PFOA serum level and TT3 was found in the majority of the small studies (Raymer et al., 2012; Shrestha et al., 2015; Byrne et al., 2018; Liu et al., 2022b). One study reported that the PFOA serum level in adult women was associated with increased TT3 level (Crawford et al., 2017). In the four small studies of adults that examined the relation between PFOA serum level and FT3 level, no association was found (Li et al., 2017c, 2021b; Byrne et al., 2018; Liu et al., 2022b).

In the six small studies in adults that have examined the relation between the PFOA level and TT4 level, no association was found (Ji et al., 2012; Raymer et al., 2012; Shrestha et al., 2015; Crawford et al., 2017; Byrne et al., 2018; Liu et al., 2022b).

No association between the PFOA serum level and FT4 was found in the majority of the small studies (Bloom et al., 2010; Lin et al., 2013; Shrestha et al., 2015; Crawford et al., 2017; Li et al., 2017c, 2021b; Byrne et al., 2018; Kang et al., 2018; Khalil et al., 2018; Caron-Beaudoin et al., 2019; Liu et al., 2022b).

No association between the PFOS serum level and TSH was found in the majority of the small studies (Bloom et al., 2010; Ji et al., 2012; Raymer et al., 2012; Lin et al., 2013; Shrestha et al., 2015; Crawford et al., 2017; Li et al., 2021b; Byrne et al., 2018; Kang et al., 2018; Khalil et al., 2018; Caron-Beaudoin et al., 2019; Liu et al., 2022b).

One study reported that the PFOS level was associated with increased TSH level (Li et al., 2017c).

Another study reported that the PFOS levels in adult men and women were associated with reduced TSH level (Dallaire et al., 2009). No association between the PFOS serum level and TT3 was found in the majority of the small studies (Shrestha et al., 2015; Crawford et al., 2017; Byrne et al., 2018; Liu et al., 2022b). One study reported that the PFOS levels in adult men and women were associated with reduced TT3 level (Dallaire et al., 2009). One study reported that the PFOS level in adult males was associated with increased TT3 level (Raymer et al., 2012). In the majority of the small studies in adults, the PFOS serum level was not associated with the FT3 level (Byrne et al., 2018; Li et al., 2021b; Liu et al., 2022b). One study reported that the PFOS levels in adult women and men were associated with reduced FT3 (Li et al., 2017c). In the majority of small studies in adults, the PFOS serum level was not associated with the TT4 level (Ji et al., 2012; Crawford et al., 2017; Byrne et al., 2018; Liu et al., 2022b). One study reported that the PFOS level in adult men was not associated with TT4 (Raymer et al., 2012), and another reported that the PFOS levels in adult women and men were associated with increased TT4 (Shrestha et al., 2015). No association between the PFOS serum level and FT4 was found in the majority of small studies (Bloom et al., 2010; Lin et al., 2013; Crawford et al., 2017; Byrne et al., 2018; Kang et al., 2018; Khalil et al., 2018; Caron-Beaudoin et al., 2019;

Li et al., 2021b). Three studies reported that the PFOS levels in adult women and men were associated with increased FT4 (Dallaire et al., 2009; Shrestha et al., 2015; Liu et al., 2022b). One study reported that the PFOS levels in adult men and women were associated with reduced FT4 (Li et al., 2017c).

[The Working Group noted that a recent meta-analysis of 12 epidemiological studies showed a significant association between blood levels of PFOS and increased FT4, although the effect size was small (pooled z value, 0.05; 95% CI, 0.03–0.08) (Kim et al., 2018). No associations were found of PFOS with TT4, TT3, or TSH; no associations were found between PFOA and TSH or thyroid hormones (Kim et al., 2018).]

Maternal exposure/maternal hormone status

A large number of studies have been conducted on serum PFOA and PFOS concentrations during pregnancy and associations with thyroid hormone outcomes in mothers and newborns. No prospective or large (n > 10 000) cross-sectional studies have been conducted; however, moderately sized cross-sectional studies (n = 1000–2000) have been conducted. [The Working Group thus considered these to be the most informative.] A cross-sectional study in Sweden tested the association of the maternal PFOA and PFOS levels (blood collected during the first and second trimester, median, 10 weeks) with TSH, FT3, TT3, FT4, and TT4. Neither PFOA nor PFOS were associated with TSH, and PFOA was only associated with higher FT4. PFOS was associated with a lower TT3 and a non-linear (inverted U) increase in TT4 (Derakhshan et al., 2022).

Similarly, a cross-sectional study conducted in Shanghai, China, which tested the associations of the maternal PFOA and PFOS levels (blood collected during the first and early second trimesters) with TSH, FT3, and FT4, reported that PFOA was associated with an increase in FT4 (Aimuzi et al., 2020).

A cross-sectional study conducted in Odense, Denmark, which examined the associations of the maternal PFOA and PFOS (blood collected during the first and second trimesters) with TSH and FT4, found that both PFOA and PFOS were associated with an increase in FT4 (Jensen et al., 2022).

In contrast, a cross-sectional study conducted in Demark that tested the associations of the maternal PFOA and PFOS levels with TSH and FT4 during the first and second trimesters reported no associations (Inoue et al., 2019). [The Working Group noted that haemodilution occurs during the early third trimester (≥ 30 weeks of gestation) (Assali and Brinkman, 1972). Moreover, because in all these studies blood was collected no later than the late second trimester (< 27 weeks of gestation), the Working Group considered that haemodilution could not be a factor that would have had an impact on the outcomes.]

Several smaller studies (n = 152–919) have been conducted to investigate associations of the maternal PFOA and PFOS serum levels with TSH and thyroid hormones. [The Working Group considered these studies to be moderately informative, because of the smaller number of participants and/or the lower PFOA or PFOS serum levels.]

In seven of the eight small studies of mothers, no association between the PFOA serum level and TSH was found (Berg et al., 2015; Kato et al., 2016; Preston et al., 2018; Itoh et al., 2019; Reardon et al., 2019; Xiao et al., 2020; Sarzo et al., 2021), but one showed an increase in TSH in women with high TPOAb titres (Webster et al., 2014).

No association was found between maternal PFOA level and TT3 in one study (Berg et al., 2015), but in another study a decrease in TT3 was reported (Sarzo et al., 2021).

In the four small studies of mothers, no association between the PFOA serum level and FT3

was found (Berg et al., 2015; Itoh et al., 2019; Reardon et al., 2019; Xiao et al., 2020). In the four small studies of adults that examined the relation between PFOA level and TT4 level, no associations were found (Berg et al., 2015; Preston et al., 2018; Xiao et al., 2020). In the seven small studies that examined the relation between PFOA level and FT4, no associations were found (Webster et al., 2014; Berg et al., 2015; Kato et al., 2016; Itoh et al., 2019; Reardon et al., 2019; Xiao et al., 2020; Sarzo et al., 2021).

Mixed results have been reported on the association between the maternal PFOS serum level and TSH. Four studies have reported no association (Preston et al., 2018; Itoh et al., 2019; Sarzo et al., 2021; Xiao et al., 2020). Three studies have reported that the maternal PFOS level was associated with an increase in TSH (Berg et al., 2015; Reardon et al., 2019; Xiao et al., 2020), although one of these only reported this association in people with high TPOAb titres (Webster et al., 2014). One study reported that the maternal PFOS level was associated with low TSH only in TPOAb-positive participants (Preston et al., 2018). One study reported that the maternal PFOS level was associated with reduced TSH (Kato et al., 2016). The two studies that investigated the relation between the maternal PFOS serum level and TT3 reported no association (Berg et al., 2015; Sarzo et al., 2021). In the four small studies that examined the relation between the maternal PFOS serum level and FT3 level, no associations were found (Berg et al., 2015; Itoh et al., 2019; Reardon et al., 2019; Xiao et al., 2020). No association between the PFOS serum level and TT4 was found in the three studies that examined this relation (Berg et al., 2015; Preston et al., 2018; Xiao et al., 2020). No association between the PFOS serum level and FT4 was found in the seven small studies that tested this relation (Webster et al., 2014; Berg et al., 2015; Kato et al., 2016; Itoh et al., 2019; Reardon et al., 2019; Xiao et al., 2020; Sarzo et al., 2021).

Maternal exposure/neonate hormone status

Studies of the associations of prenatal PFOA or PFOS serum levels (measured in the neonatal cord serum) with neonatal TSH and thyroid hormones have also been conducted.

No prospective, large (n > 10 000) or moderately sized (n > 1000) cross-sectional studies have been conducted; however, several studies with 300–700 participants have been conducted. [The Working Group selected the largest available studies and/or those with the highest PFOA or PFOS levels as being the most informative.] Three cross-sectional studies measured both PFOA or PFOS and hormone levels in neonatal cord blood, two of which were conducted in China and one in the Republic of Korea. Guo et al. (2021c) assessed the associations of PFOA or PFOS with TSH, TT3, FT3, TT4, and FT4 in cord sample samples. [The Working Group noted that the cord blood PFOA or PFOS levels may be a more reliable measure of exposure over the full course of gestation, rather than a single maternal sample that may be affected by haemodilution.] Associations were found only with thyroxine.

The neonatal cord blood PFOA level was associated with increased TT4, and the PFOS level was associated with increased TT4 and FT4 (Guo et al., 2021c). Aimuzi et al. (2019) also assessed the associations of PFOA or PFOS with TSH, FT3, and FT4 in cord blood samples. PFOA was associated with an increase in FT4 and a decrease in FT3; however, this only occurred in male neonates (Aimuzi et al., 2019). PFOS was associated with reduced TSH and increased FT3 across all the neonates and increased FT3 in the male neonates only (Aimuzi et al., 2019). In contrast, no association was reported of the neonatal cord serum PFOA or PFOS level with TSH, TT3, or TT4 in a small study (n = 43) (Kim et al., 2011).

A cross-sectional study conducted in Japan tested the association of the maternal PFOA and PFOS levels during the first trimester of pregnancy

with neonatal cord blood thyroid hormone levels (TSH, FT3, and FT4). Only one association was found: maternal PFOS was directly associated with an increase in TSH in male neonates (Itoh et al., 2019). A second study in Japan, which tested the association of the maternal PFOA and PFOS levels during the second or third trimester with neonatal TSH and FT4 in blood collected by heel puncture, also reported that maternal PFOS was associated with an increase in the cord blood TSH concentration in all the newborns (Kato et al., 2016). A cross-sectional study conducted in China tested the relationship between the maternal PFOA or PFOS levels during the second trimester and the neonatal cord blood thyroid hormone levels (TSH, TT3, FT3, TT4, and FT4). The maternal PFOA and PFOS levels were both associated with increased neonatal cord blood TT3 and FT3, but no associations were found for TSH or thyroxine (Liang et al., 2020). A cross-sectional study conducted in the USA that evaluated the relation between maternal PFOA and PFOS levels during the first and second trimesters with T4 levels in newborn heel-puncture blood found no associations (Preston et al., 2018).

The remaining studies on the relations between maternal PFOA or PFOS levels and neonatal TSH and thyroid hormones were very small (n < 200), and therefore the Working Group considered them to be of only moderate importance. In the one study that analysed associations of the maternal PFOA and PFOS levels during the third trimester with neonatal cord blood hormones (TSH, TT3, FT3, TT4, and FT4), TSH was the only hormone that was associated with the maternal PFOA and PFOS levels, with both PFOA and PFOS being associated with an increase in TSH (Xiao et al., 2020). In the one study that analysed maternal PFOA and PFOS levels during the first and second trimesters and neonatal cord blood hormones (TSH, TT3, FT3, TT4, and FT4), the only association that was found was that maternal PFOA and PFOS levels were both associated with lower FT4 levels in neonates, but only when the neonates were born to mothers with high TPOAb levels (Lebeaux et al., 2020). The cord blood PFOA level, but not that of PFOS, was positively associated with newborn heel puncture TT4 in female, but not in male, neonates (de Cock et al., 2014). Lastly, the neonatal dried blood spot PFOA and PFOS concentrations were positively associated with T4 and negatively associated with TSH (Rosen Vollmar et al., 2023).

Human cell lines and reporter assays

Few studies have investigated the thyroid hormone receptor (TR) pathway in human in vitro models. Human thyroid peroxidase activity in FTC-238 human follicular carcinoma cells (48-hour exposure) was consistently inhibited by PFOS (≥ 0.1 nM), but by only a high concentration of PFOA (1 µM) (Song et al., 2012). PFOA (IC_{50} = 1.8–3 µM, in cell-free transthyretin preincubation) and PFOS (IC_{50} = 0.6 µM) displaced T4 from transthyretin (TTR) in a TTR-TRβ CALUX assay performed in U2OS human osteosarcoma cells (Behnisch et al., 2021; Sprengel et al., 2021). PFOS (≥ 0.1 µM) antagonized TRβ activation by T3 in a one-hybrid reporter assay performed in CV-1 cells (Du et al., 2013) (see Table S4.23).

Synopsis

[The Working Group noted that the evidence for associations of PFOA and PFOS with TSH or thyroid hormones in children and adults was generally weak (see summary findings for KC8 in Table 4.27). In the most informative studies (prospective study design, high level of exposure (including occupational and contaminated community exposure), or large study size), the results were mixed, with some pattern to the reported associations of PFOA or PFOS levels with the TSH or thyroid hormone levels being identified. The strongest associations were between the PFOS serum level and increased FT4; however, the collective evidence (for TSH, TT3, FT3, and TT4) did not support an association

with the PFOA or PFOS serum level. Importantly, changes in thyroid hormones were rarely accompanied by clinically relevant changes in TSH level in adults or children. Also, there was little evidence about the effects of PFOA and PFOS on the human thyroid hormone receptor (TR) pathway in human cells in vitro.]

(ii) Steroid receptors – androgen and estrogen pathways

Exposed humans

See Table S4.23 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Child–adult exposure

Several studies have investigated the associations of PFOA and PFOS with LH, testosterone, and estrogens in children and adults. LH is responsible for stimulating testosterone production in the gonads, and testosterone is converted to estrogens through the action of aromatase. Therefore, these hormones are analysed together. [The Working Group selected the most informative studies based on a prospective study design, a high level of exposure (including occupational and contaminated community exposure), and large study size.] A single prospective cohort study of pre-adolescent girls (n = 704) in the USA found no associations between serum PFOA and estradiol pre- or post-thelarche but did show negative associations with estrone and testosterone 6 months before thelarche (Pinney et al., 2023). The same study showed no associations of serum PFOS with estradiol, estrone, or testosterone at any time point pre- or post-thelarche (Pinney et al., 2023). A cross-sectional study (n = 1041) of men aged 18–21 years in Denmark found no associations of serum PFOA or PFOS with LH, testosterone, or estradiol (Petersen et al., 2022). Similarly, a cross-sectional study (n = 920) of men aged 28–25 years in Shanghai, China, found no associations of serum PFOA with LH, testosterone, or estradiol (Luo et al., 2021). PFOS was also found to not be associated with LH or testosterone, but PFOS was reported to be negatively associated with estradiol (Luo et al., 2021). Two cross-sectional studies have been conducted of the C8 cohort, composed of participants who lived in contaminated communities in the Mid-Ohio Valley, USA. In a study of children aged 6–9 years (n = 2292), serum PFOA was positively associated with lower testosterone only in male children, whereas serum PFOS was associated with lower testosterone in both girls and boys (Lopez-Espinosa et al., 2016). In the same study, PFOA was not associated with estradiol in male or female children, but PFOS was associated with lower estradiol in boys (Lopez-Espinosa et al., 2016). In a large cross-sectional analysis of adult women in the C8 cohort (n = 29 957), serum PFOA was not found to be associated with estradiol, but serum PFOS was found to be associated with lower estradiol in women aged > 42 years (Knox et al., 2011).

In a cross-sectional study of young men (age 24–26 years; n = 263) in the Faroe Islands, Denmark, the PFOA level was not found to be associated with LH, free or total testosterone, or estradiol; however, the PFOS level was reported to be associated with an increase in LH (Petersen et al., 2018).

In a cross-sectional analysis of NHANES data (USA) regarding people aged > 12 years (n = 1682) from the 2011–2012 cohort, no association was found in any group between the PFOA or PFOS level and the total serum testosterone (Lewis et al., 2015). However, in an analysis of the NHANES 2015–2016 cohort, including people aged > 12 years (n = 1886), PFOS, but not PFOA, was found to be associated with higher testosterone levels in male participants only (Xie et al., 2021). PFOA was associated with a linear decrease in estradiol only in women aged 20–49 years, whereas PFOS concentrations in the second quartile only were found to be associated

with higher estradiol in Q2 in women aged 20–49 years (Xie et al., 2021).

In another cross-sectional study of women in the USA, in which the participants aged 47–52 years had a median serum PFOS concentration of 25 ng/mL, neither the PFOA nor the PFOS serum level was found to be associated with testosterone or estradiol (Harlow et al., 2021).

In a cross-sectional study of male and female adolescents aged 13–15 years in Taiwan, China, in whom the median serum PFOS concentration was ≥ 28 ng/mL, neither PFOA nor PFOS was associated with testosterone or estradiol in the female participants (Zhou et al., 2016). In the male participants, PFOA was associated with higher estradiol, and PFOS was associated with lower testosterone (Zhou et al., 2016).

In a further analysis of these participants within a larger study, the authors reported that specifically in adolescents with asthma, both PFOA and PFOS were associated with lower testosterone and higher estradiol (Zhou et al., 2017).

Several small cross-sectional studies (n = 59–651) have also been conducted. [The Working Group considered these studies to be moderately informative, because of the smaller number of participants and/or the lower PFOA or PFOS serum levels.] The majority of studies found no association of serum PFOA or PFOS with LH (Joensen et al., 2009; Joensen et al., 2013; Tsai et al., 2015; Cui et al., 2020). One study reported that PFOA was associated with an increase in LH in young men, whereas PFOS showed no association with LH (Raymer et al., 2012). Mixed results have been reported for the relation of serum PFOA with testosterone (free or total), including no association (Joensen et al., 2009, 2013; Tsai et al., 2015), a positive association (Raymer et al., 2012; Heffernan et al., 2018; Wang et al., 2021b), and a negative association (Cui et al., 2020).

Mixed results also have been reported for the relation of serum PFOS with testosterone (free or total), including no association (Joensen et al., 2009; Raymer et al., 2012; Heffernan et al., 2018), a positive association (Wang et al., 2021b), and a negative association (Joensen et al., 2013; Tsai et al., 2015; Cui et al., 2020). None of these studies reported an association between serum PFOA or PFOS with estradiol (Joensen et al., 2009, 2013; Raymer et al., 2012; Barrett et al., 2015; Tsai et al., 2015; Heffernan et al., 2018; Cui et al., 2020; Wang et al., 2021b).

Two cross-sectional studies in Italy investigated the associations of PFOA and PFOS serum levels with the expression of androgen and estrogen receptors in leukocytes.

In a study of fertile and infertile women (age 18–40 years; n = 111), PFOA was not found to be associated with sex hormone receptor mRNA expression, whereas PFOS was reported to be associated with higher androgen receptor (AR) expression (Caserta et al., 2013). In a study of fertile and infertile men (age 27–40 years; n = 153), only PFOA was associated with lower expression of both estrogen receptors and androgen receptors (La Rocca et al., 2015).

[The Working Group noted that a recent meta-analysis of 11 studies examined the associations of PFOA and PFOS with reproductive hormones (estradiol and total testosterone) (Li et al., 2024). PFOS was associated with reduced serum testosterone in men, although the effect size was notably small, and no association was found in women (Li et al., 2024). However, earlier systematic analyses did not report significant associations between PFOS and low testosterone (Bach et al., 2016; Petersen et al., 2020). No associations of PFOA or PFOS with estradiol were reported by Li et al. (2024).]

Prenatal exposure

Several studies of prenatal exposure to PFOA and PFOS have investigated their associations with intermediates in the sex hormone synthesis

pathway, as well as with testosterone and estradiol. No prospective cohort or large (n > 10 000) cross-sectional studies were available to the Working Group.

In a cross-sectional study of amniotic fluid in Denmark (n = 645; male fetuses only), PFOS was found to be associated with increased progesterone, 17-hydroxyprogesterone, androstenedione, and testosterone (PFOA was not analysed) (Toft et al., 2016).

In a cross-sectional analysis of the association between maternal serum PFOA and PFOS concentrations during the third trimester and the male and female neonatal cord blood hormones (Shandong, China; n = 349), neither maternal PFOA nor PFOS were reported to be associated with neonatal testosterone (Yao et al., 2021). Maternal PFOA, but not PFOS, concentration during pregnancy was found to be associated with higher levels of neonatal estradiol (Yao et al., 2021).

A second cross-sectional analysis of the association between maternal serum PFOA and PFOS concentrations during the third trimester and male and female neonatal cord blood hormones (Sapporo, Japan; n = 224) similarly reported that neither maternal PFOA nor PFOS concentrations during pregnancy were associated with neonatal testosterone (Kobayashi et al., 2021). In the study by Kobayashi and colleagues, maternal concentration of PFOS, but not PFOA, during pregnancy was reported to be associated with increased neonatal levels of estradiol (Kobayashi et al., 2021).

Two cross-sectional studies in Hubei, China, investigated the associations of neonatal cord blood PFOA and PFOS with neonatal estrogens (estrone, estradiol, and estriol) in male and females. In the larger of the two studies (n = 942), both neonatal PFOA and PFOS were positively associated with neonatal cord blood estrone and estradiol (Liu et al., 2021). However, only PFOA was associated with higher estriol (Liu et al., 2021). In the second study (n = 424), neonate PFOA was positively associated with only estrone, whereas PFOS was associated with higher estrone and estriol (Wang et al., 2019).

In a cross-sectional study of neonatal cord blood PFOA and PFOS and sex hormones in Shandong Province, China (n = 351), PFOA was reported to be positively associated with cord blood estradiol, but not total testosterone, and PFOS was positively associated with cord blood total testosterone, but not estradiol (Yao et al., 2019).

There have been a few other moderately informative, smaller (n = 72–373) cross-sectional studies of associations between maternal PFOA and PFOS levels during pregnancy and steroid hormones in neonates, children, and young adults. The maternal PFOA concentration after the second trimester was negatively associated with the testosterone precursor dehydroepiandrosterone (DHEA) in neonatal cord blood, and maternal PFOS was positively associated with neonatal DHEA (Goudarzi et al., 2017b). Neither maternal PFOA nor PFOS concentrations during pregnancy were associated with neonatal androstenedione (Goudarzi et al., 2017b). Two cross-sectional studies have tested the associations of the maternal serum PFOA and PFOS concentrations during pregnancy with anatomical biomarkers of prenatal hormone exposure: the ratio of the lengths of the second and fourth digits (2D:4D; negatively associated with testosterone exposure and positively associated with estrogen exposure) and anogenital distance (positively associated with testosterone exposure). The maternal serum PFOA concentration during the second and third trimesters, but not that of PFOS, was associated with an increased mean 2D:4D digit ratio in male, but not female, neonates with different *ESR1* genotypes (Nishimura et al., 2022).

No associations between the maternal serum PFOA or PFOS concentration during pregnancy and the anogenital distance of male or female neonates were observed (Arbuckle et al., 2020). No associations of the maternal serum PFOA or

PFOS concentration during the first and second trimesters with the infant (age 4 months) serum LH, DHEA, androstenedione, 17-hydroxyprogesterone, or testosterone were found (Jensen et al., 2020). The maternal PFOA and PFOS concentrations during the first and second trimesters were positively associated with testosterone in the adolescent daughters (Maisonet et al., 2015). However, the maternal PFOA and PFOS concentrations during the third trimester were not found to be associated with LH, total testosterone, DHEAS, or estradiol in the young adult daughters (Kristensen et al., 2013). The maternal PFOA and PFOS concentrations during the third trimester were not associated with testosterone or estradiol in the young adult sons, although PFOA was associated with higher LH (Vested et al., 2013).

In vitro effects on estrogen receptors α and β

See Table S4.24 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636)

Human primary cells

Only three studies have investigated the effects of PFOA on the estrogen receptor (ER) (ERα and ERβ) pathway in human primary cells. In human primary hepatocytes exposed for 24 hours to PFOA (≥ 25 μM), the expression of the ERα gene (*ESR1*) was significantly downregulated (Buhrke et al., 2015).

In HUVECs, PFOS (100 μM, 48 hours) significantly increased the expression of the ERα gene (*ESR1*) (Liao et al., 2012). In human primary placental cytotrophoblasts, PFOS (≥ 0.001 μM, 24 hours) reduced CYP19 protein expression and 17β-estradiol secretion (Zhang et al., 2015b).

Human cell lines

In MCF7 human breast cancer cells, PFOA (100 μM, 24–48 hours) did not affect the mRNA expression of the ER target genes *TFF1*, *EGR3*, *ESR1*, *GREB1*, or *PGR* (Behr et al., 2018; Li et al., 2020e); however, PFOA significantly suppressed the estradiol-dependent expression of *EGR3* and *TFF1* in one of these studies (Li et al., 2020e). In contrast, PFOS (50 μM, 24–48 hours) upregulated *TFF1* but not *EGR3* and suppressed the estradiol-dependent expression of these target genes (Li et al., 2020e). In the other study; however, PFOS (100 μM, 24 hours) had no effect on *TFF1*, *ESR1*, *GREB1*, *PGR*, or *CTSD* expression (Behr et al., 2018). In T47D human breast cancer cells, whereas neither PFOA (0.001 μM, 24 hours) nor PFOS (0.001 μM, 24 hours) increased the expression of known ER target genes (*PR* and *pS2*), they did increase the estradiol-dependent induction of *pS2* (Sonthithai et al., 2016). In addition, PFOS induced the expression of ERβ in HepG2 cells (Xu et al., 2017).

PFOA and PFOS have also been investigated for their ability to modulate estradiol production and secretion. In H295R human adrenocortical carcinoma cells, PFOA (48 hours) was reported to have no effect on 17β-estradiol secretion at ≤ 100 μM (Kraugerud et al., 2011; Wang et al., 2015d; Behr et al., 2018), but to reduce 17β-estradiol secretion at 1.6 μM (Rosenmai et al., 2013), and to increase its secretion at 50 μM (Rosenmai et al., 2013). In H295R cells, Kang et al. also reported increases in estradiol at 10 μM and 100 μM PFOA (Kang et al., 2016). PFOA (600 μM, 48 hours) was shown to increase aromatase activity in H295R cells (Kraugerud et al., 2011) and to reduce aromatase activity (IC_{50} = 80 μM) in JEG-3 human placental carcinoma cells (Gorrochategui et al., 2014). In H295R cells, PFOS was shown to have no effect on 17β-estradiol secretion at ≤ 100 μM (Behr et al., 2018), but to increase this at 0.03, 200, or 600 μM (Kraugerud et al., 2011; Du et al., 2013; van den Dungen et al., 2015; Kang et al., 2016).

Human binding and reporter assays

PFOA and PFOS have been shown to bind human ERα at high concentrations in competitive binding assays using the human ERα ligand-binding domain with similar binding affinities (Qiu et al., 2020). The majority of ERα reporter studies employing endogenous human ERα and estrogen response element-driven reporters performed in breast and ovarian carcinoma cell lines (BG1, ovarian; MCF7 and T47D, breast) showed that PFOA and/or PFOS did not transactivate ERα (Yao et al., 2014; Kang et al., 2016; Sonthithai et al., 2016; Evans et al., 2022). However, other studies using this approach showed that PFOA (EC_{50} = 65 μM) and PFOS (EC_{50} = 29 μM) stimulated ERα transactivation (Kjeldsen and Bonefeld-Jørgensen, 2013) or that PFOS (EC_{20} = 12 μM), but not PFOA, stimulated ERα transactivation (Li et al., 2020e), but with significantly lower efficacy than did estradiol. In addition, PFOA (10 nM to 1 μM) and PFOS (1 nM to 1 μM) increased estradiol-stimulated ERα transactivation in an endogenous ERα-driven reporter assay performed in T47D cells (Sonthithai et al., 2016).

In ER reporter studies employing the forced expression of full-length human ERα in HEK293T human kidney cells, PFOA (≥ 0.1 μM) and PFOS (≥ 0.001 μM) stimulated the transactivation of ERα (Benninghoff et al., 2011; Houck et al., 2021). ERα reporter studies using a one-hybrid approach have also been conducted. In one study of human HepG2 cells, PFOA (half-maximal activity concentration (AC_{50} = 8 μM) and PFOS (AC_{50} = 4.4 μM) stimulated ERα-mediated transactivation (Houck et al., 2021). In one study of human kidney HEK293T cells, neither PFOA (≤ 100 μM) nor PFOS (≤ 100 μM) transactivated ERα or ERβ (Behr et al., 2018). However, in experimental designs in which PFOA and PFOS were applied together with estradiol, PFOA (100 μM) was shown to enhance estradiol-driven ERβ activation, and PFOS (100 μM) was shown to enhance estradiol-driven ERα and ERβ activation in a one-hybrid assay in HEK293T cells (Behr et al., 2018).

In vitro effects on the androgen receptor

See Table S4.24 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Human primary cells

No studies available to the Working Group investigated the effects of PFOA or PFOS on androgen receptor (AR) pathways in human primary cells.

Human cell lines

In LNCaP human prostate adenocarcinoma cells (24-hour exposure), neither PFOA (≤ 100 μM) nor PFOS (≤ 100 μM) induced the mRNA expression of AR target genes (e.g. *AR* and *PSA*) (Behr et al., 2018). In H295R human adrenocortical carcinoma cells, PFOA has been reported not to have an effect on testosterone secretion (PFOA ≤ 100 μM, 48 hours) (Rosenmai et al., 2013; Wang et al., 2015d; Behr et al., 2018) or to increase testosterone secretion (PFOA ≥ 0.6 μM) (Kraugerud et al., 2011). The effect of PFOS has also been tested using the H295R steroidogenesis assay. PFOS was reported to have no significant effect on testosterone secretion in H295R cells (PFOS ≤ 200 μM) (Behr et al., 2018), to reduce testosterone production (PFOS ≥ 1 μM) (Du et al., 2013), and to increase testosterone secretion (PFOS, 0.6–600 μM) (Kraugerud et al., 2011; van den Dungen et al., 2015).

Human binding and reporter assays

PFOA (≤ 300 μM) and PFOS (≤ 300 μM) did not transactivate endogenous human AR in reporter assays performed in breast (MDA-kB2) or prostate (22Rv1/MMTV) cancer cells (Kang et al., 2016; Behr et al., 2018) or HepG2 hepatoma cells (Houck et al., 2021). PFOS (≤ 3 μM) did not

transactivate endogenous human AR in reporter assays performed in breast (MDA-kB2) cancer cells (Du et al., 2013). PFOA (≤ 100 μM) did not transactivate full-length overexpressed human AR in a reporter assay performed in CHO cells (Kjeldsen and Bonefeld-Jørgensen, 2013; Rosenmai et al., 2013). PFOS (≤ 100 μM) also did not transactivate full-length overexpressed human AR in a reporter assay performed in CHO cells (Kjeldsen and Bonefeld-Jørgensen, 2013). PFOS (> 50 μM), but not PFOA, enhanced testosterone-induced AR reporter transactivation in MDA-kB2 cells (Behr et al., 2018), but PFOA (IC_{50} = 11 μM) and PFOS (IC_{50} = 5 μM) inhibited AR reporter transactivation in CHO cells (Kjeldsen and Bonefeld-Jørgensen, 2013). No studies that used binding assays were identified.

Synopsis

[Overall, the Working Group noted that in the studies in exposed humans reviewed above there was no evidence that PFOA or PFOS were associated with LH in children and adults, and there was little evidence that PFOA was associated with testosterone. Whereas the results of a recent meta-analysis (Li et al., 2024) showed that a small but significant decrease in serum total testosterone was associated with PFOS in men, the most informative and moderately informative studies reviewed here reported no association between PFOS and testosterone.

There was also little evidence provided by either the most informative or the moderately informative studies that PFOA is associated with estradiol level (either higher or lower). There were several studies that supported an association of PFOS with lower estradiol level; however, this association was not recapitulated in the moderately informative studies. There does appear to be the potential for sex-specific differences, with men being more likely to show associations of PFOA and PFOS with testosterone and estradiol than women.

Human studies of in utero exposure to PFOA and PFOS did not show associations with sex hormones or sex hormone-dependent end-points in neonates or later in life. More studies reported no association than reported significant associations. However, most of the associations reported were between PFOA or PFOS and higher concentrations or effects of sex hormones.

In addition, PFOA and PFOS seemed not to modulate testosterone production or act as AR ligands, as measured with AR reporter assays.

Overall, inconsistent findings were reported with regard to PFOA and PFOS modulating the ER pathway-mediated effects in human primary cells, human cell lines, and human reporter assays. In systems in which ERs are expressed endogenously, high (i.e. non-human-relevant) PFOA and PFOS concentrations were required to modulate ER-dependent gene expression and transactivation, as well as estradiol production (see summary findings for KC8 in Table 4.27).]

(iii) Steroid receptors – progesterone pathway

See Table S4.23 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Exposed humans

One cross-sectional study conducted in Norway (n = 178) assessed the association between serum PFOA and PFOS and salivary progesterone in women and found that in nulliparous, but not parous, adult women, PFOS, but not PFOA, was negatively associated with salivary progesterone (Barrett et al., 2015).

One cross-sectional study conducted in Denmark (n = 545) assessed the association between PFOS and progesterone in amniotic fluid during the second trimester and reported that PFOS was associated with higher progesterone (Toft et al., 2016).

Two cross-sectional studies ($n \leq 224$ and $n = 189$, respectively) have assessed the associations of prenatal exposure to PFOA and PFOS with neonatal progesterone levels. Maternal concentration of PFOS, but not PFOA, during the third trimester was negatively associated with cord blood progesterone in the first of these (Kobayashi et al., 2021). In the second study, the maternal pregnancy (from the first to third trimester) PFOA level was negatively associated with the cord blood progesterone in male and female neonates, whereas the maternal PFOS level was not associated with progesterone in neonates of either sex (Itoh et al., 2016).

A single cross-sectional study conducted in China ($n = 374$, women and men) investigated the relations between PFOA or PFOS and progesterone in neonatal cord blood and reported that neonatal levels of PFOA and PFOS were not associated with neonatal levels of progesterone or 17-hydroxyprogesterone (Liu et al., 2020b).

A single cross-sectional study conducted in Belgium ($n = 170$, women and men) investigated the associations between PFOA or PFOS in neonatal cord blood and the expression of progesterone-receptor target genes in leukocytes and found that the blood levels of PFOA and PFOS were associated with higher levels of progesterone receptor-mediated gene expression (Remy et al., 2016).

Synopsis

[The Working Group noted that, in the few studies described above, little evidence was available on the potential association of in utero exposure to PFOA and PFOS with the progesterone pathway. PFOS appeared to be more strongly associated with progesterone and progesterone-induced pathways than PFOA, but both positive and negative associations were reported (see summary findings for KC8 in Table 4.27).]

(iv) Steroid receptors – glucocorticoid pathway

See Table S4.23 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Exposed humans

A single cross-sectional study conducted in Sweden ($n = 1048$) assessed the association between maternal PFOA or PFOS exposure during the first trimester and the urinary cortisol and cortisone concentrations during the third trimester (Dreyer et al., 2020). The maternal serum PFOA was found not to be associated with urinary cortisol or cortisone. The maternal serum PFOS was associated with lower urinary cortisone but not cortisol (Dreyer et al., 2020). One cross-sectional study conducted in Denmark ($n = 545$) assessed the association between PFOS and cortisol in amniotic fluid and reported that PFOS was associated with higher cortisol (Toft et al., 2016). Two cross-sectional studies ($n < 400$) that assessed the associations of prenatal exposure to PFOA and PFOS with neonatal corticosteroid levels have been conducted. Maternal concentration of PFOS, but not PFOA, after the second trimester was negatively associated with cord blood cortisol, cortisone, and the cortisol/cortisone ratio (Goudarzi et al., 2017b). Neonatal cord blood PFOA and PFOS were found to be positively associated with 11-deoxycortisol, but not cortisol or cortisone (Liu et al., 2020b).

In vitro – human primary cells, cell lines, and reporter assays

See Table S4.24 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

A single study has investigated the effect of PFOA or PFOS on the glucocorticoid receptor (GR) pathway in human primary cells. PFOS (0.001–1 μM, 24 hours) reduced the upregulation

of 11β-hydroxysteroid dehydrogenase (HSD)1, the enzyme responsible for the generation of cortisol, an endogenous GR ligand, in human primary decidual stromal cells (Yang et al., 2016).

Using microsomes from human and rat kidneys, PFOA and PFOS were demonstrated to inhibit the catalytic activity of 11β-HSD2 (the enzyme that deactivates cortisol), with PFOS having a lower IC_{50} than PFOA (0.05 versus 24.4 μM, respectively) (Zhao et al., 2011b, 2023). In the same studies, rat enzyme activities were measured, and the PFOS IC_{50} was 0.29 μM, whereas that of PFOA was 3.8 μM.

In H295R cells, PFOA (≤ 600 μM, 48 hours) had no effect on cortisol secretion (Kraugerud et al., 2011; Rosenmai et al., 2013; Wang et al., 2015d). In H295R cells, PFOS was shown to have no effect on cortisol secretion (PFOS ≤ 600 μM) (Kraugerud et al., 2011), nor to increase cortisol secretion (200 μM) (van den Dungen et al., 2015). In a full-length GR reporter assay performed in T47D cells, PFOS (~30–60 μM, 24 hours) enhanced cortisol-driven GR reporter activation to a modest extent (Wilson et al., 2016).

Synopsis

[The Working Group noted that there was a small number of available studies in which alteration of glucocorticoids and exposure to PFOA or PFOS during pregnancy were investigated, and few of these studies provided evidence that was supportive of a positive association. PFOS appeared to be more strongly associated than PFOA with corticosteroid levels, and the associations were generally negative (see summary findings for KC8 in Table 4.27).

A modest set of data suggested that PFOS suppressed cortisol production and deactivation but could enhance GR activation by cortisol, whereas PFOA had little effect on the GR pathway.]

(v) Other nuclear receptors and types of receptors

See Table S4.23 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Exposed humans

Several studies have investigated the association of PFOA and PFOS with vitamin D receptor-related biomarkers.

Two cross-sectional studies conducted in the USA, one of which was in adults (n = 7040) and one in children (n = 78), investigated the associations of serum PFOA and PFOS levels with total serum 25-hydroxyvitamin D (Khalil et al., 2018; Etzel et al., 2019). In adults, the PFOS level, but not that of PFOA, was associated with a lower serum vitamin D level (Etzel et al., 2019). In children, neither PFOA nor PFOS was associated with the serum vitamin D level (Khalil et al., 2018). A cross-sectional study of pregnant women in the USA (n = 442) investigated the associations of serum PFOA and PFOS levels during the first trimester with free and total serum 25-hydroxyvitamin D levels during the first and second trimesters and reported that serum PFOA level was not associated with the vitamin D level (Chang et al., 2021). In the same study, the serum PFOS level was associated with higher total serum 25-hydroxyvitamin D during both the first and second trimesters. The serum PFOS level was only associated with higher free serum 25-hydroxyvitamin D during the second trimester, and only in pregnancies with male fetuses (Chang et al., 2021).

In a cross-sectional study in China (n = 992) that investigated the associations of neonatal cord blood PFOA and PFOS levels with the total serum 25-hydroxyvitamin D, it was reported that the serum PFOS level, but not that of PFOA, was associated with higher neonatal levels of vitamin D (Liu et al., 2023c).

[The Working Group noted that PFOS appeared to be more associated with serum vitamin D level than PFOA; however, the direction of the association was negative in one study and positive in two studies.]

Three studies have investigated the associations of serum PFOA and PFOS in adult women and men with the expression of nuclear receptor and *AHR* mRNA expression in leukocytes.

In a cross-sectional study of adult women conducted in Italy (n = 154), serum PFOA was negatively associated with *PXR* and *AHR* expression in peripheral blood cells but was not associated with *PPARG* expression (Caserta et al., 2013). In the same study, the serum PFOS concentration positively correlated with *PXR* expression in peripheral blood cells (Caserta et al., 2013).

In a cross-sectional study of adult men conducted in Italy (n = 153), serum level of PFOA was negatively associated with the expression of *PXR* and *AHR* in peripheral blood cells but not with the expression of *PPARG* (La Rocca et al., 2015). In the same study, PFOS was not associated with the expression of *PXR*, *AHR*, or *PPARG* (La Rocca et al., 2015).

In a cross-sectional study of adult women and men conducted in the USA (n = 290), serum PFOA level was associated with lower expression of *LXRB*, but not *LXRA*, in peripheral blood cells, and the serum PFOS level was associated with lower expression of *LXRA*, but not *LXRB* (Fletcher et al., 2013). In the same study, no associations of PFOA or PFOS with *PPARA*, *PPARD*, or *PPARG* were reported (Fletcher et al., 2013).

[The Working Group noted that it is difficult to interpret the significance of associations between serum PFOA and PFOS levels and the expression of nuclear receptors in leukocytes, because of the small number of studies and disparate results.]

PPARα – in vitro

See Table S4.24 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

The majority of human in vitro studies have focused on the activation of PPARα. However, rodent studies have made it clear that PFOA and PFOS target more than just PPARα (Rosen et al., 2010; Attema et al., 2022; Su et al., 2022a). Table S4.24 summarizes the available studies that investigated the ability of PFOA and PFOS to bind and/or activate nuclear receptors in human systems in vitro. Evidence was gathered from transcriptional and biological assays performed in human primary hepatocytes and human liver cell models, as well as binding assays and reporter assays.

Human primary cells

Multiple studies in human primary liver cells have tested whether PFOA or PFOS activate human PPARα. In human primary hepatocytes treated with PFOA or PFOS for 24–28 hours, human *PPARA* mRNA expression was induced by PFOA (≥ 5 μM) and PFOS (≥ 25 μM) (Bjork and Wallace, 2009; Bjork et al., 2011; Rosen et al., 2013; Buhrke et al., 2015; Marques et al., 2022). Ingenuity Pathway Analysis of microarray mRNA expression data induced by PFOA identified PPARα as a predicted upstream regulator of effects on the cell cycle (Buhrke et al., 2015).

Similarly, in human primary liver spheroids composed of hepatocytes and Kupffer cells that were treated with PFOA or PFOS for 1–14 days and analysed by targeted RNA sequencing (RNASeq), the fatty acid β-oxidation pathway was significantly upregulated by PFOA (20 μM) at all time points and by PFOS (20 μM) after 1 and 4 days (Rowan-Carroll et al., 2021). PPARα was predicted to be a strongly activated upstream regulator of the gene expression changes induced by PFOA and PFOS (Rowan-Carroll et al., 2021).

When comparing full transcriptomes, the benchmark concentrations for PFOA and PFOS were similar after 14 days of exposure (5–10 μM); however, PFOS was found to regulate the transcription of more genes, in general, than PFOA (Rowan-Carroll et al., 2021).

In studies that tested the effects of PFOA or PFOS in both rodent and human primary hepatocytes, PPARα was less potently activated in human versus mouse primary hepatocytes: 5.1 times less potently for PFOA and 1.9 times less potently for PFOS (Rosen et al., 2013). Differences in efficacy have also been noted, with PFOA and PFOS more efficaciously activating PPARα in rat than human primary hepatocytes (although the authors noted that there were differences in the baseline expression of genes in rat and human hepatocytes that affected the apparent induction of expression) (Bjork et al., 2011).

Human cell lines

Similarly, studies of human liver cell lines have shown that PFOA and PFOS increase the mRNA expression of human PPARα target genes. In HepaRG human liver cells, 24-hour exposure to PFOA (100 μM) or PFOS (100 μM) upregulated the mRNA expression of known targets of PPARα, including those associated with fatty acid β-oxidation (Louisse et al., 2020, 2023; Murase et al., 2023). In addition, PFOS, but not PFOA, except at the highest concentration of 200 μM, was shown to increase lipid accumulation in HepRG cells (Louisse et al., 2020). In HepG2 human liver cancer cells, PFOA (≥ 25 μM) had similar effects on the mRNA expression of PPARα target genes to the PPARα agonists WY14 643 and GW7647, although with lower potency (Behr et al., 2020b). In contrast, in HepG2/C3a human hepatoma cells, PFOA (≤ 200 μM) and PFOS (25 μM) did not induce the mRNA expression of the PPARα target genes *ACOX1*, *ACOT*, and *CYP4A1* (Bjork and Wallace, 2009).

Human binding and reporter assays

The results obtained from human primary hepatocytes and other human cell models have been corroborated by ample evidence that PFOA and PFOS activate human PPARα derived from binding and reporter assays. In competitive binding assays performed using the human PPARα ligand-binding domain, PFOA and PFOS were shown to displace well-known PPARα ligands (Li et al., 2018b; Ishibashi et al., 2019). PFOA has been shown to activate PPARα-mediated transcription in reporter assays using full-length human PPARα (Maloney and Waxman, 1999; Nielsen et al., 2022; Sakai et al., 2022) or chimaeras of the human PPARα ligand-binding domain with the GAL4 DNA-binding domain (Vanden Heuvel et al., 2006; Takacs and Abbott, 2007; Wolf et al., 2008a, 2012; Corsini et al., 2012; Buhrke et al., 2013; Rosenmai et al., 2016, 2018; Behr et al., 2020b; Houck et al., 2021; Evans et al., 2022). PFOS also has been shown to activate PPARα reporter assays (full-length: Shipley et al., 2004; Nielsen et al., 2022; Gal-4: Wolf et al., 2008a; Behr et al., 2020b; Houck et al., 2021; Evans et al., 2022). However, some studies also showed that PFOS failed to activate PPARα reporter assays (Gal-4: Takacs and Abbott, 2007; Corsini et al., 2012; Rosenmai et al., 2018). In the reporter assays using full-length hPPARα, Cos1, Cos7, or MDA-MB-231 cells were transfected with an expression vector for the full-length hPPARα protein and a reporter construct in which reporter gene expression was driven by PPARα binding to PPAR response elements and treated with multiple concentrations of PFOA or PFOS, with reporter activity being assessed after 24 hours of exposure. In the reporter assays that used a one-hybrid approach, 3T3-L1, COS-1, THP1, HEK293, HEK293T, or HepG2 cells were transfected with an expression vector for a chimaera of the human PPARα ligand-binding domain and the GAL4 DNA-binding domain and a reporter construct in which reporter gene

expression was driven by GAL4 binding to upstream activating sequences (also known as a one-hybrid approach), and treated with multiple concentrations of PFOA or PFOS, with reporter activity being assessed after 6–24 hours of exposure. It is important to note that whereas PFOA is a full agonist of human PPARα, PFOS is a partial agonist in human PPARα reporter assays (Vanden Heuvel et al., 2006; Rosenmai et al., 2016; Behr et al., 2020b; Nielsen et al., 2022). Human PPARα is also less activated than mouse PPARα in reporter assays, with effective concentrations being 1–2.7 times higher for PFOA to activate human than mouse PPARα and 2.1–2.8 times higher for PFOS to activate human than mouse PPARα (Maloney and Waxman, 1999; Shipley et al., 2004; Vanden Heuvel et al., 2006; Takacs and Abbott, 2007; Wolf et al., 2008a).

Synopsis

[The Working Group noted that, on the basis of the studies reviewed above that were conducted in human primary cells, and various human cell lines, using binding and reporter assays, there was evidence that PFOA and PFOS transcriptionally activated PPARα. However, human PPARα was less effectively activated by PFOA or PFOS than was rodent PPARα. Also, PFOA activated human PPARα more effectively than did PFOS (see summary findings for KC8 in Table 4.27).]

CAR/PXR – in vitro

See Table S4.24 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Human primary cells

Several studies in human primary hepatocytes have tested whether PFOA or PFOS activate human constitutive androstane receptor (CAR)- and pregnane X receptor (PXR)-mediated gene transcription. These receptors are discussed together because they regulate overlapping transcriptional programmes, which can make it challenging to definitively identify receptor-specific target genes (Tojima et al., 2012). Here, we have deferred to the study authors with respect to the identification of receptor–target gene pairs.

In human primary hepatocytes exposed for 24–48 hours, PFOS (25 μM) induced the expression of the CAR target genes *CYP2B6* and *CYP2C19*, but PFOA (25 μM) only induced CAR target gene expression after 48 hours (Bjork et al., 2011; Marques et al., 2022).

In transcriptomic analyses of human primary hepatocytes, PFOA (25 μM) was shown to partially activate the PXR-dependent pathway, but not the CAR pathway (Buhrke et al., 2015). The PXR target gene *CYP3A4* has also been shown to be upregulated by PFOA and PFOS (100 μM, 48 hours) in human primary hepatocytes (Rosen et al., 2013); however, when tested at 25 μM for 24 hours, only PFOS significantly increased *CYP3A4* mRNA expression (Bjork et al., 2011).

Human cell lines

In human liver cell models, PFOA activated CAR-dependent gene expression (*CYP2B6*) in HepaRG human liver cells (30 μM, 48 hours; Abe et al., 2017) and in HepG2 cells (250 μM, 24 hours; Behr et al., 2020b). In contrast, very low-level exposure to PFOA (0.001 μM) or PFOS (0.001 μM) for 24–48 hours was shown to reduce CAR-mediated gene expression (*CYP2C19*) (Franco et al., 2020). In HepaRG human liver cells exposed for 48 hours, PFOA (≥ 50 μM) and PFOS (≥ 1 μM) induced mRNA expression of the PXR target gene *CYP3A4* (Behr et al., 2020a). Very low-level exposure to PFOA (0.001 μM) or PFOS (0.001 μM) for 24–48 hours also reduced PXR-mediated gene expression (*CYP3A4*) (Franco et al., 2020).

Table 4.27 Modulation of receptor-mediated effects: pathway characterization for PFOA and PFOS

Hormone pathway	Consistent and coherent evidence		Suggestive evidence		Paucity of data	
	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS
Exposed humans						
Thyroid pathway			X	X		
Estrogen pathway				X	X	
Androgen pathway		X	X			
Progesterone pathway			X	X		
Glucocorticoid pathway				X	X	
AHR pathway					X	X
Primary human cells						
Thyroid pathway					X	X
Estrogen pathway			X			X
Androgen pathway					X	X
Progesterone pathway					X	X
Glucocorticoid pathway					X	X
AHR					X	X
PPARα	X	X				
CAR/PXR	X	X				
PPARγ			X	X		
HNF4α					X	X
Human cell lines, binding assays and reporter assays						
Thyroid pathway					X	X
Estrogen pathway			X	X		
Androgen pathway					X	X
Progesterone pathway					X	X
Glucocorticoid pathway					X	X
AHR					X	X
PPARα	X	X				
CAR/PXR	X	X				
PPARγ			X	X		
HNF4α					X	X
Experimental systems (rodents)						
Thyroid pathway		X			X	
Estrogen pathway		X	X			
Androgen pathway	X	X				
Progesterone pathway					X	X
Glucocorticoid pathway						X
AHR					X	X
PPARα	X	X				
CAR/PXR	X	X				
PPARγ	X	X				
HNF4α					X	X

AHR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; HNF4α, hepatocyte nuclear factor 4 alpha, PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor.

Human binding and reporter assays

Reporter assays also have been employed to investigate the abilities of PFOA and PFOS to stimulate the transcriptional activities of CAR and PXR.

It has been shown that PFOS activates CAR indirectly, by modulating its phosphorylation, rather than binding to the ligand-binding domain (Abe et al., 2017); therefore, traditional reporter assays have not detected PFOA- or PFOS-induced transactivation of CAR (e.g. Behr et al., 2020b; Houck et al., 2021; Murase et al., 2023).

In reporter studies employing full-length human PXR, PFOA (0.1–300 μM, 24 hours) was shown to stimulate PXR transactivation when the host cell was a human hepatocyte model (Zhang et al., 2017b; Houck et al., 2021). In reporter assays using a one-hybrid approach, PFOA (0.1–300 μM, 24 hours) stimulated PXR-mediated transcription in only one of the two available studies (Behr et al., 2020a; Houck et al., 2021). In reporter studies employing full-length human PXR, PFOS (0.14–300 μM, 24 hours) was shown to stimulate PXR transactivation when the host cell was a human hepatocyte model (Zhang et al., 2017b; Houck et al., 2021). In reporter assays using a one-hybrid approach, PFOS (0.14–300 μM, 24 hours) stimulated PXR-mediated transcription in only one of the two available studies (Behr et al., 2020a; Houck et al., 2021).

Synopsis

[Overall, the Working Group noted that there was evidence in human primary cells and other human cell in vitro models that PFOA and PFOS stimulated CAR and PXR transcriptional activity. Reporter assays were not able to detect effects of PFOA or PFOS on CAR activity, because activation probably occurred through the modulation of phosphorylation, rather than through ligand binding. The results of PXR reporter assays suggested the transactivation of PXR by PFOA and PFOS, but the effects of PFOA and PFOS appeared to be dependent upon the type of reporter assay and host cell type (see summary findings for KC8 in Table 4.27).]

PPARγ – in vitro

See Table S4.24 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Human primary cells

Only four studies in human primary cells have examined whether PFOA and PFOS activate human PPARγ-related pathways. In HUVECs, PFOS (100 μM) exposure for either 24 or 48 hours increased the mRNA expression of *PPARG* (Liao et al., 2012). In human primary hepatocytes exposed for 48 hours, PFOA (25 μM) and PFOS (25 μM) induced the mRNA expression of *CD36* and *PPARG*, PFOA alone induced the expression of *SCD*, and neither induced the expression of *FASN* or *GPAM* (Marques et al., 2022). In transcriptomic analyses of human primary hepatocytes, PFOA (≥ 1 μM) strongly upregulated the PPARγ pathway (Buhrke et al., 2015). In primary hMSCs stimulated to undergo adipogenic differentiation (7–14 days), PFOA (≥ 0.1 μM) and PFOS (≥ 0.1 μM) stimulated the expression of *FABP4* and *PPARG* and lipid accumulation (an indicator of adipocyte differentiation, which requires PPARγ; Qin et al., 2022c). The authors reported similar findings in mammalian cells (Qin et al., 2022b). In another similar study (7–21 days of differentiation), PFOS (≥ 0.1 μM) induced *FABP4* and *PPARG* expression Gao et al. (2020). In human primary subcutaneous preadipocytes stimulated to undergo differentiation (10 days), PFOA (≥ 6 μM) induced the expression of *FABP4*, *PLIN1*, and *PPARG* and increased lipid accumulation (Li et al., 2019b). In human primary bone marrow mesenchymal stromal cells differentiating for 21 days, PFOA (≥ 0.1 μM) and PFOS (1 μM) increased lipid accumulation (Bérubé

et al., 2023). Similarly, in human primary visceral preadipocytes differentiated for 11 days, PFOS (≥ 5 μM) increased lipid accumulation (Xu et al., 2016). [The Working Group noted that none of the differentiation studies tested the ability of PFOA or PFOS to stimulate adipocyte differentiation in the absence of a hormone cocktail, which would be a stronger indicator of PPARγ agonism.]

Human cell lines

In the human liver cell HepaRG model, exposure to PFOA (100 μM) for 24 hours induced the expression of the classic PPARγ target gene *FABP4* (Attema et al., 2022).

Human binding and reporter assays

PFOA and PFOS have been shown to bind to human PPARγ at high concentrations in competitive binding assays using the human PPARγ ligand-binding domain, with PFOS having higher binding affinity than PFOA (Zhang et al., 2014b; Li et al., 2018b, 2019b). In a human PPARγ reporter assay in which full-length human PPARγ was expressed in HepG2 cells, PFOA and PFOS had similar potencies (~10 μM) for the stimulation of PPARγ transactivation (Zhang et al., 2014b). Less consistent results have been obtained using human PPARγ one-hybrid reporter assays. PFOA (at concentrations of ≥ 25 μM) and PFOS have been reported not to activate human PPARγ hosted in human kidney HEK293T cells (Behr et al., 2020b); or to activate human PPARγ in HEK293 cells with low efficacy (Li et al., 2019b); or to activate human PPARγ in INDIGO cells with very low potency (Evans et al., 2022); or to activate human PPARγ in HEK293 cells (Buhrke et al., 2013), human epithelial HeLa cells (Garoche et al., 2021), and HepG2 cells (Houck et al., 2021).

Synopsis

[The Working Group noted that there was evidence in human primary cells and other human cell in vitro models that PFOA and PFOS stimulated PPARγ transcriptional activity. Caveats remain, however, in that it is unclear if increases in PPARγ-mediated gene expression result from the direct activation of PPARγ or from PFOA and PFOS increasing the expression of *PPARG*. Additionally, the results of reporter assay studies were inconsistent with regard to the stimulation of PPARγ-transactivation by PFOA and PFOS, even when the host cell line was the same (see summary findings for KC8 in Table 4.27).]

HNF4α – in vitro

See Table S4.24 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Human primary cells, cell lines, and reporter assays

Hepatocyte nuclear factor (HNF)4α is a critical receptor for liver development and function (Hayhurst et al., 2001) and is an orphan nuclear receptor (Bogan et al., 2000). [Fatty acids are thought to become stably associated with the ligand-binding domain (Wisely et al., 2002); therefore, PFOA and PFOS are unlikely to interact with the HNF4α ligand-binding domain and are more likely to have indirect effects on HNF4α activity.] Importantly, HNF4α is essential for the activation of CAR and PXR, by acting as a coactivator (Tirona et al., 2003).

PFOA (≥ 25 μM, 24 hours) has been shown to downregulate HNF4α, as evidenced by a large decrease in *HNF1A* mRNA expression in human primary hepatocytes (Buhrke et al., 2015). Similarly, in HepG2 cells, PFOA (25 μM, 48 hours) reduced the protein expression of HNF4α and a transcriptional target (HNF1α)

(Scharmach et al., 2012). Lastly, PFOA (≥ 1 μM, 24 hours) reduced endogenous HNF4α transcriptional activity in HepG2 cells and the activity of overexpressed full-length HNF4α in HEK293 cells in reporter assays (Scharmach et al., 2012).

[The Working Group noted that PFOA was shown to suppress the activity of a critical liver transcription factor, HNF4α. However, all the evidence came from a single research group. The possibility that PFOS may interfere with the HNF4α pathway has not been investigated.]

Synopsis

[The Working Group noted that, overall, in exposed humans, the data showed some association of PFOA with modulation of the thyroid, androgen, and progesterone pathways. However, the data did not support an association between PFOA and the estrogen pathway, because the results were largely negative. There was a paucity of data regarding an association of PFOA with the glucocorticoid pathway.

In exposed humans, there was some evidence for PFOS being associated with modulation of the androgen pathway, with PFOS potentially being associated with lower testosterone levels in men, although the effect size was small. For the thyroid hormone, estrogen, progesterone, and glucocorticoid pathways, the data were inconsistent, and there was insufficient explanation for the differences, meaning that the data were only indicative of an association with PFOS.

There was a paucity of data to permit the assessment of any association of either PFOA or PFOS with the aryl hydrocarbon receptor (AHR) pathway.

In human primary cells, there was evidence that both PFOA and PFOS modulated PPARα activity (receptor activation), which was corroborated by data from human cell lines, binding, and reporter assays. There was evidence that both PFOA and PFOS modulated CAR/PXR activity (receptor activation), which was corroborated by data from human cell lines.

There was some evidence that PFOA and PFOS modulated the estrogen pathway (estradiol secretion and estrogen-receptor activation) in human primary cells, but the results were inconsistent across studies in other human cell in vitro models, with more evidence of estrogen-receptor activation coming from reporter assays in which estrogen receptor was overexpressed. There was some evidence that PFOA and PFOS modulated the PPARγ pathway (adipocyte differentiation, lipid accumulation, and PPARγ activation) in human primary cells, but the results were inconsistent across studies in other human cell in vitro models.

There was scarce evidence of associations of PFOA or PFOS with androgen-receptor activation in human primary cells, with results in other human cells in vitro (testosterone secretion and androgen-receptor activation) having largely obtained negative results. There was a paucity of data regarding any association of PFOA or PFOS with the thyroid pathway (thyroid peroxidase activity, TTR binding/T4 displacement). There was a paucity of data with which to assess the modulation of HNF4α activity (receptor activation) by PFOA and no studies examined the modulation of HNF4α by PFOS. There was a paucity of data (no studies in human primary cells) from studies examining the modulation of the progesterone receptor, the glucocorticoid receptor (and negative results regarding cortisol secretion and receptor activation in experimental human models), or the AHR by PFOA or PFOS (see summary findings for KC8 in Table 4.27).]

(b) Experimental systems

See Table S4.26 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636) and Table 4.27.

Both PFOA and PFOS have been shown to modulate receptor-mediated effects in experimental systems. Because the data were robust for many endocrine receptor outcomes, this review

primarily focuses on in vivo studies in experimental systems. The identified studies were peer-reviewed, used well-developed methods, and the quality of the study design was deemed to be adequate. Some studies used transgenic models in which the expression of one or more endocrine receptor classes was experimentally altered.

(i) Modulation of PPARs

Non-human mammalian systems in vivo

See Table S4.25 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

One of the most extensively studied effects relates to the ability of these chemicals to activate PPARs. Numerous in vivo mammalian studies that evaluated effects on PPARs were available (Table S4.24). This class of nuclear receptors has three main subtypes: PPARα, PPARβ, and PPARγ (Escher and Wahli, 2000). PPARα is highly expressed in the liver and influences the expression of genes involved in lipid metabolism, including peroxisomal β-oxidation, fatty acid uptake, and triglyceride synthesis (Rakhshandehroo et al., 2007). PFOA and PFOS are structural analogues of fatty acids, the endogenous ligands for PPARα.

In rodents, peroxisome proliferation and other metabolic effects occur in the liver after PPARα activation by either PFOA or PFOS. PFOA or PFOS exposure results in hepatomegaly in rodents (Costello et al., 2022). Studies of the exposure of PPARα-KO 129/Sv mice to PFOA or PFOS have shown that hepatomegaly was independent of PPARα activation (Filgo et al., 2015; Su et al., 2022a). PFOA or PFOS exposure in rodents also alters the activity of certain members of the cytochrome P450 (CYP) system. PPARα regulates the expression of acyl-CoA oxidase (ACOX) and CYP4A. CYP4A is involved in the oxidation of fatty acids and lipid metabolism.

The increased expression of certain CYP genes in rodents exposed to either PFOA or PFOS has been used as an indicator of the activation of the receptor subclasses. PPARα target genes include acyl-CoA oxidase (*Acox1 or Pco*), cytochrome P450 4a10 (*Cyp4a10*), and acetyl-CoA acetyltransferase 1 (*Acat1)* (Mandard et al., 2004). It has been estimated that PPARα mediates more than 75% of the changes in hepatic gene expression induced by PFOA and PFOS in mice (Rosen et al., 2008a, 2017).

Hepatic palmitoyl-CoA oxidase (PCO; alias Acox1) activity has been used as a marker of PPARα activation and peroxisome proliferation (Klaunig et al., 2003). [The Working Group noted that in the literature, acyl-CoA oxidase (*Acox1*) is also termed PCO (NCBI, 2023).]

One study in male cynomolgus monkeys (*S. cynomolgus* or *M. fascicularis*) examined the effects of 6 months of oral exposure to PFOA on PPARα (Butenhoff et al., 2002). [The Working Group noted that the increase in PPARα activation caused by PFOA, assessed by PCO activity in this study, is qualitatively similar to that seen in male rats after PFOA exposure.] Male and female rats exposed to PFOS at 20 ppm in the diet for 14 weeks developed hepatocellular hypertrophy and vacuolation, as well as increased relative liver weight, without a significant increase in hepatic PCO activity (Seacat et al., 2003). Increased relative liver weight, hepatocellular hypertrophy, and a modest (0.2-fold) increase in hepatic PCO activity occurred in rats after 28 days of exposure to PFOS at 20 ppm (Elcombe et al., 2012b).

PPARα-null mice have been studied after exposure to PFOA or PFOS. Yang et al. (2002a) reported that liver weight increased in both wildtype and PPARα-null mice treated with PFOA (0.02% in the diet) for 7 days, whereas the PCO activity was only increased in the wildtype mice. Wolf et al. (2008b) also reported that the oral PFOA exposure of wildtype and PPARα-null mice increased liver weight and hepatic cell proliferation and caused hepatocyte

hypertrophy. Increased numbers of peroxisomes were present in the livers of treated wildtype mice, whereas the livers of PPARα-null mice had numerous vacuoles without peroxisomes (Wolf et al., 2008b). [The Working Group noted that, collectively, these findings suggest that the effects of PFOS on the rat liver do not appear to primarily occur through a PPARα-dependent mode of action.]

Loveless et al. (2006) evaluated the effects of linear, branched, and a mixture of linear/branched isomers of PFOA on the relative liver weight and hepatic PCO activity of male CD rats and CD-1 mice. The liver weight of the mice increased with increasing PFOA dose and serum level, but the PCO activity was lower at the highest dose and serum level than at lower doses and serum levels. In the rats, the branched isomers of PFOA were more potent at increasing relative liver weight than the linear isomer but were less potent at increasing PCO activity. Perkins et al. (2004) reported that the subchronic dietary exposure of rats to PFOA was associated with a reversible increase in liver weight, increased hepatic PCO activity, and minimal-to-mild hepatocyte hypertrophy.

Short-term exposure to PFOS was associated with increased liver weights in wildtype and PPARα-null mice (Qazi et al., 2009). In addition, altered expression of genes related to lipid metabolism, inflammation, and xenobiotic metabolism were observed in PPARα-knockout mice exposed to PFOS, suggesting that additional pathways are activated by this chemical (Rosen et al., 2010).

Differences have been reported in the function of mouse and human PPARα (Gonzalez and Shah, 2008). The activation of mouse PPARα results in hepatocyte proliferation and dysregulation of cell-cycle genes, which does not occur in humans (Morimura et al., 2006). Transgenic mouse models that express the human PPARα gene (hPPARα) have also been used to explore the role of PPARα in PFOA and PFOS hepatotoxicity. For example, female and male hPPARα mice exposed to PFOA showed increased liver mass and histologically evident lipid accumulation (Schlezinger et al., 2020, 2021). Other studies using humanized transgenic mice have shown that hPPARα may be less responsive to either PFOA or PFOS when compared with PPARα in wildtype mice (Nakamura et al., 2009; Albrecht et al., 2013; Su et al., 2022a). [The Working Group noted that differences in the response in humanized mice are not necessarily indicative of a different response of human PPARα to PFOA, because species differences in the binding of PFOA to recognition sites on mouse DNA may exist. The Working Group also noted that the relevance to humans of the activation of PPARα with the altered hepatocyte growth and survival and clonal expansion of preneoplastic foci cells, leading to PPARα-dependent rodent liver tumour responses, has been questioned (Corton et al., 2018).]

The effects of PFOA and PFOS on PPARγ have also been examined in rodents. This pathway regulates adipocyte differentiation and lipid metabolism (Casals-Casas and Desvergne, 2011). Four studies in mice (Abbott et al., 2012; Nakagawa et al., 2012; Yan et al., 2015b; Schlezinger et al., 2020) have shown increased liver *Pparg* mRNA expression after PFOA exposure. One study (Wan Ibrahim et al., 2013) showed increased brain *Pparg* mRNA expression in neonatal mice after the PFOS exposure of pregnant dams.

Two studies in mice (Yan et al., 2015b; Li et al., 2019c) yielded mixed results regarding the liver expression of PPARβ/δ after PFOA exposure.

Non-human mammalian systems in vitro

See Table S4.26 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Cell-based in vitro studies involving either reporter assays or the expression of PPAR target genes in hepatic cells have shown that these

chemicals activated mouse, rat, and human PPARα, as well as PPARγ (Vanden Heuvel et al., 2006; Takacs and Abbott, 2007; Wolf et al., 2008b; Bjork and Wallace, 2009; Bjork et al., 2011; Houck et al., 2021). Both PFOA and PFOS exposure resulted in the transactivation of PPARγ constructs derived from humans, mice, zebrafish, and *Xenopus* in reporter assays (Garoche et al., 2021). Both PFOA and PFOS have been shown to modulate PPARγ signalling in various in vitro cell systems (Watkins et al., 2015; Liu et al., 2019; Li et al., 2021a; Modaresi et al., 2022; Qin et al., 2022c). Studies performed by Takacs and Abbott (2007) using COS-1 cells transfected with mouse or human PPARα, PPARβ/δ, or PPARγ reporter plasmids and exposed to either PFOA or PFOS showed that PFOA causes significant increases in both mouse and human PPARα reporter activity, whereas PFOS induced activation of the mouse PPARα reporter alone. PFOA and PFOS exposure also increased the activity of the mouse PPARβ/δ, but not the human PPARβ/δ reporter construct. Neither PFOA nor PFOS activated the mouse or human PPARγ reporter (Takacs and Abbott, 2007).

(ii) Modulation of CAR/PXR

Non-human mammalian systems in vivo

See Table S4.25 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

In vivo studies using CAR-null mice (Abe et al., 2017) or mice expressing hPPARα (Schlezinger et al., 2020) have shown that PFOA exposure modulates the CAR and PXR pathways. These studies reported effects on the expression of CAR target genes, which include *Cyp2b10*, *Cyp2c29*, *Cyp2c55*, *Akr1b7*, and *Ugt2b34* (Dusek et al., 2019). The PXR target genes examined in some studies included *Oatp1a4*, *CYP3A4*, *CYP2B6*, *MDR1*, and *MRP2* (Smutny et al., 2022). Two studies performed in male C57BL/6 mice showed increased hepatic expression of CAR after PFOA exposure (Cheng and Klaassen, 2008; Li et al., 2019c).

Increased liver expression of the CAR target gene *Cyp2b1* has been reported to occur in male Sprague-Dawley rats after either a 7- or 28-day (Elcombe et al., 2012a, b) exposure to PFOS at 100 ppm. Increased liver activity of the PXR target gene *Cyp3a1was* also identified after 28 days (Elcombe et al., 2012b). Three other rodent studies (Bijland et al., 2011; Dong et al., 2016; Lai et al., 2017a) also reported PFOS effects on the PXR pathway.

Non-human mammalian systems in vitro

In vitro studies using transcriptome profiling in rodent and human hepatocytes showed that PFOA and PFOS may regulate PXR target gene expression and, to lesser extent, that of CAR (Bjork et al., 2011; Buhrke et al., 2015; Abe et al., 2017; Houck et al., 2021).

(iii) Modulation of thyroid-receptor function

Non-human mammalian systems in vivo

See Table S4.25 (Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

One of the most extensively studied endocrine responses seen after PFOA or PFOS exposure relates to the effects of these chemicals on thyroid hormone function. In rats, PFOS exposure reduced serum T4 and T3 levels without increasing TSH secretion (Chang et al., 2008; Davidsen et al., 2022). In mice, PFOS had less consistent effects on thyroid hormone levels (Table S4.25). One study in cynomolgus monkeys reported no effects of PFOS on the serum TSH, FT4, or TT3 concentrations (Chang et al., 2017). Monkeys exposed to PFOS had low serum TT4 concentrations, which was not deemed to be clinically significant. [However, the Working Group noted that the exposure level in this study

was limited and that long delays between exposure and the measurement of thyroid hormone concentrations occurred.]

The exposure of rodents to PFOS during pregnancy was often not associated with altered maternal or pup thyroid hormone levels (Fuentes et al., 2006; Chang et al., 2009), although one study reported reduced maternal serum TT3 and TT4 concentrations after exposure during gestation (Conley et al., 2022), and this finding was replicated in pups (Lau et al., 2003).

Adult exposure to PFOS was generally associated with reduced serum thyroid hormone concentrations, whereas the TSH level was often unaffected (see Table S4.26, Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636).

Putative mechanisms for the PFOS-induced disruption of thyroid hormone function include inhibition of the sodium/iodide symporter, altered synthesis of thyroglobulin, effects on thyroperoxidase, and the displacement of thyroid hormones from TTR (Weiss et al., 2009; Yu et al., 2009; Dong et al., 2016; Ren et al., 2016; Coperchini et al., 2021a; Davidsen et al., 2022).

In vivo studies examining the effects of PFOA on thyroid hormone function have been more limited in number (Table S4.26, Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636), with mixed effects reported in exposed non-human primates, mice, and rats (Butenhoff et al., 2002, 2012a; Blake et al., 2020).

Non-human mammalian systems in vitro

Several studies have shown that PFOA and PFOS affect the binding of thyroid hormones to the thyroid hormone transport protein TTR (Weiss et al., 2009; Ren et al., 2016; Behnisch et al., 2021). Both PFOA and PFOS have been shown to modulate thyroid hormone signalling in rat cell systems (Croce et al., 2019; Selano et al., 2019; De Toni et al., 2022).

(iv) Modulation of estrogen-receptor function

Non-human mammalian systems in vivo

Data concerning the direct effects of PFOA or PFOS on the estrogen receptor are limited to those obtained in single studies with negative or mixed findings (Yao et al., 2014; Xu et al., 2017). The estradiol concentrations in mice and rats after either PFOA or PFOS exposure can be variable, with some studies reporting elevated levels, and others showing a reduction in serum estrogen concentration (Table S4.26, Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636). Male mice exposed to PFOS did not demonstrate altered serum estradiol concentrations (Wang et al., 2014b; Qu et al., 2016; Qiu et al., 2021; Huang et al., 2022a), whereas female ICR mice exposed to PFOS had reduced estradiol concentrations (Wang et al., 2018b). Reductions in serum estradiol concentrations occurred at exposure levels that did not affect male mice (Qiu et al., 2021; Huang et al., 2022a). However, male rats exposed to PFOS had reduced serum estradiol concentrations in two studies (López-Doval et al., 2015; Salgado et al., 2015).

One study conducted in male cynomolgus monkeys (*S. cynomolgus* or *M. fascicularis*) showed no effect on serum estradiol concentration after a 26-week exposure to PFOA (Butenhoff et al., 2002). Several rodent studies also reported no effect of PFOA on serum estradiol concentration (Perkins et al., 2004; Zhao et al., 2010; Tucker et al., 2015; Su et al., 2022b; Yang et al., 2022). Other studies reported increased serum estradiol concentrations in male rats upon PFOA exposure (Biegel et al., 2001; Han et al., 2022) and in female rats upon PFOS exposure (Qiu et al., 2020).

Zhang et al. (2020b) reported lower serum estradiol concentrations in female ICR mice exposed to PFOA.

Short-term studies using zebrafish have shown that PFOS acts as an estrogen-receptor agonist (Du et al., 2013).

Non-human mammalian systems in vitro

In vitro studies using ER reporter assays performed in trout or human HEK293T cells and transcription factor activation bioassays performed in human HepG2 cells have shown that PFOA and PFOS regulate ERα target gene expression (Benninghoff et al., 2011; Buhrke et al., 2015; Qiu et al., 2020; Houck et al., 2021).

(v) Modulation of androgen-receptor function

Non-human mammalian systems in vivo

Several studies performed in mice have shown that PFOS exposure results in reduced serum or testicular testosterone concentrations (Table S4.25, Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636). However, other studies in mice showed no effects of PFOS exposure (Wang et al., 2014b; Li et al., 2022e). Most studies performed in rats have also shown reduced serum testosterone concentration after PFOS exposure, with fewer demonstrating no effect or increased testosterone concentration (Table S4.25). One study conducted in cynomolgus monkeys showed no effect of a 26-week exposure to PFOA (see also above) (Butenhoff et al., 2002). PFOS exposure in rodents has also been shown to reduce epididymal sperm count and alter the expression levels of several key steroidogenic enzymes in the testes (Wan et al., 2011). Effects of PFOS on testosterone synthesis in Leydig cells have been linked to the CREB/transcription coactivator 2 (CRTC2)/steroidogenic acute regulatory protein (StAR) signalling pathway (Qiu et al., 2021).

Two studies performed in Sprague-Dawley rats showed increased liver testosterone 6β-hydroxylase activity after PFOS administration (Elcombe et al., 2012a, b).

PFOA also showed effects on the androgen receptor pathway. Reduced serum or plasma testosterone concentrations were reported in male rats and mice after PFOA exposure (Li et al., 2011; Owumi et al., 2021b). Reduced serum testosterone concentrations were identified in the male offspring of Kunming mice exposed during gestation (Song et al., 2018). Increased serum testosterone concentrations were identified in female CD-1 mice (Yang et al., 2022) and male CD rats (Biegel et al., 2001) after PFOA administration. However, one study reported no effect of PFOA on the circulating testosterone concentration of exposed male CD rats (Perkins et al., 2004).

(vi) Other receptor systems

Non-human mammalian systems in vivo

Effects of PFOA and PFOS on prolactin and insulin function have been reported in studies that evaluated the function of multiple hormones (Table S4.25, Annex 5, Supplementary material for Sections 4.1 and 4.2, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/636). Hines et al. (2009) reported that exposure to PFOA at 0.01 mg/kg bw significantly increased serum insulin in mice. Wan et al. (2014) reported that perinatal exposure to PFOS resulted in elevated levels of glucose and insulin in F_1 pups and adult CD-1 mice. Combined gestational and lactational exposure to PFOS elevated fasting serum insulin concentrations and impaired glucose tolerance in rat offspring (Lv et al., 2013).

Neither PFOS nor PFOA altered AHR transcriptional activity in transfected mouse Hepa1.1 2cR cells (Long et al., 2013). [The Working Group noted that a positive control, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, was included with this

AHR–luciferase reporter gene bioassay.] Other studies examined whether PFOA or PFOS altered the expression of *Cyp1a1*, which encodes an enzyme and is regulated by AHR. One study performed in juvenile Atlantic salmon (*Salmo salar*) fed either PFOA or PFOS at 0.2 mg/kg for < 1 week showed no relation between PFOA or PFOS treatment and the hepatic expression of CYP1A1 and AHR isoforms; however, a temporary but significant increase in *Cyp1a1* expression was identified in PFOA-fed salmon after 5 days and PFOS-fed salmon after 2 days (Mortensen et al., 2011). The exposure of rare minnow (*Gobiocypris rarus*) to PFOA increased the *Ahr* mRNA level in the gills and was associated with the downregulation of *Cyp1a* mRNA (Liu et al., 2008). One study examined the effects of PFOS on intestinal immunity and infection in mice with *Citrobacter rodentium* infection (Suo et al., 2017). PFOS prevented the replication of *C. rodentium* by promoting IL-22 production by group 3 innate lymphoid cells through the activation of AHR. The large intestinal lamina propria lymphocyte mRNA expression of *Cyp1a1* was also elevated after PFOS treatment (Suo et al., 2017).

In the liver, HNF4α regulates multiple genes and lipid metabolism and is involved in hepatocellular differentiation (Yeh et al., 2019). In mice, the conditional hepatocyte-specific deletion of HNF4α results in hepatomegaly and hepatic steatosis. This phenotype is similar to that induced in rodents exposed to PFOA or PFOS (Bonzo et al., 2012). However, only one study has examined the role of HNF4α after PFOA or PFOS exposure in experimental systems (Yan et al., 2015b). This showed reduced hepatic mRNA expression of *Hnf4a* in mice exposed to PFOA at 1.25 or 5 mg/kg per day. The high PFOA dose used in this study was not associated with altered *Hnf4a* expression. Finally, an in vitro study using proteomic profiling in human hepatocytes showed that PFOA alters the expression of genes regulated by HNF4α (Scharmach et al., 2012).

Synopsis

[The Working Group noted that, overall, the studies described above in experimental systems in vivo reported evidence that both PFOA and PFOS could modulate PPARα activity. In addition, there was evidence that PFOA modulated PPARγ activity. In one rodent study PFOS was shown to modulate PPARγ activity.

There was a paucity of available data for the effects of either PFOA or PFOS on PPARβ/δ. There was evidence that both PFOA and PFOS modulated CAR/PXR function.

PFOS modulated thyroid function in exposed animals. However, there were limited data suggesting that thyroid effects were induced by PFOA exposure. There was some evidence that PFOA and PFOS altered serum estradiol concentrations in rodents; however, both non-significant and significant findings were reported.

Evidence that PFOS altered the testosterone concentrations of rodents was available from multiple studies, but there was more limited evidence for the effects of PFOA on androgen-receptor function and testosterone concentration. Finally, there was a paucity of information on the effects of both PFOA and PFOS on other receptor systems in experimental systems (see summary findings for KC8 in Table 4.27).]

4.2.9 Causes immortalization

(a) Humans

(i) Exposed humans

See Table 4.28.

The shortening or lengthening of telomeres has been associated with some types of cancers and may contribute to carcinogenesis. Telomere shortening may increase genetic instability, whereas telomere lengthening may promote deleterious cell survival and proliferation and serve as a marker of immortalization.

Table 4.28 End-points relevant to immortalization in humans exposed to PFOA or PFOS

End-point, assay	Biosample type	Location, setting, study design	Exposure level[a] No. of study participants	Response (significance)	Covariates adjusted for	Comments	Reference
Telomere length, by Sigma TeloTAGGG Telomere Length assay	Peripheral blood leukocytes and serum	Firefighters from San Francisco Fire Department and office workers, City and County of San Francisco, USA	163 participants (84 firefighters and 79 office workers) Women PFOA, 1.16 ± 1.76 ng/mL PFOS, 4.18 ± 2.08 ng/mL	Linear regression models (minimally adjusted model, Model 1, and a fully adjusted model, Model 2, used to assess the associations between continuous log-transformed PFAS and TL In Model 1, a doubling of PFOA concentration was associated with a 273 (95% CI, 54–493) bp increase in TL; in Model 2, a doubling in PFOA was associated with a 240 (95% CI, 25–455) bp increase in TL A doubling in PFOS concentration was associated with a 183 (95% CI, 15–352) bp increase in TL in Model 1, and a 172 (95% CI, 5–340) bp increase in TL in Model 2	Age, occupation, the number of times dairy products were eaten per week, and the number of times eggs were eaten per week. Covariates assessed include demographic variables such as race/ethnicity and education; health variables such as BMI, stress, and sleep metrics; and food frequency variables.	Potential confounders were selected a priori, on the basis of results from previous literature and prior analyses performed on these data. No tables provided for the above statistics.	Clarity et al. (2021)

Table 4.28 (continued)

End-point, assay	Biosample type	Location, setting, study design	Exposure level[a] No. of study participants	Response (significance)	Covariates adjusted for	Comments	Reference
Telomere length, by qPCR	Maternal whole blood and delivery cord blood	Chemicals in Our Bodies cohort	Mothers (n = 125) and newborns (n = 326) PFOA, 0.8 ± 2.0 ng/mL PFOS, 2.0 ± 2.1 ng/mL	Quantile g-computation PFAS mixture alone was associated with a modest increase in maternal TL (mean change in maternal TL per quartile increase, 0.04; 95% CI, −0.01 to 0.09) In the analysis restricted to maternal–fetal paired samples, an increase in the PFAS mixture of one quartile was positively associated with umbilical cord blood TL (mean change in umbilical cord blood TL per quartile increase, 0.11 (95% CI, 0.01–0.22) Individual PFOA (β = 0.01; 95% CI, −0.03–0.06), and PFOS (β = −0.01; 95% CI, −0.05 to 0.03)	Maternal education, race/ethnicity, maternal age, gestational age at delivery, pre-pregnancy BMI, parity, and infant sex		Eick et al. (2021)
Telomere length, by qPCR	Leukocytes, serum	US NHANES 1999–2000 Cross-sectional data set	773 participants; 389 men and 384 women PFOA mean (10th–90th percentile), 5.63 (2.24–8.76) ng/mL PFOS, 33.97 (13.54–57.68) ng/mL	Fully adjusted multiple variable linear regression Each increment of one SD in the $\log_{10}$ PFOS level was associated with a 21-bp increase in the TL (P = 0.033), after adjustment for potential confounders No associations of the TL with the concentrations of PFOA or other PFAS	Age, sex, race, BMI, educational level, leukocyte count, C-reactive protein, and PIR status		Huang et al. (2019b)

Table 4.28 (continued)

End-point, assay	Biosample type	Location, setting, study design	Exposure level[a] No. of study participants	Response (significance)	Covariates adjusted for	Comments	Reference
Telomere length, by qPCR	Cord blood leukocytes, cord serum	Wuhan Maternal and Child Health Hospital in Wuhan, China	916 cord blood samples PFOA, 1.81 ± 2.33 ng/mL PFOS, 5.77 ± 10.32 ng/mL	Weighted quantiles of exposure and linear regression Negative association in the adjusted model: % change in neonatal TL, −5.19% (95% CI, −9.44 to −0.73%; $P = 0.040$) for each unit increase in WQS index of PFAS mixture The weights of PFOS and PFOA were 13.16% and 10.75%, respectively	Maternal sociodemographics, age, education, alcohol drinking habits, smoking status, pre-pregnancy BMI, parity, birth weight, gestational age, neonatal sex, and neonatal birth season Alcohol consumption and active smoking were not adjusted for, because no participants drank or smoked during pregnancy		Li et al. (2023a)
Telomere length, by qPCR	Buffy coat from cord blood, cord plasma	Shanghai Allergy Cohort, Shanghai, China; 2012–2013	581 participants PFOA, 7.65 ± 3.88 ng/mL PFOS, 2.93 ± 3.28 ng/mL	Generalized linear models adjusted for covariates LTL was significantly shorter in the female newborns whose PFOS, but not PFOA, concentrations were in the highest quartile, compared with those in the lowest quartile, after adjusting for potential confounders (0.926 ± 0.053 vs 0.945 ± 0.054, respectively (mean ± SD); $P = 0.023$)	Maternal and paternal ages, maternal education, maternal pre-pregnancy BMI, mode of delivery, gestational age at birth, infant sex, birth weight, and antepartum obstetric risk		Liu et al. (2018b)

Table 4.28 (continued)

End-point, assay	Biosample type	Location, setting, study design	Exposure level[a] No. of study participants	Response (significance)	Covariates adjusted for	Comments	Reference
Telomere length, by qPCR	Buffy coat containing leukocytes, serum	Flemish Environment and Health Study, Flanders, Belgium	175 participants PFOA geometric mean (25th–75th percentile): 2.78 (2.13–3.65) µg/L PFOS: 7.52 (5.31–10.9) µg/L	sPLS regression model followed by OLS regression Serum PFOA level was inversely associated with LTL; sPLS model (raw coefficient, −0.017; 95% CI, −0.032 to −0.002; $P = 0.03$; and OLS model (relative change, −3.64%; 95% CI, −6.60 to −0.60%; $P = 0.02$	In linear regression models the a priori covariates selected from among socioeconomic status, lifestyle, smoking habits, and ethnicity were age, sex, BMI, smoking habits, household education, and ethnicity		Vriens et al. (2019)
Telomere length, by qPCR	Leukocytes from cord blood, maternal serum	Birth cohort study in Guangxi, China, 2015–2018	PFOA, geometric mean, 2.379; 25th–95th percentile, 1.716–5.599 ng/mL PFOS geometric mean, 0.983; 25th–95th percentile, 0.662–3.736 ng/mL 499 mother–umbilical cord blood pairs	Multivariable linear regression: each ln-transformed unit concentration increase in PFOA was associated with 20.41% (95% CI, −30.44 to −8.93%) shorter LTL in spring-born infants but not in those born in other seasons	Cigarette smoking, passive exposure to tobacco, alcohol consumption, pre-pregnancy BMI, maternal age, occupation, parity, pregnancy complications, gestational age, date of birth (birth season), infant sex, birth weight		Pan et al. (2022)

BMI, body mass index; bp, base pair; CI, confidence interval; LTL, leukocyte telomere length; NHANES, National Health and Nutrition Examination Survey; OLS, ordinary least-squares; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid: PIR, poverty income ratio; qPCR, quantitative polymerase chain reaction; SD, standard deviation; sPLS, sparse partial least-squares; TeloTAGGG, commercial telomere length assay; TL, telomere length; vs, versus; WQS, weighted quantile sum.

[a] PFOS and PFOA concentrations are expressed as mean ± SD or geometric mean ± geometric SD, unless otherwise stated in the table.

Several cross-sectional studies examined potential links of PFOA and PFOS exposure in humans with changes in telomere length (TL).

Clarity et al. (2021) conducted a study on blood collected from female firefighters from the San Francisco Fire Department and office workers in the City and County of San Francisco, USA. The minimally adjusted model (Model 1) and fully adjusted model (Model 2) assessed the association between the continuous log-transformed PFOA and PFOS serum concentrations and leukocyte telomere length (LTL). Statistically significant positive associations of LTL with PFOA (β = 0.57; 95% CI, 0.12–1.02) and PFOS (β = 0.44; 95% CI, 0.05–0.83) were observed in both cohorts, and especially in firefighters in Model 1, after adjustment for age. In Model 2, after adjustment for age, dairy consumption, and egg consumption, only PFOA was found to be significantly associated with LTL in the firefighters. [The Working Group noted that firefighters were occupationally exposed to many different chemicals, including benzene, PAHs, formaldehyde, dioxins, and polybrominated diphenyl ethers, but the effect estimate differences did not account for unmeasured chemical co-exposure in the firefighters that may have affected TL.]

A strong positive association between the blood PFOS concentration and TL was shown mainly in the female participants from among 773 adults in the NHANES cycle of 1999–2000 (Huang et al., 2019b).

In another study, prenatal exposure to PFAS mixtures that included PFOA and PFOS showed a modest association with LTL in umbilical cord blood (mean change in TL per quartile increase, 0.11; 95% CI, 0.01–0.22), and only when maternal–fetal unit pairs were considered (Eick et al., 2021). No significant association was observed of individual PFOA and PFOS blood levels with TL in maternal whole blood (Spearman correlations of 0.09 and 0.05, respectively) or umbilical cord blood (Spearman correlations of 0.22 and −0.06, respectively) (Eick et al., 2021).

In contrast to these findings, Li et al. (2023a) showed a modest negative association between PFOS concentration and umbilical cord blood TL in male newborns only, and Liu et al. (2018b) identified shorter TL in the umbilical cord blood of female newborns exposed to higher levels of PFOS. In both studies there was no significant association between the level of PFOA and TL in umbilical cord blood.

No significant associations of umbilical cord blood TL with the maternal serum levels of PFOA or PFOS were observed in a birth cohort study of 499 mother–newborn pairs conducted in Guangxi, China, between June 2015 and May 2018 (Pan et al., 2022).

In addition, serum PFOA level was inversely associated with TL in 175 adults aged 50–65 years in the cross-sectional Flemish Environment and Health study (Vriens et al., 2019). [The Working Group noted that TL displays large inter-individual variation at birth and throughout the human lifespan and may depend on the differentiation and activation status of leukocytes. All the available studies were cross-sectional, and the lack of prospective studies investigating the effects of PFOA and PFOS exposure on TL in humans was a notable research gap.]

(ii) Human cells in vitro

The inhibition of gap junctional intercellular communication (GJIC) may lead to a loss of intercellular communication and play a role in mitogenic activation, promoting tumour formation and cellular proliferation, migration, and invasion. Some carcinogens may inhibit GJIC and promote cancer development.

Saejia et al. (2019) showed that treatment with PFOA did not affect cell migration, but enhanced cell invasion, adhesion, and the activity of matrix metalloproteinase-2 through the activation of the NF-κB signalling pathway in human follicular thyroid carcinoma (FTC133) cells. Treatment

of non-tumorigenic human breast epithelial MCF-10A cells with 10 μM PFOS for 72 hours induced cell-cycle progression, cell migration, and invasion, which may lead to breast cancer initiation and development (Pierozan and Karlsson, 2018).

(b) Experimental systems

Non-human mammalian cells in vitro

It has been shown that PFOA and PFOS inhibit GJIC in rat liver epithelial cells (Upham et al., 1998). The activation of extracellular receptor kinase and phosphatidylcholine-specific phospholipase C in an oxidation-dependent manner was suggested to be a mechanism of GJIC dysregulation (Upham et al., 2009).

The morphological transformation of Syrian hamster embryo cells in vitro assay is recommended by the Organisation for Economic Co-operation and Development for the detection of genotoxic and non-genotoxic carcinogens. Seven days of exposure to non-cytotoxic concentrations of PFOS alone or PFOA in combination with benzo[*a*]pyrene (B[*a*]P, 0.4 μM) pre-treatment induced cell transformation (measured as colony forming unit frequency) in a non-genotoxic manner (Jacquet et al., 2012a, b). PFOS and its acid form induced cell transformation only at 0.37 and 3.7 μM concentrations, in a non-dose-dependent manner without an initiator, whereas PFOA at concentrations from 3.7×10^{-4} to 37.2 μM induced cell transformation in B[*a*]P-sensitized cells (Jacquet et al., 2012a, b). [The Working Group noted that immortalization-specific studies of primary or cultured human cells were not available.]

Synopsis

[The Working Group noted that the evidence that PFOA or PFOS induces cell immortalization was sparse for all test systems.]

4.2.10 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

(i) Cell proliferation

Exposed humans

Xie et al. (2023) identified positive correlations of PFOA and PFOS concentrations with the expression of molecular markers of glioma, specifically Ki-67 and p53. A total of 137 glioma tissue and 40 non-glioma tissue samples were collected. The study showed that PFOA and PFOS is commonly present in cancerous and noncancerous brain tissue, but higher concentrations of PFOA and PFOS were present in glioma samples from the brain than in non-glioma samples, albeit without statistical significance. The data revealed a positive correlation between PFOA and PFOS concentrations and tumour grade. Positive correlations of PFOA and PFOS with the expression of the glioma molecular markers Ki-67 or p53 were also observed. Significant correlations were observed of tumour grade with Ki-67 and p53 expression ($r^2 = 0.33$ and $r^2 = 0.10$, respectively). Ki-67 expression significantly correlated with the level of PFOA (univariate linear regression; $n = 97$; $r^2 = 0.24$; $P < 0.05$). A multiple regression model indicated that approximately 30% of Ki-67 expression could be explained by variation in the PFOS and PFOS concentrations, and predictor importance analysis suggested that Ki-67 expression was mainly driven by the PFOA concentration. No significant univariate regression associations were observed between the concentrations of individual PFAS and p53 (Xie et al., 2023). [The Working Group noted several limitations to this study. PFOA and PFOS were measured in the glioma tissue; however, it was not specified which part(s) of the brain was sampled and compared. The PFAS concentrations varied substantially in different brain areas (Di Nisio et al., 2022). Paired glioma and non-glioma

samples were available for 18 patients, and no statistically significant differences in the concentrations of PFAS were observed between the 18 pairs of glioma and non-glioma tissue samples, possibly because of the limited sample numbers. The 137 glioma and 40 non-glioma brain tissue samples included in the study were from patients from whom only one of the two tissue types was collected. It was not informative to compare PFAS levels in tumour and non-tumour brain tissue from different participants, because PFOA and PFOS exposure varied substantially among the participants. In addition, it was noted that the expression of Ki-67 was absent in 40 out of 137 glioma samples and that the relevance of cell proliferation measurements in tumour tissues as an end-point for this KC is questionable.]

Bassler et al. (2019) explored the mechanisms of the associations of PFOA and PFOS with the development of NAFLD. Two hundred adult samples from a cross-sectional study of participants from districts with PFOA contamination of drinking-water were analysed. The serum concentrations of biomarkers of hepatocyte death/apoptosis were altered. A univariate analysis showed that serum cytokeratin 18 M30 (a marker of hepatocyte apoptosis) and cytokeratin C18 M65 (a marker of hepatocyte total cell death, necrosis and apoptosis) were both positively associated with serum PFOA and PFOS. In a multivariate analysis, M30 was positively associated with PFOA, and there was a similar trend for PFOS [The Working Group noted that although caspase-cleaved cytokeratin 18 fragments (CK-18 forms both M30 and M65 antigens) are markers of hepatocyte death, they have also been considered to be candidate markers for the detection of non-alcoholic steatohepatitis and fibrosis (Feldstein et al., 2009).] [The Working Group also noted that this study observed associations of PFOA and PFOS with markers of inflammation. See Section 4.2.6.]

Human primary cells in vitro

Human primary normal prostate epithelial cells were cultivated to form a 3D spheroid model and exposed to PFOA or PFOS at 10 nM for 3–4 weeks. PFOA and PFOS significantly increased the total number of spheroids and their size, indicating elevated stem cell self-renewal and progenitor cell proliferation. Transcriptome analyses showed an upregulation of genes encoding signalling pathway intermediates involved in cell proliferation (G2-M checkpoint, mitotic spindle, E2F targets) and oncogenesis (kRAS and MYC signalling, TNF-α via NF-κB, IL-6/JAK/STAT3, TGFβ, and inflammatory signalling). Metabolomic analysis of PFOA and PFOS-exposed prostaspheres (spheroids) revealed upregulation of glycolytic pathways, including those involved in the Warburg effect, in response to PFOA or PFOS exposure (Hu et al., 2022).

In addition, transcriptomic analysis of human primary hepatocytes treated with non-cytotoxic doses (10 nM–10 μM PFOA or PFOS, potassium salt) for 48 or 96 hours revealed that PFOA predominantly changed the expression of genes involved in lipid metabolism and hepatic steatosis, whereas PFOS predominantly induced changes in the expression of genes involved in carcinogenesis and cell death signalling. PFOA and PFOS caused a decrease in *CLDN1* mRNA. Claudin-1 protein is involved in maintaining cellular adhesion and the formation of cell junctions. PFOA and PFOS also caused an induction of the *AKR1B10* gene, which is associated with the progression of hepatocellular carcinoma (Matkowskyj et al., 2014).

The effect of PFOS on human primary oesophageal epithelial cell proliferation was assayed using a cell counting kit (CCK)-8. PFOS at 10 nM had no significant effect on proliferation but increased the migration and invasion of the oesophageal squamous cell carcinoma cell lines KYSE150, KYSE140, and KYSE70 by regulating

the transcription and protein stability of ZEB1 (Liu et al., 2022c).

Human cell lines in vitro

Exposure to PFOS (10 μM) or PFOA (100 μM) for 72 hours induced breast epithelial cell (MCF-10A cell line, expected to be ERα- and β-negative) proliferation and the alteration of regulatory cell-cycle proteins (cyclin D1, CDK6, p21, p53, p27, ERK 1/2, and p38), and this persisted after multiple cell divisions. Interestingly, PFOA and PFOS increased proliferation and caused a persistent increase in cyclin D1 levels, but through two different mechanisms. PFOS activated the ERK pathway and PFOA acted by inactivating p38 and reducing cyclin D1 degradation. Both compounds promoted cell migration and invasion (Pierozan et al., 2020). [The Working Group noted that this study also observed associations of PFOA and PFOS with alterations in epigenetic end-points (see Section 4.2.4).]

Gimenez-Batista et al. investigated the effects of several concentrations of PFOA (100, 72.5, 7.25, 3.6, and 0.36 μM) or PFOS (100, 60, 6, and 0.6 μM) on the growth of a human colon myofibroblast (CCD-18Co) cell line using an MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay, in the presence or absence of the pro-inflammatory cytokine IL-1b. After 96 hours of treatment, CCD-18Co myofibroblast proliferation was significantly induced in the presence or absence of IL-1b (Giménez-Bastida et al., 2015).

Exposure of a human non-small cell lung carcinoma cell line (A549) to one of several concentrations of PFOA or PFOS (10, 200, or 400 μM) for 48 hours caused alterations to the cell cycle and apoptosis. Lower doses of these compounds caused the cell balance to shift towards cell proliferation, whereas exposure to higher concentrations shifted the balance towards apoptosis, as evaluated by MTT assay and confirmed by measurement of the mRNA expression of genes involved in the cell cycle and proliferation. Increases in cellular proliferation were noted at 100 and 200 μM PFOA and PFOS exposure levels; however, significant reductions in cell viability at ≥ 600 μM PFOA and 400 μM PFOS were also observed (Jabeen et al., 2020).

Spheroids produced from COV434 and KGN human ovary granulosa tumour cell lines were exposed to PFOA (0.02, 0.2, 2, 20, or 200 ng/mL, or 2 mg/mL) or PFOS (0.08, 0.8, 8, 80, or 800 ng/mL, or 8 mg/mL). The proliferation of the spheroid cultures was estimated by the measurement of ATP using the CellTiter-Glo 3D cell viability assay. PFOA and PFOS increased COV434 and KGN cell proliferation in a dose-dependent manner, compared with untreated control cells (Gogola et al., 2019).

In other studies, lower viability or induction of apoptosis was observed after PFOA or PFOS exposure in primary cultures of normal human thyroid cells (NHT) (Coperchini et al., 2021b), in a human hepatoma cell line (HepG2) (Hu and Hu, 2009), and in monolayers (2D) and spheroids (3D) of neuronal cells (N2a) (Choi et al., 2013).

Two cancer cell lines, prostate (DU145) and breast (MCF7), were treated with very low doses of PFOA (10^{-6} to 10^{-12} M), for 48 hours. PFOA increased the proliferation of DU145 and MCF7 cells, as monitored by real-time imaging. PFOA acted through distinct signalling pathways in these two cell lines (AKT/mTORC1 and plexin D1-dependent pathways in MCF7 and DU145 cells, respectively) (Charazac et al., 2022).

PFOA at 50 or 100 μM after 72 hours significantly promoted viability, migration, and invasion of RD cells (a human embryonal rhabdomyosarcoma cell line), and significantly inhibited apoptosis. Higher concentrations (500 and 1000 μM) inhibited cell viability. Treatment with a PI3K inhibitor antagonized the effects of PFOA on migration, invasion, and apoptosis. The authors concluded that PFOA promoted RD cell migration and invasion and inhibited apoptosis through the PI3K/AKT signalling pathway (Zhang et al., 2019).

Trophoblast cells HTR-8/SVneo were exposed to 100–1000 μM PFOA for 72 hours. Low doses increased trophoblast viability, whereas concentrations of > 400 μM reduced cell viability. The activation of ROS-dependent ERK signalling at low doses triggered trophoblast proliferation, whereas the activation of UPR signalling triggered trophoblast apoptosis under conditions of severe ERS (Du et al., 2022).

Immortalized human granulosa HGrC1cells (a cell type of ovarian follicles) were exposed to PFOA at 1–100 μM for 24–96 hours. Low doses (up to 10 μM) caused increased viability and proliferation, whereas the highest dose (100 μM) caused reductions in these parameters. YAP1-mediated proliferation was confirmed by adding a YAP1 inhibitor, which prevented the effects of PFOA. Thus, PFOA was shown to induce proliferation, migration, and invasion of HGrC1 cells, potentially via upregulation of the Hippo pathway effector YAP1 and of cell-cycle regulators such as cyclin D1 (Clark et al., 2022).

PFOA (≥ 100 nM) treatment also stimulated A2780 ovarian cancer cell invasion and migration, and increased the expression of the matrix metalloproteinases MMP-2 and 9 (Li et al., 2018c).

Human normal HL-7702 hepatocyte line was exposed to PFOA at 0–800 μM for 48 or 96 hours. Low doses (50–100 μM) increased cell proliferation by promoting a shift from the G1 to S phase, whereas at higher doses (200–400 μM), the cell numbers were comparable with those of the control, mainly because of cell-cycle arrest in the G0/G1 phase. Notably, no apoptosis was detected, even at 400 μM. Corroborating these data, proteomic analysis detected 111 significantly expressed proteins, of which 46 were related to cell proliferation and apoptosis. The induction of cyclin D1, CDK6, cyclin E2, cyclin A2, and CDK2 in the low-dose PFOA groups was associated with larger numbers of cells in S phase, as well as fewer cells in the G0/G1 phase, indicating that PFOA shifted cells from G1 to S phase (Zhang et al., 2016b).

The viability of L-02 cells increased slightly after 24 hours of treatment with PFOA at 8 μM, but decreased at higher PFOA concentrations (16–512 μM) after 24 hours (Wang et al., 2022a).

In an experiment performed in HepG2 cells, which were treated with 200–500 μM PFOA for 12–48 hours, it was observed that 50 μM PFOA increased the number of cells in the G2/M phase, while reducing the number in S phase; 100 and 150 μM PFOA increased the G0/G1 cell percentages and reduced the S/G2/M cell percentages; and ≥ 400 μM reduced the proportion of G0/G1 cells (Shabalina et al., 1999).

In contrast, cell proliferation was not altered in other cell types, for example in human choriocarcinoma (Jeg-3) and in endometrial adenocarcinoma (RL95-2) cells treated with 0.01–100 μM PFOA for 24 hours (Tsang et al., 2013), in ovarian cancer cells (A2780 cells) treated with 0–200 nM PFOA for different periods of time, and in human ovarian GC cells (KGN) treated with 0.03–300 μM PFOA for 12–48 hours (Zhou et al., 2020).

To examine the effects of PFOA during human endometrial carcinogenesis, Ishikawa cells were treated with 50 nM PFOA for 48 hours. This treatment did not affect proliferation, but it promoted both the migration and invasion of these endometrial cancer cells (Ma et al., 2016).

Pierozan and Karlsson (2018) showed that PFOS-induced cell proliferation and cell death are dependent on the exposure time and concentration. The breast cell line MCF-10A was treated with 0–1 mM PFOS for 24, 48, or 72 hours. Significantly lower cell viability was observed at ≥ 250 μM PFOS at all time points. In contrast, exposure to 10 μM PFOS for 48 hours, or 1 or 10 μM PFOS for 72 hours, increased MTT production and the number of cells, reduced the percentage of cells in the G0/G1 phase, and increased the percentage of cells in S phase at all time points. There were also decreases in the mean fluorescence intensities associated with p27, p21, and p53 staining and an increase in the mean

fluorescence intensity associated with CKD4 staining in PFOS-treated cells, compared with the controls. In addition, treatment with 10 μM PFOS for 72 hours also stimulated MCF-10A cell migration and invasion.

However, PFOS at concentrations ranging from approximately 1 nM to 100 μM did not induce significant effects on cell viability and/or cell death in hMSCs (Gao et al., 2020), human primary placental cytotrophoblasts isolated from placenta at full-term pregnancy (Zhang et al., 2015b), human embryo liver cells (L-02) (Zeng et al., 2021; Dong et al., 2022), human choriocarcinoma cell lines HTR-8/SVneo and JEG-3, embryonic stem cell-derived cardiomyocytes (Cheng et al., 2013; Li et al., 2021a), and renal proximal tubular epithelial cells NRK-52E (Wen et al., 2016).

Notably, ferroptosis, a process of regulatory cell death that is induced by excessive lipid peroxidation, was investigated in two studies. Cui et al. (2022) investigated the role of ferroptosis in HUVECs exposed to 180 μM PFOS for 12–48 hours. The results showed that the viability of the HUVECs was significantly reduced by the PFOS treatment after 12 hours. PFOS increased the expression of the ferroptosis-related protein ACSL4 and reduced the expression of GPX4, HO-1, and FTH1. The results were confirmed using a ferroptosis inhibitor (Cui et al., 2022).

In the human proximal tubular epithelial cell line HK-2, treatment with PFOS at 50–250 μM reduced cell viability after 12 hours in a dose-dependent manner and induced ferroptosis and apoptosis (Wang et al., 2022b).

(ii) Cell death inhibition

Human hepatocarcinoma HepG2 cells exposed to 200 μM PFOA for ≤ 72 hours showed autophagosome accumulation. [The Working Group noted that the autophagosome accumulation may have been the result of autophagy activation or have been caused by the inhibition of autophagy at the degradation step.] The proteomic analysis performed in this study suggested the inhibition of autophagy. The same study observed autophagosome accumulation in mouse livers (Yan et al., 2017). [The Working Group noted that these results are helpful in understanding the potential mechanisms of the hepatotoxicity induced by PFOA. In addition, this study showed that the PFOA-dependent reduction in HepG2 cell viability may not be directly attributable to a dysfunction of autophagy.]

(iii) Angiogenesis

In HTR8/SVneo cells, an embryonic trophoblast cell type that is able to form vessel-like vascular networks in 3D matrices, exposure to PFOA at 100 μg/mL affected morphological parameters of the pseudo-vascular network in a dose-dependent manner, including the number of pseudo-vascular junctions and the total lengths of non-branching segments. In contrast to PFOA, PFOS did not have significant effects on angiogenesis (Poteser et al., 2020).

(b) Experimental systems

(i) Non-human mammalian systems in vivo

In the chronic carcinogenicity study by Butenhoff et al. (2012a) (see Section 3.1.2), a significantly increased incidence of tubular hyperplasia of the ovaries of rats was observed in groups treated with PFOA, compared with controls – 0/48 (0%), 7/50 (14%), and 15/47 (33%) at 0 (control), 30, and 300 ppm, respectively. There were also increases in the incidence of hepatocellular hypertrophy in male and female rats. [The Working Group noted that although the differences were not statistically significant, the incidence of pancreas acinar hyperplasia in male rats was 0/46 (0%), 2/46 (4%), and 2/49 (4%) for the groups at 0 (control), 30, and 300 ppm, respectively).]

Dietary administration of PFOA at 300 ppm for 2 years to CD rats resulted in increased Leydig cell proliferation (46% versus 14% for the control

group) and a higher incidence of adenoma (11% versus 0% for the control groups) in the testes. Also, PFOA showed a tendency to increase pancreatic acinar cell proliferation at 15, 18, and 21 months, when compared with the control groups. The incidence of acinar cell hyperplasia was 39% versus 18% or 10% for the ad libitum or pair-fed control groups, respectively; and that of adenoma was 9% versus 0% or 1% for the ad libitum or pair-fed control groups, respectively. No cell proliferation was observed in the livers (Biegel et al., 2001).

In a review of the pancreatic lesions in male rats in the studies by both Biegel et al. (2001) and Butenhoff et al. (2012a), using the same diagnostic criteria as those applied in the study by Biegel et al. (2001), a significant positive trend ($P < 0.05$, Cochran–Armitage trend test) in the incidence of pancreatic acinar cell hyperplasia was observed, with the incidence being significantly increased [$P = 0.0382$, Fisher exact test] at the highest dose (3/46 (7%), 1/46 (2%), and 10/47 (21%) for 0, 30, and 300 ppm PFOA, respectively) (Caverly-Rae et al., 2014). In addition, histopathological examination of the pancreas revealed focal ductal hyperplasia in C57Bl/6 mice treated with PFOA at 2.5 or 5.0 mg/kg orally for 7 days (Kamendulis et al., 2014).

In a 2-year carcinogenicity study with and without perinatal exposure (NTP, 2020) (see Section 3.1.2), exposure to PFOA resulted in increases in the incidence of hepatocellular hypertrophy in male and female rats and increases in the incidence of papillary urothelium hyperplasia at the 16-week interim time point of the feeding study. In this 2-year feeding study of PFOA, increases in the incidence of hepatocyte hypertrophy, bile duct hyperplasia, hyperplasia of the renal papillary epithelium, and epithelial hyperplasia of the forestomach were observed in female rats at 300 or 1000 ppm. In the male rats, increases in the incidence of hepatocyte hypertrophy and bile duct hyperplasia were also observed. In addition, acinus hyperplasia was also significantly increased in all the postweaning-only exposure groups in the chronic study and in the groups at 300 ppm in the perinatal and postweaning study, and this lesion was considered to be potentially preneoplastic.

Filgo et al. (2015) (see Section 3), described non-neoplastic lesions in four strains of mice (age, 18 months) after exposure to PFOA during gestation. Significant increases in the incidence of non-neoplastic liver lesions were observed in CD-1 mice after PFOA exposure, including oval cell hyperplasia, Ito cell hypertrophy, and centrilobular hepatocyte hypertrophy. Several non-neoplastic changes were observed after PFOA exposure in PPARα-KO mice, but not in 129/Sv WT mice, including significant dose-related increases in the incidence of both bile duct hyperplasia and bile duct inclusion bodies (hyaline droplets). In addition, the incidence of centrilobular hepatocyte hypertrophy and of haematopoietic cell proliferation was significantly increased by PFOA exposure in PPARα-KO mice, but not in 129/Sv WT mice. The incidence of Ito cell hypertrophy was reduced by PFOA treatment in PPARα-KO mice (Filgo et al., 2015). [The Working Group noted that this study demonstrated that PPARα is not essential for the liver effects of PFOA in mice, because these were observed in PPARα-KO mice.]

Male Sprague-Dawley rats were exposed orally to PFOA (300 ppm) in the diet for 1, 7, or 28 days. The results showed that the hepatic bromodeoxyuridine (BrdU) labelling index was increased by approximately three-fold 1 day after the start of treatment. The increase in the hepatic BrdU labelling index versus the control group was largest after 8 days (approximately five-fold compared with controls). The labelling index values were near the background levels for male rats after 2 and 29 days, even though they were statistically significantly higher than those for the respective controls. The administration of PFOA to rats led to hepatomegaly, characterized by hypertrophy and hyperplasia, as a result

of early increases in cell proliferation, which would ultimately lead to liver tumour formation. (Elcombe et al., 2010). [The Working Group noted that technical problems occurred with fixation and immunostaining for BrdU, so a second study was conducted to evaluate cell proliferation. The results were those of both studies together.]

Male Sprague-Dawley rats were treated intravenously via a tail vein with methylpalmitate, a Kupffer cell inhibitor, 24 hours before a single oral dose of PFOA (100 mg/kg) and were killed 24 hours later. PFOA significantly increased the BrdU labelling index in the liver, and pre-treatment with methylpalmitate reduced PFOA-induced labelling by 57% (Alsarra et al., 2006).

Thottassery et al. (1992) investigated the role of adrenal hormones in the hepatomegaly induced by PFOA. Male Sprague-Dawley rats underwent adrenalectomy and received a single dose of PFOA at 150 mg/kg by oral gavage. In intact rats and in rats that had undergone adrenalectomy, PFOA caused increases in ornithine decarboxylase activity and significantly reduced hepatic DNA levels (by 30%; $n = 4–10$; $P < 0.05$) (Thottassery et al., 1992). [The Working Group noted that the reduced hepatic DNA concentration was inversely proportional to the hepatomegaly observed. In rats that had undergone adrenalectomy, however, hepatomegaly was mostly caused by hyperplasia.]

Thirty-six adult Sprague-Dawley rats received ethane dimethyl sulfonate to eliminate Leydig cells, then were treated with PFOA at 0, 25 or 50 mg/kg per day by oral gavage for 9 consecutive days. The number of proliferating cell nuclear antigen (PCNA)-positive Leydig cells in testes sections was reduced after 21 days, indicating that PFOA exposure may reduce the proliferation of stem Leydig cells (Lu et al., 2019).

Male C57BL/6 mice were fed a low-fat control diet or a high-fat diet (HFD) for 16 weeks to model normal and steatotic livers, respectively. After 16 weeks on these diets, the mice were treated with PFOA (1 mg/kg bw per day) for 2, 8, or 16 weeks. PFOA induced hepatocyte hypertrophy, regardless of the diet, as indicated by histological examination. In rats consuming the low-fat control diet, PFOA induced an increase of 3.30-fold in hepatocyte DNA synthesis compared with vehicle-treated control diet-fed mice by week 2. HFD and PFOA had a synergistic effect on the BrdU labelling index by week 2. By week 8, HFD significantly increased DNA synthesis in hepatocytes compared with the vehicle-treated control diet-fed group. The control diet + PFOA and the HFD + PFOA groups also showed elevated DNA synthesis levels of 11.23-fold and 15.72-fold, respectively, compared with the vehicle-treated control diet-fed group (Li et al., 2019c). [The Working Group noted that, taken together, these results suggest that pre-existing NAFLD enhanced PFOA-stimulated hepatocyte hyperplasia only at the early time point studied. The Working Group also noted that this study shows that PFOA activates PPARα, CAR, and PXR and that PFOA reverses HFD-induced steatosis and reduces the size of adipose tissue depots.]

Gestational intrauterine exposure to PFOA at 5 mg/kg in female Kunming mice was used to characterize the potential effects of prenatal PFOA exposure on the cerebral cortex cells of offspring at PND21. PFOA-treated PND21 mice demonstrated increased levels of nerve growth factor (NGF) in serum and cortex cells. In addition, PFOA-exposed cerebral cortex cells showed higher NGF and PCNA expression, and exposure to PFOA and an NGF-specific inhibitor downregulated the expression (Qin et al., 2018). In contrast, in other studies in PFOA-exposed Kunming mice, increases in apoptotic markers were observed in the liver (Liu et al., 2015b) and uterus (Li et al., 2018c; Zhang et al., 2021b).

Adult male and female cynomolgus monkeys (*S. cynomolgus* or *M. fascicularis*) were treated with PFOS (potassium salt) at 0, 0.03, 0.15, or 0.75 mg/kg per day for 182 days and were monitored for 1 year after the treatment. Hepatocellular hypertrophy and lipid vacuolation were present

at term in the group at 0.75 mg/kg per day. The treatment had no significant effect on cell proliferation in the liver, pancreas, or testes after 182 days of treatment, as determined using the PCNA immunohistochemistry cell labelling index (Seacat et al., 2002).

Male and female Sprague-Dawley rats were exposed to PFOS (potassium salt) at a dietary concentration of 0.5, 2, 5, or 20 ppm for up to 104 weeks (Butenhoff et al., 2012b). A 20-ppm dose recovery group received 20 ppm PFOS in the diet for up to 53 weeks, after which it was fed control diet. The male rats at 20 ppm that were killed during week 53 had an increased incidence and severity of centrilobular hepatocytic hypertrophy and vacuolation, whereas the female rats had only centrilobular hypertrophy and the changes were less severe. These alterations were also observed in rats killed at the scheduled end of the study. There were no statistically significant increases in hepatocellular S-phase labelling index (cell proliferation index), as measured using BrdU immunohistochemistry, in any of the experimental groups.

Hepatocellular hypertrophy, increased cell proliferation, and reduced liver apoptotic index were observed in Sprague-Dawley rats fed PFOS (potassium salt; 20 or 100 ppm) for 28 days, and this was reported to be mediated by the nuclear receptors PPARα, CAR, and PXR (see also Section 4.8) (Elcombe et al., 2012c). A subsequent study showed that after a recovery period of 84 days after treatment, increased liver proliferative index, reduced liver apoptotic index, and a lower number of hepatocellular glycogen vacuoles were observed (Elcombe et al., 2012a).

Han et al. (2018b) treated male Sprague-Dawley rats daily by gavage with PFOS (1 or 10 mg/kg) for 28 days. PFOS exposure triggered Kupffer cell activation and significantly upregulated the expression of PCNA, c-Jun, c-MYC, and cyclin D1 in the liver (Han et al., 2018b). [The Working Group noted that this study showed inflammatory cell infiltration; see Section 4.2.6.]

Groups of 25 pregnant Sprague-Dawley rats were given daily oral doses of PFOS (potassium salt) at 0.1, 0.3, or 1.0 mg/kg per day from GD0 to PND20. The mean number of Ki-67$^+$ thyroid follicular epithelial cells in female fetal thyroids from the 1.0 mg/kg per day group was higher by 2.1-fold than for the control group ($P < 0.05$) (Chang et al., 2009).

The effects of PFOS on GJIC in vivo were studied using Sprague-Dawley rats (n = 4–6) exposed to PFOS (5 mg/kg per day) orally for 3 days or 3 weeks. GJIC was significantly reduced in the livers of PFOS-treated rats, with an EC_{50} of 30 μM (15 mg/L) after 3 days of exposure, and the magnitude of inhibition was the same for the longer exposure period of 21 days (no statistical significance was shown) (Hu et al., 2002).

Male Sprague-Dawley rats were treated with PFOS (0, 5, or 10 mg/kg per day) by gavage for 7 days and injected intraperitoneally with ethane dimethyl sulfonate the next day to eliminate Leydig cells, so that the Leydig cell regeneration process could be investigated. PFOS pre-treatment significantly lowered the serum testosterone level and reduced the number of regenerated Leydig cells (Mao et al., 2021). [The Working Group noted that transverse sections of the testes immunohistochemically stained for CYP11A1 and 11β-HSD1 were used to analyse cell number. In addition, in a 3D seminiferous tubule culture system, PFOS inhibited stem Leydig cell proliferation and differentiation, as well as the hedgehog signalling pathway.]

Qu et al. (2016) showed alterations in testicular ER expression, together with reduced proliferation and increased apoptosis of germ cells, which might be involved in the PFOS-induced testicular toxicity. In male ICR mice, oral administration of PFOS (0–10 mg/kg bw) for 4 weeks caused reduced sperm count, testosterone level, and CRTC2/StAR expression, and damage to the testicular interstitium, paralleled by increases in phosphorylated PKA, CREB, and p38 in the testes (Qiu et al., 2021). PFOS impaired normal

placental angiogenesis in female CD-1 mice by disrupting the lncRNA Xist/miR-429/VEGF-A pathway, adversely affecting fetal development (Chen et al., 2018).

(ii) Non-human mammalian systems in vitro

The 3T3-L1 preadipocyte culture system has been used to test numerous compounds that influence adipocyte differentiation or function. Cells were treated with PFOA (5–100 µM) or PFOS (50–300 µM), the PPARα agonist WY-14 643, or the PPARγ agonist rosiglitazone. The cells were assessed morphometrically and biochemically for number, size, and lipid content. There was a significant concentration-related increase in cell number and reduced cell size after exposure to PFOA or PFOS (Watkins et al., 2015). However, Fischer rat thyroid line-5 (FRTL-5) cells exposed to 10^5 nM PFOA or PFOS showed significant inhibition of cell proliferation. In particular, the percentage of proliferating FRTL-5 cells was 14.2% of the total number of cells in the medium, and this was reduced to 7.5% by PFOA and to 3.9% by PFOS (Coperchini et al., 2015).

PND4 neonatal ovaries from CD-1 mice were cultured in control medium (dimethyl sulfoxide < 0.01% final concentration) or PFOA (50 µM or 100 µM). The results showed that exposure to PFOA at 50 µM for 96 hours increased the number of secondary follicles, the expression of Ki-67 and the protein and gene expression of *Ccna2*, *Ccnb2*, *Ccne1*, *Ccnd1*, *Ccnd2*, *and Ccnd3*. PFOA also induced the expression of the Hippo pathway components *Mst1/2*, *Lats1*, *Mob1b*, *Yap1*, *and Taz*, as well as the downstream Hippo pathway targets *Areg*, *Amotl2*, *and Cyr61*, although it reduced the expression of the anti-apoptotic gene *Birc5*. Inhibition of the Hippo pathway effector YAP1 with verteporfin resulted in the attenuation of PFOA-induced follicular growth and proliferation (Clark et al., 2022). [The Working Group noted that PFOA can disrupt the Hippo pathway, leading to changes in the cell cycle, increased cell growth, and enhanced follicle development.]

Rat liver epithelial cells were cultured with PFOA at 10 µM, 50 µM, or 100 µM for 38 weeks and compared with passage-matched control cells. PFOA-treated cells showed increases in MMP-9 secretion and cell migration, and they developed more and larger colonies in soft agar. Microarray data showed Myc pathway activation at 50 µM and 100 µM, associated with Myc upregulation and PFOA-induced morphological transformation. Western blotting confirmed that PFOA caused significant increases in c-MYC protein expression in a time- and concentration-related manner. The tumour invasion indicators MMP-2 and MMP-9, the cell-cycle regulator cyclin D1, and the oxidative stress protein GST were all significantly upregulated at 100 µM (Qu et al., 2023).

The role of Wnt/β-catenin signalling in PFOS-induced neurotoxicity has also been investigated. C17.2 neural stem cells (mouse-derived multipotent neural stem cells isolated from the cerebellum) were treated with PFOS at 12.5, 25, 50, 100, or 200 nM for ≤ 48 hours (Dong et al., 2016). The CCK-8 assay was used to count the cells and indicated that PFOS exposure impaired the proliferation of the cells in a dose-dependent and time-dependent (at 50 nM) manner. Furthermore, flow cytometry analyses of the cell-cycle distribution revealed a decrease in the number of cells in S phase. The protein levels of cyclin D1 and PCNA were significantly reduced after PFOS exposure for 12 hours, compared with the control group. Gene expression of *Myc* and *Cox2* and survival were significantly impaired in a dose- and time-dependent manner after PFOS exposure, suggesting the involvement of β-catenin signalling (Dong et al., 2016).

In ovine primary theca cells, PFOS at 50 ng/mL was not cytotoxic after 24 hours and had no effect on GJIC (Gingrich et al., 2021).

Synopsis

[The Working Group noted that the above studies reported some evidence that PFOA increased cell proliferation in human primary cells. Transcriptomic analyses in human primary cells suggested that PFOA modulated gene signalling pathways involved in cell proliferation. PFOA induced cell proliferation, migration, or invasion in human cell lines and cell proliferation or hyperplasia in multiple tissues in rodents, including in PPARα-null mice.

Similarly, PFOS seemed to increase cell proliferation, migration, or invasion in human cell lines and cell proliferation or hyperplasia in the liver of rats.]

4.2.11 Multiple key characteristics of carcinogens

See Tables 4.29 to 4.31.

Data from transcriptomic and metabolomic studies were assessed for quality with respect to study design parameters, quality control of the raw data, data processing criteria, and differential analysis and information content regarding the 10 KCs of carcinogens. The results of the studies were mapped/associated to the KCs. To establish the association, the Working Group mapped genes, metabolites, or enriched pathways to each KC, based on the known association of the transcript or metabolite with a biological process underlying the KC.

(a) Humans

(i) Exposed humans

Chang et al. (2022) used a meet-in-the-middle approach to investigate the interrelationships between serum PFOA and PFOS concentrations, maternal metabolomic perturbations, and fetal growth. The authors showed an association of maternal serum PFOA level with reduced fetal growth in a population of 313 African-American women. Changes in amino acid, lipid and fatty acid, and bile acid metabolism were associated with PFOA, and to a lesser extent PFOS, exposure. Uric acid was suggested to be a potential intermediate biomarker of the early response to PFOA exposure and to predict reduced fetal growth (see Table 4.30). This study was in good agreement with a previous cross-sectional study performed in the US adult population (NHANES 2009–2014; $n = 4917$), which showed associations of PFOA and PFOS exposure with serum uric acid and gout (Scinicariello et al., 2020).

Rhee et al. (2023) identified several metabolites that were significantly associated with both *n*-PFOA and *n*-PFOS in eight nested case–control serum metabolomic profiling studies as part of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. The strongest associations were observed for sphingolipids, fatty acid metabolites, and androgenic steroids. [The Working Group noted that sphingolipids, derivatives of the amino alcohol sphingosine, are biologically active components of cell membranes that play a significant role in intracellular signal transduction, regulate cellular processes (e.g. proliferation, maturation, and apoptosis), and are involved in cellular stress responses.] The associations of specific metabolites with *n*-PFOS remained significant after the model was adjusted for serum *n*-PFOA, but the *n*-PFOA–metabolite associations were substantially attenuated after adjustment for *n*-PFOS. [The Working Group noted that differences in the associations of PFOA or PFOS levels with metabolites in patients with cancer and controls were not discussed in this study.]

A metabolome-wide association study using non-targeted ultra-high-resolution mass spectrometry identified metabolites associated with serum PFOA and PFOS levels in 115 children aged 8 years (Kingsley et al., 2019). In this cross-sectional study, serum PFOA and PFOS concentrations correlated with lipid metabolism and with arginine, proline, aspartate, asparagine, and butanoate metabolism. In addition,

Table 4.29 Omics data relevant to multiple key characteristics of carcinogens in humans exposed to PFOA or PFOS

End-point	Location, setting, study design	Sample, tissue, or cell type No. of study participants	Methods	Type of exposure	Results	Covariates adjusted for	Potentially related KCs	Comments	Reference
MWAS with pathway enrichment analysis	Emory University African American Vaginal, Oral, and Gut Microbiome in Pregnancy Study, Atlanta, Georgia, USA Prospective birth cohort study	Serum 313 pregnant African-American women at 8–14 wk of gestation	Isotope dilution calibration Untargeted, high-resolution metabolomic profiling using hydrophilic interaction LC with positive ESI and reverse-phase (C18) chromatography with negative ESI Multiple linear regression models	General population PFOA, Q1: < LOD–0.45; Q4: 1.07–4.42 ng/mL PFOS, Q1: < LOD–1.44; Q4: 3.24–12.4 ng/mL	Significant association was found between serum PFOA (OR, 1.20; 95% CI, 0.94–1.49) and small-for-gestational age birth; and correlations with level 1 biomarkers (uric acid and ferulic acid) and level 2 biomarkers (unsaturated fatty acid C18:1, parent bile acid, and bile acid–glycine conjugate) PFOS correlated with 2-hexyl-3-phenyl-2-propenal and parental bile acid	Maternal age, education, BMI, parity, tobacco use, marijuana use, and infant sex	KC5; lipid metabolism		Chang et al. (2022)

Table 4.29 (continued)

End-point	Location, setting, study design	Sample, tissue, or cell type No. of study participants	Methods	Type of exposure	Results	Covariates adjusted for	Potentially related KCs	Comments	Reference
MWAS	One of 8 nested case–control studies conducted within the PLCO cohort, 1993–2001, collected at 10 screening centres across the USA Cross-sectional	Serum 3647 participants (1818 patients in the cancer study and 1829 controls)	UHPLC-MS/MS, multiple linear regression modelling and meta-analysis using DerSimonian and Laird random effects models were used to combine findings from the various studies. Bonferroni-corrected significance threshold applied	General population. PFOA: < 2.9–> 6.5 µg/L PFOA: < 19.1–> 47.12 µg/L	38 *n*-PFOA-associated metabolites, including 17 lipids, two nucleotides (uric acid, 3-methylcytidine), one carbohydrate (1,5-anhydrosorbitol), and one cofactor/vitamin 51 *n*-PFOS-associated metabolites, including 20 lipids (sphingolipids, fatty acid metabolites, and bile acid metabolites), five cofactors/vitamins, three amino acids, two nucleotides (guanosine, 3-methylcytidine), and one carbohydrate (D-glucose)	Estimated glomerular filtration rate, educational attainment, BMI, sex	KC4, KC10, lipid metabolism	Study used semi-targeted metabolomic analyses, measuring relative metabolite levels, which made direct comparison between populations difficult. Use of non-fasted serum samples may lead to short-term changes in metabolite levels and introduce bias. The majority of participants were postmenopausal women.	Rhee et al. (2023)
MWAS	Cincinnati, Ohio, USA HOME Study, a prospective pregnancy and birth cohort Cross-sectional	Serum 115 children aged 8 yr	Non-targeted, high-resolution metabolomic profiling using LC and Fourier transform high-resolution MS	General population PFOA, mean ± SD, 2.6 ± 1.0 ng/mL PFOS, 4.4 ± 3.2 ng/mL	Serum PFOA and PFOS concentrations correlated with TCA cycle, pyrimidine and purine metabolism, changes in de novo fatty acid biosynthesis	Age, sex, race/ethnicity	KC10, lipid metabolism	Results based on enriched pathways in network-based metabolome-wide correlation analysis.	Kingsley et al. (2019)

Table 4.29 (continued)

End-point	Location, setting, study design	Sample, tissue, or cell type No. of study participants	Methods	Type of exposure	Results	Covariates adjusted for	Potentially related KCs	Comments	Reference
MWAS	CHDS (1959–1967), Oakland, California, USA Cross-sectional	Maternal perinatal serum 397 participants	C18 chromatography coupled with quadruple orbitrap MS Metabolic features were annotated with xMSannotator using the Human Metabolome Database	General population PFOA median (25th–75th percentile), 0.4; 0.25–0.6 ng/mL PFOS, 33.9; 16.05–61 ng/mL	301 metabolites were significantly associated with serum PFOS concentration. Pathway enrichment analyses: glycine, threonine, alanine, and serine metabolism, and urea cycle/amino group metabolism, carnitine shuttle, lysine metabolism, and branched-chain amino acid metabolism	Total cholesterol (continuous variable), age (continuous variable), and *p,p′*-DDE level (continuous variable)	KC10	Study included 50 women whose daughters developed breast cancer, but the association with metabolomic changes was not discussed. Linear regression model used.	Hu et al. (2019)
Transcriptomics	C8 Health Project (2005–2006), Mid-Ohio Valley and Parkersburg, West Virginia, USA Cross-sectional	Serum and whole blood 290 participants	TaqMan Low-density array (quantitative RT-PCR), solid-phase extraction, followed by reverse-phase HPLC-MS/MS	General population PFOA GM (95% CI): 40.9 (33.7–49.5) ng/mL in men; 25.5 (20.7–31.2) ng/mL in women PFOS: 8.3 (7.3–9.45) ng/mL in men; 5.5 (20.7–31.2) ng/mL in women	In men, inverse associations of PFOA level were identified with *ABCG1*, *NPC1*, and *PPARA* transcripts; no associations with PFOS In women, an inverse association between the *NR1H2* (LXRB) transcript with PFOA and a positive association of PFOA with *NCEH1* expression were identified; *NCEH1* and *PPARA* expression were positively correlated with the level of PFOS	Age, sex, BMI, household family income, smoking status	KC8	Adjusted linear regression model.	Fletcher et al. (2013)

Table 4.29 (continued)

End-point	Location, setting, study design	Sample, tissue, or cell type No. of study participants	Methods	Type of exposure	Results	Covariates adjusted for	Potentially related KCs	Comments	Reference
Transcriptomics	Postmenopausal Norwegian women (age 48–62 years), part of Norwegian Women And Cancer Study Cross-sectional	Serum and whole blood RNA 270 healthy participants	HPLC-QTOF-MS and AB Human Genome Survey Microarray V2.0	General population PFOA median (range), 4.4 (0.79–0.21) ng/mL PFOS median range, 19 (5.7–84) ng/mL	Higher levels of PFOS were associated with the TCA cycle pathway ↓ *NNT*, *PDHB*, *SDHD*, *SDHC*, *SUCLA2*, *IDH3A*, *MDH1*, and *SUCLG2* expression ↑ *ACO2*	None	KC10	No covariates adjusted for	Rylander et al. (2011)

BMI, body mass index; CHDS, Child Health and Development Studies; CI, confidence interval; *p,p′*-DDE, dichlorodiphenyldichloroethylene; ESI, electrospray ionization; GM, geometric mean; HOME, Health Outcomes and Measures of the Environment; HPLC-MS/MS, high-performance liquid chromatography-tandem mass spectrometry; HPLC-QTOF-MS, high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry; KC, key characteristic of carcinogens; LC, liquid chromatography; LOD, limit of detection; MS, mass spectrometry; MWAS, metabolome-wide association study; OR, odds ratio; PFOA, perfluorooctanoic acid; *n*-PFOA, linear isomer of perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; Q, quartile; RT-PCR, reverse transcription-polymerase chain reaction; SD, standard deviation; TCA, tricarboxylic acid; UHPLC-MS/MS, ultra-high performance liquid chromatography-tandem mass spectrometry; USA, United States of America; wk, weeks(s); yr, year(s).

in reversed-phase chromatography with ESI operated in negative mode (C18-negative mode), tyrosine, galactose, vitamin A (retinol), and lysine metabolism pathways, de novo fatty acid biosynthesis, tricarboxylic acid (TCA) cycle, pyrimidine, and purine metabolism pathways were enriched for both PFOA and PFOS. Thus, this study indicated that the serum levels of PFOA and PFOS were associated with a common set of biological pathways related to energy production and catabolism (Kingsley et al., 2019).

Hu et al. (2019) investigated the metabolite profiles associated with the serum PFOS levels of 397 participants in the Child Health and Development Studies (CHDS) cohort. Using a linear regression model, the authors identified 301 metabolites that were significantly associated with the serum PFOS concentration. Pathway enrichment analysis showed that these metabolites were associated with glycine, threonine, alanine, and serine metabolism; the urea cycle/amino group metabolism; the carnitine shuttle; lysine metabolism, which generates carnitine; and branched-chain amino acid metabolism (i.e. valine, leucine, and isoleucine degradation). This cluster of pathways suggested a mechanistic link of PFOS with energy production and lipid regulation. Metabolites involved in the urea cycle and amino group metabolism, such as β-alanine, creatinine, pipecolate, lysine, arginine, creatine, and adrenochrome, were increased in the samples with high PFOS levels; and homocysteine and betaine negatively correlated with the PFOS concentration in serum. [The Working Group noted that there were associations between PFOA or PFOS levels and several common metabolites across multiple human studies, identified using a metabolomic approach.]

Guo et al. (2022b) summarized the application of non-targeted metabolomics in epidemiological studies that assessed metabolite and metabolic pathway alterations associated with exposure to PFOA (11 studies) and PFOS (10 studies) in a scoping review that included human studies involving children and adolescents (three studies), non-pregnant adults (five studies), and pregnant women (three studies). Alterations in tryptophan metabolism and the urea cycle were associated with PFOA or PFOS exposure in multiple studies. Lipid metabolites involved in glycerophospholipid metabolism, which is critical for biological membrane function, and fatty acids and carnitines, which are relevant to the energy supply pathway of fatty acid oxidation, were also associated with PFOA and PFOS exposure. Secondary significant metabolome changes associated with PFOA and PFOS exposure included the components of the TCA cycle, which is involved in energy generation, and purine and pyrimidine metabolism, which are cellular energy pathways. [The Working Group noted that there were commonalities in the associations of PFOA or PFOS level with non-targeted metabolomic alterations. Because of the cross-sectional nature of the studies, the authors were unable to rule out the temporality of the observed associations and minimize confounding bias and measurement errors. The studies had limitations in power, with sample sizes of < 1000 participants and relatively low PFOA and PFOS exposure levels.]

Fletcher et al. (2013) conducted transcriptional profiling of 13 genes in whole-blood samples from 290 out of 69 000 participants in the C8 Health Project that was carried out during 2005–2006 to examine the potential health effects of PFAS on residents of the Mid-Ohio Valley, USA, who lived in six contaminated water districts surrounding a chemical plant. The data showed inverse associations of serum PFOA concentration with the *NR1H2* (*LXRB*), *NPC1*, and *ABCG1* genes, which are involved in cholesterol transport; a positive association between serum PFOS level and the cholesterol mobilization-related *NCEH1* gene, and a negative association with the *NR1H3* gene, which is involved in cholesterol transport, were also identified. Moreover, the authors noted sex-specific differences in the expression of genes related to

cholesterol mobilization and transport and serum PFOA and PFOS levels. Inverse associations of PFOA level with *ABCG1*, *NPC1*, and *PPARA* transcripts was shown in male participants, but no associations of PFOS with any transcript. In women, the authors showed an inverse association of *NR1H2* (*LXRB*), but a positive association of *NCEH1* gene expression, with the PFOA level. The levels of the *NCEH1* and *PPARA* transcripts also positively correlated with the level of PFOS in women.

In another study, the applicability of peripheral blood transcriptomics for exploration of the effects of PFOA and PFOS exposure on 270 healthy postmenopausal Norwegian women (age 48–62 years) was investigated (Rylander et al., 2011). The authors identified two significantly dysregulated gene sets related to the TCA cycle in the "PFOS high" group (> 30 ng/mL; n = 42), compared with the "PFOS low" group (< 30 ng/mL; n = 228), but no significantly enriched genes in the tested sets were detected in the groups with different PFOA levels. Eight key genes (*NNT*, *PDHB*, *SDHD*, *SDHC*, *SUCLA2*, *IDH3A*, *MDH1*, and *SUCLG2*) were downregulated, and one, *ACO2*, was upregulated, in the group with higher level of PFOS. [The Working Group noted that the presence of PFOA and PFOS in the blood of all the participants may have reduced the chance of detecting differentially expressed single genes or metabolites.]

(ii) Human cells in vitro

See Table 4.30.

Buhrke et al. (2015) conducted a study to investigate the potential PFOA-mediated alterations in the transcriptome of human primary hepatocytes. PFOA exposure affected the PPARα pathway, influenced by substantial gene expression alterations, including upregulation of *PPARA*, *JUN*, and *FOS*, and downregulation of *ER1* and *HNF4a*, which is an important factor for liver development and embryogenesis. Activation of the PPARα network and the inhibition of copper-transporting ATPase 2 (ATP7B), sterol regulatory element-binding transcription factors 1 and 2 (SREBF 1 and SREBF 2), sterol regulatory element-binding protein cleavage-activating protein (SCAP), and insulin receptor (INSR) networks of differentially expressed genes was shown in 3D human primary hepatocyte spheroids treated with PFOA or PFOS for 14 days (Rowan-Carroll et al., 2021).

In addition, Rosen et al. (2013) treated human primary hepatocytes with 12 different perfluoroalkyl acids, including PFOA and PFOS, and showed that *CYP2B6*, *CYP3A4*, *PLIN2*, and *FABP1* were among the most upregulated genes.

Proteomic studies showed that the inhibition of GRP78, HSP27, CTSD, HNRNPC, HUWE1, UBQLN1, and hnRNPC, and the activation of PAF1, may be involved in the activation of p53, which triggered the apoptotic process in human hepatic L-02 cells treated with PFOA (Huang et al., 2013, 2014).

Li et al. (2023b) combined metabolomic and proteomic analyses to investigate the altered profiles in metabolite and protein levels in human primary hepatocytes exposed to PFOS at human exposure-relevant concentrations. The authors showed that an alteration in glycerophospholipid metabolism was the most significant lipid metabolism dysregulation induced by PFOS in hepatocytes and was associated with the intracellular transport process.

A transcriptomic analysis performed in HepaRG cells exposed to PFOS showed dose-dependent dysregulation of genes involved in a PPARα-regulated network, cholesterol biosynthesis, ATF4-activated genes in response to ERS, cytosolic tRNA aminoacylation, and amino acid transport across the plasma membrane (Louisse et al., 2023) (see also Section 4.2.8).

PFOA induced changes in the levels of lipid metabolites, arachidonic acid, myristic acid, and oleic acid in L-02 cells; precursors associated with nucleic acid synthesis (e.g. adenine and guanosine diphosphate) in DLD-1 cells; and

Table 4.30 Omics data relevant to multiple key characteristics of carcinogens in human cells in vitro exposed to PFOA or PFOS

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Transcriptomics	Primary human hepatocytes	1, 25, or 100 µM PFOA, 24 h	Human genome GeneChips HG-U133 plus 2.0 (Affymetrix) Statistically significant dysregulated genes were identified by one-way ANOVA ($P < 0.05$)	43 genes were significantly dysregulated (FC, > 2; $P < 0.05$) after 1, 25–109, or 100–215 µM PFOA exposure The PPARα-dependent signalling pathway was identified by IPA to be the most significantly dysregulated pathway Gene expression: ↑ *PPARA*, ↓ *ESR1*, ↓ *HNF4A*, ↑ *JUN*, and *FOS*	KC8, KC10		Buhrke et al. (2015)
Gene expression	3D human primary hepatocytes	0.02–100 µM PFOA or 0.02–100 µM PFOS, 14 days	RNA sequencing human TempO-Seq S1500 panel DESeq2 v1.30, FDR-adjusted $P < 0.05$ and FC > 1.5	PFOA and PFOS-induced changes in gene expression related to cholesterol biosynthesis and lipid metabolism, and PPARα activation network	KC8	Small number of genes analysed.	Rowan-Carroll et al. (2021)
Gene expression	Human primary hepatocytes	0–200 µM PFOA or 0–225 µM PFOS, 48 h	Custom 48-gene TaqMan low-density RT-PCR arrays; the $2^{-\Delta\Delta Ct}$ method was used; dose–response data were evaluated using SAS jmp	*CPT1A*, *ANGPTL4*, *PLIN2*, and *APOA2* were the most dose–dependently responsive genes to PFOA and PFOS exposure Upregulation of *CYP2B6*, *CYP3A4*, and *FABP1* after PFOA or PFOS exposure	Lipid metabolism	Small number of genes were analysed; analysis performed in context of other PFAA.	Rosen et al. (2013)

Table 4.30 (continued)

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Proteomics	Human non-tumour hepatocytes (L-02)	0, 25, or 50 mg/L, 72 h PFOA	2D fluorescence DIGE coupled with ultrafleXtreme MALDI-TOF/TOF MS Results with $P < 0.05$, which is equal to a Mascot score > 33 were considered to be a positive identification	~1500 protein spots were detected in the DIGE gels at pH 4–7, and 28 protein spots were statistically significantly changed (1.5-fold increase or decrease, $P < 0.05$) 24 spots were downregulated and 4 were upregulated by PFOA The identified proteins were associated with cancer, cell death and survival, and cellular development networks; TP53 (p53), ERK1/2, and STAT3 were the key regulators of these networks	KC10	Small number of proteins detected.	Huang et al. (2013)
Proteomics	Human non-tumour hepatocytes (L-02)	0, 25, or 50 mg/L, 72 h PFOS	iTRAQ labelling and 2D nanoLC-MS/MS analysis. The acquired peak lists for all the MS/MS spectra were searched using the Mascot search engine (Matrix Science, 2023); IPA network analysis of proteins	~1300 proteins were identified and quantified at more than a 95% CI with an FDR < 0.99% 18 proteins were significantly differentially expressed (11 upregulated and 7 downregulated) in a dose-dependent manner by PFOS exposure Differentially expressed *HNRNPC*, *HUWE1*, *UBQLN1*, *RPL21*, and *PAF1* shown to be involved in p53 and c-myc networks, which are associated with DNA replication, recombination, and repair, RNA post-transcriptional modification, and the cell cycle	KC10	Small number of proteins detected.	Huang et al. (2014)

Table 4.30 (continued)

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Metabolomics and proteomics	Primary human hepatocytes	10 μM PFOS, 48 h	UHPLC-MS/MS coupled with quadrupole-Orbitrap high-resolution MS for lipidomics and metabolomics; U3000nano RSLC nanoLC interfaced with a high-resolution MS system and XCalibur 4.3 software for proteomics; DEPs were defined using $\log_2$ FC, ≥ 1.0; $P < 0.05$	82 metabolites present at different concentrations were placed into 13 classes after PFOS treatment; these were involved in glycerophospholipid metabolism and phosphatidylethanolamine biosynthesis 55 proteins (27 upregulated and 28 downregulated) were significantly changed by PFOS treatment; intracellular transport, nuclear lumen, and ribonucleoprotein complex pathway were significantly upregulated; and amide transport and establishment of protein localization to organelle pathway were significantly downregulated after PFOS treatment	KC5, KC10		Li et al. (2023b)
Transcriptomics	HepaRG cells	6.25, 12.5, 25, 50, 100, 200, or 400 μM PFOS, 24 h	Whole-genome gene expression microarray and BMDExpress as the software tool; ANOVA was used, $P < 0.05$; Benjamini–Hochberg applied; FC filter, 1.0	18 Reactome gene sets were upregulated and 90 downregulated. 10 genes were selected that showed clear concentration–response curves for PFOS and were involved in diverse biological processes (*ATF4*, *SLC7A11*, *YARS1*, *PDK4*, *ANGPTL4*, *LSS*, *HMGCR*, *OAT5*, *THRSP*, and *CXCL10*	KC8, KC10		Louisse et al. (2023)

Table 4.30 (continued)

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Metabolomics	A549 (lung), DLD-1 (intestine) and L-02 (liver) cells	0, 100, or 300 μM PFOA, 48 h	UHPLC with MS system and quadruple orbitrap MS with switching positive and negative mode electrospray ionization; Compound Discoverer 2.1 software and Optimal Scaling analysis using SPSS 20.0 software were used for metabolomic analysis; one-way ANOVA was conducted to compare the relative abundances of metabolites between PFOA-treated groups and the controls	Number of different metabolites (DM) L-02: 10 DLD-1: 12 A549: 67 Most DMs were changed in dose-dependent manner L-02: lipid metabolites: arachidonic acid, myristic acid, and oleic acid DLD-1: precursors associated with nucleic acid synthesis (e.g. adenine and GDP) A549: lipids, amino acids, and carbohydrates In A549 and L-02, PFOA induced the production of pro-inflammatory interleukins (IL-1β, IL-6, IL-8, and IL-13)	KC6, KC10	Small number of metabolites detected.	Zhang et al. (2021c)

Table 4.30 (continued)

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Transcriptomics and lipidomics	Primary human lymphocytes	50 μM PFOS, 72 h	mRNA sequencing, edgeR was used to identify DEGs and $\log_2$ FC, ≥ 1.0, $P < 0.05$ applied; HPLC-triple quadrupole time-of-flight MS and XCMS software were used for lipidomic analysis	530 DEGs (247 upregulated and 283 downregulated) were found in lymphocytes after PFOS treatment PFOS exposure dysregulated genes (*BHLHE41*, *DCSTAMP*, *FCRLA*, *MYO7B*, *NOTCH3*, *NTRK2*, *RARRES2*, *SDC2*, *SORT1*, *SPIB*, and *SPP1*) and lipids that play important roles in immune functions, such as lymphocyte differentiation, inflammatory response, and immune response PFOS induced changes in 96 metabolites, including 37 lipids associated with glycerophospholipid, sphingolipid, glycerolipid metabolism; adipocytokine signalling pathway; regulation of autophagy, and arachidonic acid metabolism	KC7, KC10	Single concentration tested.	Li et al. (2020c)

Table 4.30 (continued)

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Transcriptomics and metabolomics	PrECs	10 nM PFOA or PFOS, 3–4 weeks	Single-cell RNA sequencing using 10X Cellranger pipeline and Seurat package (Satija Lab, 2023). Agilent GC/MS system was used for metabolite detection. AMDIS 2.71 (NIST, 2023) database and MetaboAnalyst software were used for metabolite analysis. Principal components analysis and uniform manifold approximation and projection were used for cluster visualization. GSEA was performed on normalized gene expression data using Molecular Signatures Database (Broad Institute, 2023).	UMAP plots revealed cell 5 clusters in the PFOS-, and 8 clusters in the PFOA-, exposed prostaspheres. Both chemicals induced changes in expression of the luminal keratin genes *KRT8*/*KRT18* The cells lacked stemness and showed basal keratin gene expression Significant enrichment of pathways involved in cell replication including, E2F targets, G2/M checkpoint, and mitotic spindle; increased TNFα via NFκB pathway and k-RAS signalling; IL-2, IL-6, and TGFβ inflammatory response; and metabolic pathways (glycolysis, oxidative phosphorylation) were shown in PFOA and PFOS-treated spheroids; the top enriched metabolites were involved in glycine and serine metabolism, with an enhancement of glucose metabolism through the Warburg effect; the top individual metabolites significantly induced by PFOA and PFOS exposure included glycerol, glutamic acid, citric acid, urea, serine, alanine, and glucose	KC8, KC9, KC10	Single concentration tested.	Hu et al. (2022)

Table 4.30 (continued)

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Transcriptomics	Human Burkitt lymphoma cell line (Namalwa)	100 µM PFOA, 48 h	RNA sequencing, DEGs identified using generalized linear models that incorporated empirical Bayesian methods; canonical pathways identified using IPA	The four most significantly downregulated genes were *RAG1*, *RAG2*, *TCL1A*, and *TFRC*. PFOA affected two cellular processes related to immune function, B-cell development and primary immunodeficiency signalling RT-PCR confirmed that PFOA and PFOS caused a time-dependent reduction in *RAG1* and *RAG2*	KC7	Single concentration tested.	Janssen et al. (2022)
Metabolomics and proteomics	MLTC-1 Leydig cells	0.1, 1, or 10 µM PFOA, 48 h	Proteomic data acquisition using NanoLC-MS/MS and the MaxQuant software (Max Planck Institute of Biochemistry, 2023) were used for protein identification and quantification. UPLC system coupled to a quadruple orbitrap MS and SIMCA-P software (v14.0) were used for multivariate analysis of metabolites; MetaboAnalyst software and IPA software were used for metabolic and molecular network analysis	The expression levels of 67 proteins were significantly changed in PFOA-treated cells ($P < 0.05$; FC, ≥ 1.5 in 10 µM PFOA) involved in lipid and fatty acid metabolic processes, catabolic processes, and steroid hormone regulation Metabolic pathway analysis of 17 DMs showed that these metabolites were involved in lipid and fatty acid, amino acid, and carbohydrate metabolism and steroidogenesis IPA showed that ERK1/2, p38 MAPK, and cAMP were key regulators of 18 proteins and 7 metabolites related to steroid hormone regulation, and that fatty acid and lipid metabolism were affected by PFOA exposure	KC10		Huang et al. (2022b)

Table 4.30 (continued)

End-point	Tissue, cell type or line	Exposure concentration or range, duration	Methods	Results[a]	Potentially related KCs	Comments	Reference
Transcriptomics and proteomics	Human breast cancer cells (SKBr3)	10 or 50 μM PFOA, 48 h	RNA sequencing LC-MS/MS analysis using Flex Binary UHPLC System connected to a Hybrid quadrupole–Orbitrap mass spectrometer; the FC thresholds (> 1.05 or < 0.95) and $P < 0.05$ were used to identify DEGs or DEPs	PFOA induced 1390 DEGs and 136 of the DEGs were associated with DEPs; 14 genes/proteins were associated with calcium metabolism cAMP signalling pathway was identified as a key network dysregulated by PFOA exposure; ADORA1 was suggested to be a target for PFOA binding that may have induced the Gi-cAMP-PKA pathway and reduced the concentration of cAMP Low concentrations of PFOA inhibited ADORA2A expression, whereas a high concentration (50 μM) induced its expression, causing opposite cellular effects	KC8, KC10	The suggestion that PFOA can bind to ADORA1 or ADORA2A receptors was not experimentally confirmed.	Li et al. (2022g)

ADORA1, adenosine A1 receptor; ANOVA, analysis of variance; cAMP, cyclic adenosine monophosphate; CI, confidence interval; DEGs, differentially expressed genes; DEPs, differentially expressed proteins; 2D DIGE, 2D fluorescence difference gel electrophoresis; 2D nanoLC-MS/MS, two dimensional nanoliquid chromatography-tandem mass spectrometry; DMs, differential metabolites; ESI, electrospray ionization; FC, fold change; FDR, false discovery rate; GC-MS, gas chromatography-mass spectrometry; GDP, guanosine diphosphate; GSEA, gene set enrichment analysis; IL, interleukin; IPA, Ingenuity Pathway Analysis; IQR, interquartile range; iTRAQ, isobaric tags for relative and absolute quantitation; KC, key characteristic of carcinogens; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOD, limit of detection; MALDI-TOF/TOF MS, matrix-assisted laser desorption-ionization-time of flight/time-of-flight mass spectrometry; MS, mass spectrometry; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PCA, principal components analysis; PFAA, perfluoroalkyl acids; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PKA, protein kinase A; PPAR, peroxisome proliferator-activated receptor; PrECs, primary normal human prostate epithelial cells; RT-PCR, reverse transcription-polymerase chain reaction; STAT, signal transducer and activator of transcription; UMAP, uniform manifold approximation and projection; UPLC, ultra-high performance liquid chromatography.

[a] ↑, increase; ↓, decrease.

lipids, amino acids, and carbohydrates in A549 cells. Regardless of the cell type, A549 or L-02, PFOA induced the production of pro-inflammatory IL-1β, IL-6, IL-8, and IL-13, as determined using a non-targeted metabolomic approach (Zhang et al., 2021c).

Li et al. (2020c), using a transcriptomic analysis in human primary lymphocytes, showed that PFOS exposure induced changes in the expression of genes and lipids that play important roles in immune function, such as in lymphocyte differentiation, the inflammatory response, and immune responses (see Sections 4.2.6 and 4.2.7).

To investigate carcinogenic mechanisms in the prostate after chronic exposure to PFOA and PFOS, Hu et al. (2022) conducted a study in primary normal human prostate epithelial cells in the form of a serial passage of prostasphere cultures that were treated with 10 nM PFOA or PFOS for 3–4 weeks. Exposure to either PFAS caused a significant increase in the total number of spheres, indicating augmentation of stem cell symmetric self-renewal. Transcriptome analysis using single-cell RNA sequencing showed that both chemicals induced changes in the expression of the luminal keratin genes KRT8/18, and the cells lacked stemness and basal keratin gene expression. Significant enrichment of pathways involved in cell replication, including E2F targets, G2M checkpoint and mitotic spindle, increased TNF-α via the NF-κBpathway, kRAS signalling, IL-2, IL-6, TGFβ, the inflammatory response, and metabolic pathways (glycolysis and oxidative phosphorylation), were shown in PFOA and PFOS-treated spheroids. Metabolomic gas chromatography-MS analysis of PFOA- and PFOS-exposed prostaspheres showed an enrichment in metabolites involved in glycine and serine metabolism, with enhancement of anaerobic glucose utilization through the Warburg effect. The top individual metabolites that were significantly induced by PFOA and PFOS exposure included glycerol, glutamic acid, citric acid, urea, serine, alanine, and glucose (Hu et al., 2022).

A transcriptomic-based approach was also used to investigate the carcinogenic potential of PFOA and PFOS in several other in vitro systems, including the bladder (Ye et al., 2022) and breast cancer (Li et al., 2022f), and their immunosuppressive properties in human B lymphoma cells (Janssen et al., 2022).

(b) Experimental systems

Non-human mammalian in vivo

See Table 4.31.

A recent study investigated the effects of PFOA on the hepatic transcriptome of PPARα$^{-/-}$ and wildtype mice fed an HFD and treated with PFOA at 0.05 mg/kg or 0.3 mg/kg bw per day for 20 weeks (Attema et al., 2022). In the wildtype mice, the largest effects were observed in the high-dose PFOA group, with a total of 788 genes being significantly changed. Although the overall effects of high-dose PFOA were substantially reduced in PPARα$^{-/-}$ mice, 294 genes were still significantly altered by high-dose PFOA in the absence of PPARα. Of the genes induced by high-dose PFOA in the wildtype mice, 88% were dependent on PPARα. This result was confirmed by gene set enrichment analysis. Significant positive enrichment was observed for pathways related to xenobiotic metabolism, steroid hormone biosynthesis, and omega-6 fatty acid metabolism in wildtype and PPARα$^{-/-}$ mice. Also, in PPARα$^{-/-}$ mice, many of the genes that were significantly upregulated were regulated by the rodent-specific PXR agonist pregnenolone 16a-carbonitrile, as well as by the CAR agonist 1,4-bis (2-(3,5-dichloropyridyloxy)) benzene, suggesting that they are PXR and CAR target genes.

Rosen et al. (2008a) compared the transcript profiles of the livers of wildtype and PPARα-null mice exposed to PFOA and concluded that the majority of the genes were dependent on PPARα. The independent genes were involved in lipid homeostasis and xenobiotic metabolism. The expression of many of the identified xenobiotic

Table 4.31 Omics data relevant to multiple key characteristics of carcinogens in non-human mammalian systems in vivo exposed to PFOA and PFOS

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	Mouse, C57BL/6J, male (wildtype and PPARα$^{-/-}$)	Liver	PFOA in drinking-water, 0.05 or 0.3 mg/kg bw per day, 20 wk	RNA-Seq, Limma analysis with cut-off $P \leq 0.001$; FC, > 1.5	Effects of PFOA were mostly PPARα-dependent. PXR and CAR could be involved in the absence of PPARα.	KC8, lipid metabolism		Attema et al. (2022)
Transcriptomics	Mouse, wildtype and PPARα-null 129S1/SvlmJ, male	Liver	PFOA by gavage, 0, 1, or 3 mg/kg bw per day, 7 days	Applied Biosystems Mouse Genome Survey Microarrays, a two-way ANOVA across dose ($P \leq 0.03$); post-hoc *t*-test of the least-square means was used to evaluate individual treatment effects ($P \leq 0.0025$)	In PFOA-treated mice, the changes in transcripts related to fatty acid metabolism, inflammation, xenobiotic metabolism, and cell cycle regulation were PPARα-independent. Involvement of other PPAR isoforms in fatty acid metabolism and inflammation suggested.	KC6, KC8, KC10, and lipid metabolism	In PPARα-null mice, the number of DEGs was ~5 times as low as that in wildtype mice, which may have led to misinterpretation of the data.	Rosen et al. (2008a)
Proteomics	Mouse, BALB/c, male and female	Liver	PFOA by gavage, 0, 0.05, 2.5, or 5 mg/kg bw per day, 28 days	iTRAQ labelling, FC, ≥ 1.5	Dose-dependent proteomic changes: mitochondrial dysfunction, oxidoreductase activity, peroxisome proliferator activity, ion 236 binding, and transferase activity.	KC5, KC8, KC10	Livers of 10 mice pooled per group (not biological replicates).	Li et al. (2017b)

Table 4.31 (continued)

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	ICR mice	Liver	PFOA by gavage, 0 or 10 mg/kg bw per day, 7 days	RNA sequencing, edgeR 3.0.8. $\log_2$ (FC) > 1 and $P < 0.05$	PFOA induced 2426 DEGs associated with fatty acid and lipid metabolism, oxidative stress, alterations of liver cell proliferation and apoptosis of hepatocytes, liver inflammation, necrosis, hepatic steatosis, and steatohepatitis.	KC5, KC6, KC8, KC10, and lipid metabolism	Single dose.	Li et al. (2022g)
Transcriptomics and lipidomics	Mouse, CD-1, female	Neonatal testes (PND1) and male offspring at PND63	PFOS by gavage, 0, 0.3, or 3 mg/kg bw per day, throughout gestation	RNA sequencing, edgeR package. $\log_2$ (FC) > 0.3 and FDR < 0.05; LC-MS/MS using Kinetex C18 column	56 (low dose) and 319 (high dose) DEGs were associated with lipid metabolism, oxidative stress, and cell junction signalling in testes. Levels of adrenic acid, docosahexaenoic acid, and eicosapentaenoic acid were reduced in testes at PND1 by PFOS treatment; and LOX-mediated 5-HETE and 15-HETE derived from arachidonic acid were increased. Male offspring at PND63 showed reductions in serum testosterone and epididymal sperm count.	KC5, KC8, KC10, lipid metabolism		Lai et al. (2017b)

Table 4.31 (continued)

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	Rat, Sprague-Dawley	Liver	PFOA by gavage, 0, 1, 3, 5, 10, or 15 mg/kg bw per day, 21 days	Affymetrix rat genome 230 2.0 GeneChip For upregulated genes change *P*-value < 0.0025; downregulated genes change *P*-value < 0.9975	> 500 genes significantly altered ($P < 0.0025$, FC > 2) after exposure to PFOA at any dose, with the largest number being at 10 mg/kg (813 genes) and 15 mg/kg (667 genes) PFOA. Dysregulated genes were associated with fatty acid synthesis and degradation, mitochondrial fatty acid β-oxidation (7 genes), apoptosis, cell communication and adhesion, growth and cell cycle, signal transduction and regulation of hormones.	KC10, KC6, KC5, lipid metabolism		Guruge et al. (2006)
Transcriptomics	Rat, Sprague-Dawley (Crl:CD(SD) IGS BR), male	Liver	PFOA or PFOS by gavage (10 mL/kg) for 1, 3, or 5 consecutive days The PFOA and PFOS groups received 20 and 10 mg/kg bw per day, respectively	Microarray, differentially expressed genes filtered at $P < 0.05$; pathway perturbations were visualized by DrugMatrix	PFOA and PFOS exhibited PPARα agonist-like effects on genes associated with fatty acid homeostasis. PFOA and PFOS exposure also resulted in the downregulation of cholesterol biosynthesis genes.	KC8, lipid metabolism		Martin et al. (2007)

Table 4.31 (continued)

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	Mouse, C57BL/6, male	Liver	PFOA HFD for 24 wk + 8 wk PFOA, gavage, 1 mg/kg bw per day	RNA sequencing, DESeq2; $\log_2$ (fFC) > 1; and a Benjamini–Hochberg-corrected $P < 0.1$	1233 and 835 DEGs were detected in PFOA-treated chow and HFD groups, respectively. 11 lipid metabolism related pathways were increased by PFOA and most were correlated with the "clearance" (oxidation, hydrolysis, catabolism) of lipids, causing significant downregulation of the hepatic steatosis pathway in HFD-fed mice. KEGG pathway analysis showed pathways including "PPAR signalling pathway," "Fatty acid degradation," "Biosynthesis of unsaturated fatty acids," "Peroxisome," and "Chemical carcinogenesis" were enriched by PFOA exposure, regardless of diet, leading to activation of the PPARα target genes *Cyp4a10*, *Lpl*, and *Cd36*, and the CAR target genes *Cyp2b10* and *Cyp3a11*, but inhibited Pparγ and Pparδ signalling.	KC8, KC10, lipid metabolism		Li et al. (2019c)

Table 4.31 (continued)

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	Mouse, athymic nude, male	Prostate tumour xenografts	PFOS and an HFD; gavage of 0 or 10 mg/kg bw per day, 40 days	RNA sequencing of RWPE-kRAS xenografts	Synergistic effect on xenograft growth of PFOS and HFD was observed. Genes involved in pyruvate metabolism, glycolysis pathways, PPARα network, and chromatin organization were significantly upregulated by PFOS in tumours from mice fed an HFD.	KC8, KC4	Single dose; histone modification analysis was not coherent.	Imir et al. (2021)
Transcriptomics	Mouse, CD-1, female	Fetal liver, lung	PFOA Gavage, 0, 1, 3, 5, or 10 mg/kg bw per day on GD1–GD17	Affymetrix mouse 430 2.0 expression GeneChips, two-way ANOVA across dose ($P \leq 0.05$); *t*-test of the least square means was used as a post-hoc test to evaluate individual treatment effects ($P \leq 0.0025$)	Clear dose–response effects in both the fetal liver and lung, with more extensive gene expression changes in liver. In the fetal liver, the DEGs were associated with lipid transport, ketogenesis, glucose metabolism, lipoprotein metabolism, cholesterol biosynthesis, steroid metabolism, bile acid biosynthesis, phospholipid metabolism, retinol metabolism, proteosome activation, and inflammation. These were associated with PPARα (except bile acid and glucose metabolism). Genes related to fatty acid catabolism were changed in both the fetal liver and lung	KC5, KC6, KC10, lipid metabolism		Rosen et al. (2007)

Table 4.31 (continued)

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	Mouse, CD-1 female	Fetal liver, lung	PFOS Gavage, 0, 5, or 10 mg/kg bw per day, GD1–GD17	Affymetrix mouse 430 2.0 expression GeneChips, two-way ANOVA across dose ($P \leq 0.05$); *t*-test of the least square means was used as a post-hoc test to evaluate individual treatment effects ($P \leq 0.0025$)	PFOS induced similar gene expression changes in liver and lung to PFOA (Rosen et al., 2007), which were primarily related to PPARα activation. In fetal lung: *Cyp4a14*, enoyl-coenzyme A hydratase (*Ehhadh*), and fatty acid binding protein 1 (*Fabp1*).	KC5, KC6, KC10, lipid metabolism		Rosen et al. (2009)
Transcriptomics	Mouse, CD-1	Fetal liver	PFOS Corn oil gavage, from mating to GD18.5, 0.3mg/kgbwper day (equivalent to human tolerable daily intake of 150 ng/kg bw per day)	RNA sequencing, edgeR 3.0.8. $\log_2$ (FC) > 1 and a Benjamini–Hochberg-corrected $P < 0.05$	PFOS activated the synthesis and metabolism of fatty acids and lipids, caused liver damage, and affected liver development in the fetus. Wnt/b-catenin, Rac, and TGF-b pathways activated.	KC8, KC10, lipid metabolism	1 pool (3 samples) per group.	Lai et al. (2017a)
Transcriptomics	Rat, Sprague-Dawley	Fetal liver	PFOS Gavage, 0 or 0.3 mg/kg bw per day, GD2–GD20	Affymetrix RAE 230A microarray, $P \leq 0.05$	225 upregulated and 220 downregulated genes. Peroxisomal proliferation pathway was dysregulated, but no change in *Ppara* gene. *Cyp7a1* reduced.	KC8, lipid and bile acid metabolism	Single dose.	Bjork et al. (2008)

Table 4.31 (continued)

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	Rat, Sprague-Dawley	Blood	PFOS Gavage, 0, 2.5, or 5 mg/kg bw per day, 28 days	RNA sequencing, DEGseq R package. $\log_2$ (FC) > 1/ < –1 and Q-value < 0.001	DEGs in blood of treated rats were associated with spliceosome, B-cell receptor signalling pathway, acute myeloid leukaemia, protein processing in the endoplasmic reticulum, NF-κB signalling pathway, and Fc gamma R-mediated phagocytosis.	KC6, KC8, KC10	No transcriptomic data for the liver or kidney.	Wang et al. (2023b)
Transcriptomics and metabolomics	Rat, Sprague-Dawley	Livers of rat mothers	PFOS Gavage, 0, 0.03, or 0.3 mg/kg bw per day, during pregnancy (GD1–GD18)	RNA sequencing, DESeq and Q-values < 0.05; UPLC/MS Progenesis QI software, OPLS-DA model, FC > 1.5 or ≤ 0.66, and VIP ≥ 1	DEGs were related to several metabolic pathways, such as PPAR signalling, ovarian steroid synthesis, arachidonic acid metabolism, insulin resistance, cholesterol metabolism, unsaturated fatty acid synthesis, and bile acid secretion. Untargeted metabolomics identified 164 and 158 DMs These were enriched with respect to α-linolenic acid metabolism, glycolysis/ gluconeogenesis, glycerolipid metabolism, glucagon signalling pathway, and glycine, serine, and threonine metabolism.	KC8, lipid metabolism		Yu et al. (2023)

Table 4.31 (continued)

End-point	Species, strain, sex	Tissue	Route, dose duration, dosing regimen	Methods	Results	Relevant KCs	Comments	Reference
Transcriptomics	Mouse, BALB/c, male	Spleen	PFOA Gavage, 0, 0.4, 2, or 10 mg/kg bw per day, 28 days	RNA sequencing, weighted gene co-expression network analysis	7043 DEGs, with enrichment in cell cycle, autoimmunity, and anaemia in the spleen after PFOA.	KC6, KC10	Only one dose (10 mg/kg) was investigated.	Guo et al. (2021b)
Transcriptomics	Mouse, wildtype and PPARα-null 129S1/SvlmJ, male	Liver	PFOS Gavage, PFOS 0, 3, or 10 mg/kg bw per day, 7 days	Applied Biosystems Mouse Genome Survey Affymetrix 430 2.0 GeneChips, two-way ANOVA across doses ($P \leq 0.03$); *t*-test of the least square means was used as a post-hoc test to evaluate individual treatment effects ($P \leq 0.0025$)	The PPARα-dependent DEGs were associated with lipid metabolism, peroxisome biogenesis, proteasome activation, and inflammation; PPARα-independent DEGs were related to lipid metabolism and xenobiotic metabolism. Modest activation of CAR, and possibly PPARγ and/or PPARβ/δ was noted.	KC6, KC8, lipid metabolism		Rosen et al. (2010)

ANOVA, analysis of variance; CAR, constitutive androstane receptor; DEG, differentially expressed gene; DMs, differential metabolites; FC, fold change; FDR, false discovery rate; GD, gestational day; HETE, hydroxyeicosatetraenoic acid; iTRAQ, isobaric tags for relative and absolute quantitation; HFD, high-fat diet; KC, key characteristic of carcinogens; KEGG, Kyoto Encyclopedia of Genes and Genomes; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOX, LOX, lipoxygenase; OPLS-DA, orthogonal partial least squares discriminant analysis; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PND, postnatal day; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; UPLC-MS, ultra-high performance liquid chromatography-mass spectrometry; VIP, variable importance in the projection; wk, week(s).

metabolism genes is known to be under the control of the nuclear receptor CAR and the transcription factor NRF2 (Rosen et al., 2008a).

Exposure of male and female Balb/c mice to PFOA at 0.05, 0.5, or 2.5 mg/kg per day for 28 days induced dose-dependent changes in proteins associated with mitochondrial dysfunction, oxidoreductase activity, peroxisome proliferator activity, ion binding, and transferase activity (Li et al., 2017a). In another study, ICR mice treated with PFOA for 7 days showed transcriptomic changes associated with the metabolism of many fatty acids and lipids, and particularly fatty acid β-oxidation, long-chain fatty acid transport, and the acyl-CoA metabolic process (Li et al., 2022g).

Lai et al. (2017b) conducted transcriptomic and targeted lipidomic analyses using neonatal testes in an effort to identify molecular targets and lipid markers associated with in utero PFOS exposure at doses of 0.3 or 3 μg/g bw, corresponding to the general population and occupational exposure levels, respectively. Analysis of male offspring at PND63 showed significant reductions in serum testosterone and epididymal sperm count. After PFOS exposure, the levels of adrenic acid and docosahexaenoic acid in the testes were significantly reduced by the low and high PFOS concentrations, respectively. Exposure to PFOS significantly induced the generation of 5-HETE and 15-HETE from arachidonic acid by LOX in the testes. Pathway analysis of the transcriptomic data highlighted that PFOS exposure induced changes in redox responses and oxidation–reduction processes in neonatal testes (Lai et al., 2017b).

Guruge et al. (2006) showed that male Sprague-Dawley rats exposed to PFOA at 1, 3, 5, 10, or 15 mg/kg bw per day for 21 days were characterized by a moderately dose-dependent number of significantly dysregulated genes. The upregulated genes are involved in the metabolism of lipids, cell communication, adhesion, growth, apoptosis, hormone regulatory pathways, proteolysis and peptidolysis, and signal transduction. The downregulated genes are related to the transport of lipids, inflammation, cell adhesion, apoptosis, the regulation of hormones, metabolism, and G-protein-coupled receptor protein signalling pathways.

Martin et al. (2007), using transcriptomic analysis, showed that PFOA and PFOS exposure resulted in the downregulation of cholesterol biosynthesis genes and alterations to thyroid hormone metabolism genes. These effects were associated with a decrease in serum cholesterol and serum thyroid hormone depletion, respectively, in the livers of male rats after 1, 3 and 5 days of exposure.

NAFLD could be considered to be a risk factor and potentiate the toxic carcinogenic effects of chemicals. Based on these considerations, Li et al. (2019c) investigated the hepatic effects of PFOA in mice in which NAFLD had been induced. PFOA activated xenobiotic nuclear receptors, inflammation, and cell proliferation in the livers of mice fed an HFD. Transcriptomic analysis showed that PFOA activated PPARα, CAR, and PXR in the livers of mice fed a control diet or an HFD, but reduced the severity of hepatic steatosis and hepatic triglyceride levels, enhanced lipid oxidation pathways, and attenuated HFD-induced hepatic fibrosis.

Imir et al. (2021) investigated the impact of metabolic alterations induced by an HFD combined with PFOS exposure on prostate tumour progression by analysing prostate RWPE–kRAS xenograft tumour growth in vivo. PFOS exposure of athymic nude male that were fed an HFD-induced RWPE–kRAS xenograft tumour growth and caused alterations in metabolites associated with glucose metabolism via the Warburg effect, the transfer of acetyl groups into mitochondria, and the TCA cycle, and in particular pyruvate and acetyl-CoA. Gene set enrichment analysis identified genes involved in pyruvate metabolism and glycolysis pathways to be significantly upregulated by PFOS exposure in tumours in mice fed an HFD. These data

indicate that metabolic alterations induced by HFD combined with PFOS exposure may play a significant role in prostate tumour growth and progression.

Rosen et al. (2007, 2009) conducted a transcriptomic microarray analysis of the lungs and livers of fetuses from pregnant CD-1 mice exposed to PFOA and PFOS to investigate the mechanism whereby they induce developmental toxicity. The expression of genes related to fatty acid catabolism was altered in both the fetal liver and lung. In the fetal liver, exposure to PFOA or PFOS caused significant alterations in the expression of genes associated with lipid transport, ketogenesis, glucose metabolism, lipoprotein metabolism, cholesterol biosynthesis, steroid metabolism, bile acid biosynthesis, phospholipid metabolism, oxidative phosphorylation, retinol metabolism, proteosome activation, and inflammation. Interestingly, PFOA and PFOS altered the expression of genes related to lipid metabolism, inflammation, and xenobiotic metabolism in both wildtype and PPARα-null CD-1 mice, which was consistent with modest activation of CAR, and possibly PPARγ and/or PPARβ/δ (Rosen et al., 2008b, 2010).

Similarly, prenatal PFOS exposure induced transcriptomic changes that may activate the synthesis and metabolism of fatty acids and lipids, leading to liver damage and interference with liver development in the fetuses of CD-1 mice (Lai et al., 2017a) and Sprague-Dawley rats (Bjork et al., 2008).

A transcriptomic analysis of blood samples from rats exposed to PFOS identified differentially expressed genes that were associated with the spliceosome, the B-cell receptor signalling pathway, acute myeloid leukaemia, protein processing in the ER pathway, NF-κB signalling pathway, and Fc gamma R-mediated phagocytosis (Wang et al., 2023b).

Using transcriptome sequencing combined with non-targeted metabolomic assays, Yu et al. (2023) identified differentially expressed genes in the livers of Sprague-Dawley rats given PFOS at 0.03 or 0.3 mg/kg bw per day that were related to several metabolic pathways, such as PPAR signalling, ovarian steroid synthesis, arachidonic acid metabolism, insulin resistance, cholesterol metabolism, unsaturated fatty acid synthesis, and bile acid secretion. Non-targeted metabolomics identified 164 and 158 metabolites present at different concentrations in 0.03 and 0.3 mg/kg bw per day exposure groups, respectively, which could be associated with α-linolenic acid metabolism, glycolysis/gluconeogenesis, glycerolipid metabolism, glucagon signalling pathway, and glycine, serine, and threonine metabolism.

Yu et al. (2016) conducted a high-throughput targeted metabolomic study of 278 metabolites to investigate the effects of PFOA exposure for 28 days on the brains and livers of male Balb/c mice. This study aimed to link the metabolic profiles of the livers and brains of mice exposed to PFOA with alterations in the transcriptome and proteome, and PFOA-induced hepatomegaly and neurobehavioural effects. PFOA treatment induced metabolic changes in the brain and liver that were associated with the metabolism of amino acids, lipids, and carbohydrates. The energy and lipid metabolism pathways were more susceptible to PFOA exposure. Lipidomic data in mice exposed to PFOA suggested that the β-oxidation and biosynthesis of fatty acids and inflammation are involved in PFOA-induced hepatomegaly. An iTRAQ labelling quantitative proteomic technology was used for the global characterization of the liver proteome in mice exposed to PFOS at 1.0, 2.5, or 5.0 mg/kg bw for 24 hours (Tan et al., 2012). Seventy-one of 1038 unique detectable proteins were significantly dysregulated in the mouse livers after PFOS exposure, and these were involved in lipid metabolism, transport, biosynthetic processes, and the response to a stimulus. Long-chain acyl-CoA synthetase, acyl-CoA oxidase 1, bifunctional enzyme, 3-ketoacyl-CoA thiolase A, CYPs, and GSTs were identified as key enzymes that regulate

peroxisomal β-oxidation and the metabolism of xenobiotic compounds that were affected by PFOS exposure.

In the study by Guo et al. (2021b), male mice were fed diet containing PFOA at a dose of 0, 0.4, 2, or 10 mg/kg per day for 28 days to investigate the splenic atrophy induced by PFOA. The authors demonstrated that mice exposed to PFOA reduced spleen weight and relative spleen weight and lower iron levels in the spleen and serum. Weighted gene co-expression network analysis of 7043 genes showed enrichment in those involved in cell cycle, autoimmunity, and anaemia in the spleen of PFOA-treated mice. PFOA exposure resulted in an increase in the ratio of the total number of macrophages to M1 macrophages in the spleen, the phagocytic ability of macrophages, and the levels of cytokines such as TNF-α, IL-1β, and IL-6. These findings suggest that overactivation of macrophages may play an important role in the splenic atrophy induced by PFOA exposure.

[The Working Group noted that these transcriptomic alterations provide an insight into PFOA and PFOS exposure in relation to the KCs of carcinogens. In addition, the Working Group also noted that transcriptomic, metabolomic, and lipidomic data collected after PFOA or PFOS exposure provide information associated with KCs, such as inflammation, cell proliferation, stress responses, and lipid metabolism.]

Synopsis

[The Working Group noted that the above studies showed some evidence that human exposure to PFOA and PFOS alters pathways related to nutrient and energy supply. Metabolomic analyses in exposed humans have suggested that PFOA and PFOS increase the activities of glycolytic pathways. Transcriptomic analyses in human primary cells have shown alterations in cell proliferation pathways, and transcriptomic data in experimental systems have shown alterations in lipid metabolism pathways.]

4.3 Evaluation of high-throughput in vitro screening data

See Tables 4.32 to 4.35.

An analysis of the in vitro bioactivities of PFOA and PFOS was informed by data from high-throughput screening assays generated by the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018). PFOA, its ammonium salt APFO, PFOS, and its salt potassium perfluorooctanesulfonate, were among the thousands of chemicals tested in the large assay battery of the Tox21 and ToxCast research programmes of the US EPA and the US NIH. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2021). A supplementary table (Annex 6, Supplementary material for Section 4.3, Evaluation of high-throughput in vitro toxicity screening data, online only, available from: https://publications.iarc.who.int/636) provides a summary of the findings, including the assay names, the corresponding KCs, the resulting "hit calls", both positive and negative, and any reported caution flags for PFOA. The results were generated with the software "kc-hits" (key characteristics of carcinogens – high-throughput screening discovery tool) (Reisfeld et al., 2022; available from: https://gitlab.com/i1650/kc-hits); the evaluations discussed in the present monograph were performed using the US EPA ToxCast and Tox21 assay data and the curated mapping of KCs to assays available at the time. The findings and interpretation of these high-throughput assays for PFOA and PFOS are discussed below. [The Working Group noted that for PFOA, its ammonium salt, PFOS, and its potassium salt, the chemical purity quality control rating was "Unknown/Inconclusive", so the results should be interpreted with caution.]

Table 4.32 Number of assays available for the evaluation of high-throughput in vitro screening data for PFOA

		PFOA	
		Active (no flag)	Active with flag, or inactive
APFO	Active (no flag)	13	1
	Active with flag or inactive	2	270

APFO, ammonium perfluorooctanoate; PFOA, perfluorooctanoic acid.

4.3.1 PFOA

After mapping against the KCs of carcinogens, the ToxCast/Tox21 database contained 289 assay end-points in which PFOA was tested, and 288 assay end-points in which APFO was tested (US EPA, 2023a). Of these, PFOA and APFO were found to be active and without caution flags in 15 and 14 assay end-points, respectively, relevant to the KCs of carcinogens, with 13 assay end-points active without flags for either (see Tables 4.32 and 4.33).

PFOA and APFO were active in two assay end-points mapped to KC5, "induces oxidative stress". Both of these assays were performed in the HepG2 cell line, and both were related to transcription factor activity mapping to endogenous human NRF2, which regulates antioxidant response elements.

In addition, PFOA and APFO were both active in nine assay end-points mapped to KC8, "modulates receptor-mediated effects", with a tenth assay end-point for which PFOA was active with < 50% efficacy, and APFO was active without flags. Three assay end-points were for ER, one for antagonist activity in a human embryonic kidney cell line (HEK293T), and two for inducible changes in transcription in a human liver cancer cell line (HepG2). One assay using a human liver cancer cell line (HepaRG) was active for CYP2B6 induction, which is considered to be a marker for PXR-mediated metabolism. The remaining assays indicated activity for PPARα and PPARγ, PPRE, and PXR response element, all of which are indicative of nuclear receptor activation.

Finally, PFOA and APFO were both active in three assays mapped to KC10, "alters cell proliferation, cell death, or nutrient supply", with a fourth assay end-point for which APFO was active with less than 50% efficacy, and PFOA perfluorooctanoate was active without flags in two of them. Two assay end-points measured cell viability and one measured reduced mitochondrial membrane potential, all in HepG2 cells. The fourth assay end-point was only active without flags for the effect of PFOA on cell viability in a human cervical cell line (ME-180).

4.3.2 PFOS

After mapping against the KCs of carcinogens, the ToxCast/Tox21 database contained 292 assay end-points in which PFOS was tested and 289 assay end-points in which potassium perfluorooctanesulfonate was tested (US EPA, 2023b). Of these, PFOS and potassium perfluorooctanesulfonate were found to be active and without caution flags in 32 and 31 assay end-points, respectively, relevant to the KCs of carcinogens, with 24 assay end-points active without flags for either (see Tables 4.34 and 4.35), the results of which are summarized below.

PFOS and potassium perfluorooctanesulfonate were active in two assay end-points mapped to KC2, "is genotoxic", with a third assay end-point being active with flags for PFOS and without flags for potassium perfluorooctanesulfonate. The first two of these were in HepG2 cell lines and related to p53 activation, but at different time points (24 and 72 hours, respectively),

Table 4.33 High-throughput in vitro screening data for PFOA

Assay ID	Assay name	PFOA		APFO	
		AC_{50} (μM)	Flags	AC_{50} (μM)	Flags
KC5: Induces oxidative stress					
1110	TOX21_ARE_BLA_agonist_ratio	29.06		43.91	
97	ATG_NRF2_ARE_CIS_up	47.89		116	
KC8: Modulates receptor-mediated effects					
786	TOX21_ERa_BLA_Antagonist_ratio	51.93		46.87	
117	ATG_ERa_TRANS_up	7.561		44.7	
75	ATG_ERE_CIS_up	10.36		162.6	
132	ATG_PPARa_TRANS_up	21.83		14.23	
134	ATG_PPARg_TRANS_up	124.1		114.7	
719	NVS_NR_hPPARg	23.35	[a]	26.36	
969	LTEA_HepaRG_CYP2B6_up	23.76		5.641	
142	ATG_RXRb_TRANS_up	65.13		37.67	
103	ATG_PXRE_CIS_up	35.28		40.99	
102	ATG_PPRE_CIS_up	116.2		86.49	
KC10: Alters cell proliferation, cell death, or nutrient supply					
2066	TOX21_HRE_BLA_Agonist_viability	23.43		48.35	[b]
64	ATG_AP_1_CIS_up	139.3		124.7	
51	APR_HepG2_MitoMembPot_72h_dn	116.1		111.2	
45	APR_HepG2_CellLoss_72h_dn	123.2		114.2	[c]

AC_{50}, concentration that elicits a half-maximal response; APFO, ammonium perfluorooctanoate; ID, identifier; KC, key characteristic of carcinogens; PFOA, perfluorooctanoic acid.

[a] Less than 50% efficacy.

[b] Only the highest concentration above baseline, active, < 50% efficacy.

[c] Unspecified flag.

and the third assay was related to DNA repair in a chicken lymphoblast cell line (DT40). Additionally, activity for PFOS was reported for one active and unflagged assay end-point for KC4, "induces epigenetic alterations". This was also performed in HepG2 cells and was related to transcription factor activity of the cis-acting elements in the reporter *Pax* genes. The same assay end-points were reported to be active, but only for one concentration above baseline for potassium perfluorooctanesulfonate.

PFOS was active in two assays mapped to KC5, "induces oxidative stress". Both of these assays were performed in HepG2 cells. One indicated transcriptional activation of the NRF2 promoter, which regulates antioxidant response elements, and this was also active for potassium perfluorooctanesulfonate. The other indicated transcriptional activation that induces metallothioneins, and was reported to be active, but only for one concentration above baseline for potassium perfluorooctanesulfonate. Potassium perfluorooctanesulfonate was also reported to be active for a third assay end-point indicating increased stress kinases in HepG2 cell line, in which PFOS was not tested.

In addition, PFOS was active without flags in 10 assays mapped to KC8, "modulates receptor-mediated effects", seven of which were also active, without flags, for potassium perfluorooctanesulfonate. Three assays for PFOS were active for ER/PR, including progesterone antagonism in a kidney cell line (HEK293T) and two for transcriptional activation in a human liver cancer cell

Table 4.34 Number of assays available for the evaluation of high-throughput in vitro screening data for PFOS data

		PFOS	
		Active (no flag)	Active with flag or inactive
KPFOS	Active (no flag)	24	7
	Active with flag or inactive	8	249

KPFOS, potassium perfluorooctanesulfonate; PFOS, perfluorooctanesulfonic acid.

Table 4.35 High-throughput in vitro screening data for PFOS

Assay ID	Assay name	PFOS		KPFOS	
		AC_{50} (μM)	Flags	AC_{50} (μM)	Flags
KC2: Is genotoxic					
2131	TOX21_DT40_657	67.02	[a]	65.28	
60	APR_HepG2_p53Act_72h_up	5.435		111	
40	APR_HepG2_p53Act_24h_up	109.3		123.6	
KC4: Induces epigenetic alterations					
100	ATG_Pax6_CIS_up	76.27		231.1	[b]
KC5: Induces oxidative stress					
97	ATG_NRF2_ARE_CIS_up	10.22		30.7	
91	ATG_MRE_CIS_up	41.98		165.9	[b]
62	APR_HepG2_StressKinase_72h_up	NA		112	
KC8: Modulates receptor-mediated effects					
804	TOX21_TR_LUC_GH3_Antagonist	86.54	[a]	65.05	
2127	TOX21_PR_BLA_Antagonist_ratio	35.5		63.53	[a, c]
75	ATG_ERE_CIS_up	150.3		32.63	
117	ATG_ERa_TRANS_up	23.05		35.92	[b]
132	ATG_PPARa_TRANS_up	58.85		88.24	
719	NVS_NR_hPPARg	20.9		20.28	
963	LTEA_HepaRG_CYP1A1_up	21.51		NA	
102	ATG_PPRE_CIS_up	179.7		179.5	
135	ATG_PXR_TRANS_up	18.01		14.01	
103	ATG_PXRE_CIS_up	11.32		30.38	
134	ATG_PPARg_TRANS_up	167		73.43	
KC10: Alters cell proliferation, cell death, or nutrient supply					
2066	TOX21_HRE_BLA_Agonist_viability	41.58		26.33	
1195	TOX21_PPARd_BLA_Agonist_viability	57.77		32.44	
1121	TOX21_FXR_BLA_antagonist_viability	31.45		31.86	
2120	TOX21_ERb_BLA_Antagonist_viability	30.28		42.46	
2116	TOX21_ERb_BLA_Agonist_viability	25.45		42.18	
1188	TOX21_FXR_BLA_agonist_viability	30.13		29.07	[d]
2128	TOX21_PR_BLA_Antagonist_viability	32.85		55.94	
2082	TOX21_RT_HEK293_FLO_40hr_viability	27.66	[c]	29.09	
2080	TOX21_RT_HEK293_FLO_32hr_viability	27.93	[c]	28.81	
2078	TOX21_RT_HEK293_FLO_24hr_viability	28.14	[c]	29.16	

Table 4.35 (continued)

Assay ID	Assay name	PFOS		KPFOS	
		AC_{50} (μM)	Flags	AC_{50} (μM)	Flags
2077	TOX21_RT_HEK293_FLO_16hr_viability	29.81	[c, d]	23.96	
2124	TOX21_PR_BLA_Agonist_viability	27.65		40.5	
64	ATG_AP_1_CIS_up	18.83		23.71	
55	APR_HepG2_NuclearSize_72h_dn	8.097		105.1	
54	APR_HepG2_MitoticArrest_72h_up	113.8		107.5	
49	APR_HepG2_MitoMass_72h_dn	104.3		112.9	
45	APR_HepG2_CellLoss_72h_dn	110.6		110.9	
1326	TOX21_p53_BLA_p4_viability	81.43		45.36	
29	APR_HepG2_MitoMass_24h_dn	114.2		120.2	
251	BSK_hDFCGF_Proliferation_down	10		60	[b, f]
112	ATG_TGFb_CIS_up	107.6		101.5	

AC_{50}, concentration that elicits a half-maximal response; ID, identifier KC, key characteristic; KPFOS, potassium perfluorooctanesulfonate; NA, not tested or not available; PFOS, perfluorooctanesulfonic acid.
[a] Only the highest concentration above baseline, active.
[b] Only one concentration above baseline, active.
[c] Efficacy, < 50%.
[d] Borderline active.
[e] Multiple points above baseline, inactive.
[f] Hit-call potentially confounded by overfitting.

line (HepG2), although for potassium perfluorooctanesulfonate two of these had activity flags. Multiple assays were active for other nuclear receptors, including transcriptional activation of PPARα in HepG2 cells, PPARγ in HepG2 cells and a biochemical (cell-free, using extracted gene-proteins) assay, CYP1A1 induction in HepaRG cells, and the transcriptional activation of PXR in HepG2 cells. Additionally, thyroid hormone modulation was indicated in one assay end-point performed in a rat pituitary gland cell line (GH3) for potassium perfluorooctanesulfonate, but only at the highest concentration of PFOS.

Finally, PFOS and potassium perfluorooctanesulfonate were active without flags in 17 and 18 assay end-points, respectively, mapped to KC10, "alters cell proliferation, cell death, or nutrient supply", 14 of which overlapped. Many of these assay end-points measured cell viability in ME-180, HEK293T, HepG2, and HCT116 cells. Reduced proliferation, which is also indicative of a loss of viability under pro-inflammatory conditions, was detected in one assay for PFOS using human foreskin fibroblasts, with potassium perfluorooctanesulfonate being active at only one concentration above baseline. Reduced nuclear size 72 hours after treatment, reduced mitochondrial mass after 24 and 72 hours, and increased mitotic arrest after 72 hours were also detected in HepG2 cells. Increased transactivation of TGFβ in HepG2 cells was also detected in one assay.

References

Abbott BD, Wood CR, Watkins AM, Tatum-Gibbs K, Das KP, Lau C (2012). Effects of perfluorooctanoic acid (PFOA) on expression of peroxisome proliferator-activated receptors (PPAR) and nuclear receptor-regulated genes in fetal and postnatal CD-1 mouse tissues. *Reprod Toxicol.* 33(4):491–505. doi:10.1016/j.reprotox.2011.11.005 PMID:22154759

Abdellatif A, Al-Tonsy AH, Awad ME, Roberfroid M, Khan MN (2003–2004). Peroxisomal enzymes and 8-hydroxydeoxyguanosine in rat liver treated with perfluorooctanoic acid. *Dis Markers*. 19(1):19–25. doi:10.1155/2003/135859 PMID:14757943

Abe T, Takahashi M, Kano M, Amaike Y, Ishii C, Maeda K, et al. (2017). Activation of nuclear receptor CAR by an environmental pollutant perfluorooctanoic acid. *Arch Toxicol*. 91(6):2365–74. doi:10.1007/s00204-016-1888-3 PMID:27832320

Abraham K, Mielke H, Fromme H, Völkel W, Menzel J, Peiser M, et al. (2020). Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. *Arch Toxicol*. 94(6):2131–47. doi:10.1007/s00204-020-02715-4 PMID:32227269

Abraham K, Monien BH (2022). Transdermal absorption of $^{13}C_4$-perfluorooctanoic acid ($^{13}C_4$-PFOA) from a sunscreen in a male volunteer – what could be the contribution of cosmetics to the internal exposure of perfluoroalkyl substances (PFAS)? *Environ Int*. 169:107549. doi:10.1016/j.envint.2022.107549 PMID:36191486

Abudayyak M, Öztaş E, Özhan G (2021a). Assessment of perfluorooctanoic acid toxicity in pancreatic cells. *Toxicol In Vitro*. 72:105077. doi:10.1016/j.tiv.2021.105077 PMID:33422635

Abudayyak M, Öztaş E, Özhan G (2021b). Determination of perflourooctanoic acid toxicity in a human hepatocarcinoma cell line. *J Health Pollut*. 11(31):210909. doi:10.5696/2156-9614-11.31.210909 PMID:34434601

Ahmad S, Wen Y, Irudayaraj JMK (2021). PFOA induces alteration in DNA methylation regulators and SARS-CoV-2 targets *Ace2* and *Tmprss2* in mouse lung tissues. *Toxicol Rep*. 8:1892–8. doi:10.1016/j.toxrep.2021.11.014 PMID:34853776

Aimuzi R, Luo K, Chen Q, Wang H, Feng L, Ouyang F, et al. (2019). Perfluoroalkyl and polyfluoroalkyl substances and fetal thyroid hormone levels in umbilical cord blood among newborns by prelabor caesarean delivery. *Environ Int*. 130:104929. doi:10.1016/j.envint.2019.104929 PMID:31228788

Aimuzi R, Luo K, Huang R, Huo X, Nian M, Ouyang F, et al.; Shanghai Birth Cohort Study (2020). Perfluoroalkyl and polyfluroalkyl substances and maternal thyroid hormones in early pregnancy. *Environ Pollut*. 264:114557. doi:10.1016/j.envpol.2020.114557 PMID:32388293

Ait Bamai Y, Goudarzi H, Araki A, Okada E, Kashino I, Miyashita C, et al. (2020). Effect of prenatal exposure to per- and polyfluoroalkyl substances on childhood allergies and common infectious diseases in children up to age 7 years: the Hokkaido study on environment and children's health. *Environ Int*. 143:105979. doi:10.1016/j.envint.2020.105979 PMID:32717646

Alam MN, Han X, Nan B, Liu L, Tian M, Shen H, et al. (2021). Chronic low-level perfluorooctane sulfonate (PFOS) exposure promotes testicular steroidogenesis through enhanced histone acetylation. *Environ Pollut*. 284:117518. doi:10.1016/j.envpol.2021.117518 PMID:34261222

Albrecht PP, Torsell NE, Krishnan P, Ehresman DJ, Frame SR, Chang SC, et al. (2013). A species difference in the peroxisome proliferator-activated receptor α-dependent response to the developmental effects of perfluorooctanoic acid. *Toxicol Sci*. 131(2):568–82. doi:10.1093/toxsci/kfs318 PMID:23143925

Alharthy SA, Hardej D (2021). The role of transcription factor Nrf2 in the toxicity of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in C57BL/6 mouse astrocytes. *Environ Toxicol Pharmacol*. 86:103652. doi:10.1016/j.etap.2021.103652 PMID:33812015

Alsarra IA, Brockmann WG, Cunningham ML, Badr MZ (2006). Hepatocellular proliferation in response to agonists of peroxisome proliferator-activated receptor alpha: a role for Kupffer cells? *J Carcinog*. 5(1):26. doi:10.1186/1477-3163-5-26 PMID:17129391

Ambroz A, Rossner P Jr, Rossnerova A, Honkova K, Milcova A, Pastorkova A, et al. (2022). Oxidative stress and antioxidant response in populations of the Czech Republic exposed to various levels of environmental pollutants. *Int J Environ Res Public Health*. 19(6):3609. doi:10.3390/ijerph19063609 PMID:35329296

Amstutz VH, Cengo A, Gehres F, Sijm DTHM, Vrolijk MF (2022). Investigating the cytotoxicity of per- and polyfluoroalkyl substances in HepG2 cells: a structure–activity relationship approach. *Toxicology*. 480:153312. doi:10.1016/j.tox.2022.153312 PMID:36075290

Andersen ME, Mallick P, Clewell HJ 3rd, Yoon M, Olsen GW, Longnecker MP (2021). Using quantitative modeling tools to assess pharmacokinetic bias in epidemiological studies showing associations between biomarkers and health outcomes at low exposures. *Environ Res*. 197:111183. doi:10.1016/j.envres.2021.111183 PMID:33887277

Andersson AG, Lundgren A, Xu Y, Nielsen C, Lindh CH, Pineda D, et al. (2023). High exposure to perfluoroalkyl substances and antibody responses to SARS-CoV-2 mRNA vaccine – an observational study in adults from Ronneby, Sweden. *Environ Health Perspect*. 131(8):87007. doi:10.1289/EHP11847 PMID:37578904

Arbuckle TE, MacPherson S, Foster WG, Sathyanarayana S, Fisher M, Monnier P, et al. (2020). Prenatal perfluoroalkyl substances and newborn anogenital distance in a Canadian cohort. *Reprod Toxicol*. 94:31–9. doi:10.1016/j.reprotox.2020.03.011 PMID:32283250

Assali NS, Brinkman CR 3rd (1972). Pathophysiology of gestation. Volume I: maternal disorders. Cambridge (MA), USA: Academic Press; p.278.

Attema B, Janssen AWF, Rijkers D, van Schothorst EM, Hooiveld GJEJ, Kersten S (2022). Exposure to low-dose perfluorooctanoic acid promotes hepatic steatosis and disrupts the hepatic transcriptome in mice. *Mol Metab*. 66:101602. doi:10.1016/j.molmet.2022.101602 PMID:36115532

Austin ME, Kasturi BS, Barber M, Kannan K, Mohan Kumar PS, Mohan Kumar SM (2003). Neuroendocrine effects of perfluorooctane sulfonate in rats. *Environ Health Perspect*. 111(12):1485–9. doi:10.1289/ehp.6128 PMID:12948888

Bach CC, Vested A, Jørgensen KT, Bonde JP, Henriksen TB, Toft G (2016). Perfluoroalkyl and polyfluoroalkyl substances and measures of human fertility: a systematic review. *Crit Rev Toxicol*. 46(9):735–55. doi:10.1080/10408444.2016.1182117 PMID:27268162

Badr MZ, Birnbaum LS (2004). Enhanced potential for oxidative stress in livers of senescent rats by the peroxisome proliferator-activated receptor alpha agonist perfluorooctanoic acid. *Mech Ageing Dev*. 125(1):69–75. doi:10.1016/j.mad.2003.10.006 PMID:14706239

Barrett ES, Chen C, Thurston SW, Haug LS, Sabaredzovic A, Fjeldheim FN, et al. (2015). Perfluoroalkyl substances and ovarian hormone concentrations in naturally cycling women. *Fertil Steril*. 103(5):1261–70. e3. doi:10.1016/j.fertnstert.2015.02.001 PMID:25747128

Bartell SM, Calafat AM, Lyu C, Kato K, Ryan PB, Steenland K (2010). Rate of decline in serum PFOA concentrations after granular activated carbon filtration at two public water systems in Ohio and West Virginia. *Environ Health Perspect*. 118(2):222–8. doi:10.1289/ehp.0901252 PMID:20123620

Barton KE, Zell-Baran LM, DeWitt JC, Brindley S, McDonough CA, Higgins CP, et al. (2022). Cross-sectional associations between serum PFASs and inflammatory biomarkers in a population exposed to AFFF-contaminated drinking water. *Int J Hyg Environ Health*. 240:113905. doi:10.1016/j.ijheh.2021.113905 PMID:35065522

Basini G, Bussolati S, Torcianti V, Grasselli F (2022). Perfluorooctanoic acid (PFOA) induces redox status disruption in swine granulosa cells. *Vet Sci*. 9(6):254. doi:10.3390/vetsci9060254 PMID:35737306

Bassler J, Ducatman A, Elliott M, Wen S, Wahlang B, Barnett J, et al. (2019). Environmental perfluoroalkyl acid exposures are associated with liver disease characterized by apoptosis and altered serum adipocytokines. *Environ Pollut*. 247(April):1055–63. doi:10.1016/j.envpol.2019.01.064 PMID:30823334

Bastos Sales L, Kamstra JH, Cenijn PH, van Rijt LS, Hamers T, Legler J (2013). Effects of endocrine disrupting chemicals on in vitro global DNA methylation and adipocyte differentiation. *Toxicol In Vitro*. 27(6):1634–43. doi:10.1016/j.tiv.2013.04.005 PMID:23603478

Beesoon S, Martin JW (2015). Isomer-specific binding affinity of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) to serum proteins. *Environ Sci Technol*. 49(9):5722–31. doi:10.1021/es505399w PMID:25826685

Beesoon S, Webster GM, Shoeib M, Harner T, Benskin JP, Martin JW (2011). Isomer profiles of perfluorochemicals in matched maternal, cord, and house dust samples: manufacturing sources and transplacental transfer. *Environ Health Perspect*. 119(11):1659–64. doi:10.1289/ehp.1003265 PMID:21757419

Behnisch PA, Besselink H, Weber R, Willand W, Huang J, Brouwer A (2021). Developing potency factors for thyroid hormone disruption by PFASs using TTR-TRβ CALUX® bioassay and assessment of PFASs mixtures in technical products. *Environ Int*. 157:106791. doi:10.1016/j.envint.2021.106791 PMID:34364217

Behr A-C, Kwiatkowski A, Ståhlman M, Schmidt FF, Luckert C, Braeuning A, et al. (2020a). Impairment of bile acid metabolism by perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in human HepaRG hepatoma cells. *Arch Toxicol*. 94(5):1673–86. doi:10.1007/s00204-020-02732-3 PMID:32253466

Behr AC, Lichtenstein D, Braeuning A, Lampen A, Buhrke T (2018). Perfluoroalkylated substances (PFAS) affect neither estrogen and androgen receptor activity nor steroidogenesis in human cells in vitro. *Toxicol Lett*. 291:51–60. doi:10.1016/j.toxlet.2018.03.029 PMID:29601859

Behr AC, Plinsch C, Braeuning A, Buhrke T (2020b). Activation of human nuclear receptors by perfluoroalkylated substances (PFAS). *Toxicol In Vitro*. 62:104700. doi:10.1016/j.tiv.2019.104700 PMID:31676336

Benninghoff AD, Bisson WH, Koch DC, Ehresman DJ, Kolluri SK, Williams DE (2011). Estrogen-like activity of perfluoroalkyl acids in vivo and interaction with human and rainbow trout estrogen receptors in vitro. *Toxicol Sci*. 120(1):42–58. doi:10.1093/toxsci/kfq379 PMID:21163906

Benskin JP, De Silva AO, Martin LJ, Arsenault G, McCrindle R, Riddell N, et al. (2009). Disposition of perfluorinated acid isomers in Sprague-Dawley rats; part 1: single dose. *Environ Toxicol Chem*. 28(3):542–54. doi:10.1897/08-239.1 PMID:18939892

Berg V, Nøst TH, Hansen S, Elverland A, Veyhe A-S, Jorde R, et al. (2015). Assessing the relationship between perfluoroalkyl substances, thyroid hormones and binding proteins in pregnant women; a longitudinal mixed effects approach. *Environ Int*. 77:63–9. doi:10.1016/j.envint.2015.01.007 PMID:25647630

Bernstein AS, Kapraun DF, Schlosser PM (2021). A model template approach for rapid evaluation and application of physiologically based pharmacokinetic models for use in human health risk assessments: a case study on per- and polyfluoroalkyl substances.

Toxicol Sci. 182(2):215–28. doi:10.1093/toxsci/kfab063 PMID:34077538

Berntsen HF, Bjørklund CG, Audinot JN, Hofer T, Verhaegen S, Lentzen E, et al. (2017). Time-dependent effects of perfluorinated compounds on viability in cerebellar granule neurons: dependence on carbon chain length and functional group attached. *Neurotoxicology*. 63:70–83. doi:10.1016/j.neuro.2017.09.005 PMID:28919516

Bérubé R, LeFauve MK, Heldman S, Chiang Y-TT, Birbeck J, Westrick J, et al. (2023). Adipogenic and endocrine disrupting mixture effects of organic and inorganic pollutant mixtures. *Sci Total Environ*. 876:162587. doi:10.1016/j.scitotenv.2023.162587 PMID:36871739

Biegel LB, Hurtt ME, Frame SR, O'Connor JC, Cook JC (2001). Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. *Toxicol Sci*. 60(1):44–55. doi:10.1093/toxsci/60.1.44 PMID:11222872

Bijland S, Rensen PC, Pieterman EJ, Maas AC, van der Hoorn JW, van Erk MJ, et al. (2011). Perfluoroalkyl sulfonates cause alkyl chain length-dependent hepatic steatosis and hypolipidemia mainly by impairing lipoprotein production in APOE*3-Leiden CETP mice. *Toxicol Sci*. 123(1):290–303. doi:10.1093/toxsci/kfr142 PMID:21705711

Bjork JA, Butenhoff JL, Wallace KB (2011). Multiplicity of nuclear receptor activation by PFOA and PFOS in primary human and rodent hepatocytes. *Toxicology*. 288(1–3):8–17. doi:10.1016/j.tox.2011.06.012 PMID:21723365

Bjork JA, Lau C, Chang SC, Butenhoff JL, Wallace KB (2008). Perfluorooctane sulfonate-induced changes in fetal rat liver gene expression. *Toxicology*. 251(1–3):8–20. doi:10.1016/j.tox.2008.06.007 PMID:18692542

Bjork JA, Wallace KB (2009). Structure–activity relationships and human relevance for perfluoroalkyl acid-induced transcriptional activation of peroxisome proliferation in liver cell cultures. *Toxicol Sci*. 111(1):89–99. doi:10.1093/toxsci/kfp093 PMID:19407336

Blake BE, Cope HA, Hall SM, Keys RD, Mahler BW, McCord J, et al. (2020). Evaluation of maternal, embryo, and placental effects in CD-1 mice following gestational exposure to perfluorooctanoic acid (PFOA) or hexafluoropropylene oxide dimer acid (HFPO-DA or GenX). *Environ Health Perspect*. 128(2):27006. doi:10.1289/EHP6233 PMID:32074459

Blake BE, Pinney SM, Hines EP, Fenton SE, Ferguson KK (2018). Associations between longitudinal serum perfluoroalkyl substance (PFAS) levels and measures of thyroid hormone, kidney function, and body mass index in the Fernald Community Cohort. *Environ Pollut*. 242(Pt A):894–904. doi:10.1016/j.envpol.2018.07.042 PMID:30373035

Blanc M, Rüegg J, Scherbak N, Keiter SH (2019). Environmental chemicals differentially affect epigenetic-related mechanisms in the zebrafish liver (ZF-L) cell line and in zebrafish embryos. *Aquat Toxicol*. 215:105272. doi:10.1016/j.aquatox.2019.105272 PMID:31442592

Bloom MS, Kannan K, Spliethoff HM, Tao L, Aldous KM, Vena JE (2010). Exploratory assessment of perfluorinated compounds and human thyroid function. *Physiol Behav*. 99(2):240–5. doi:10.1016/j.physbeh.2009.02.005 PMID:19419669

Bogan AA, Dallas-Yang Q, Ruse MDJ Jr, Maeda Y, Jiang G, Nepomuceno L, et al. (2000). Analysis of protein dimerization and ligand binding of orphan receptor HNF4alpha. *J Mol Biol*. 302(4):831–51. doi:10.1006/jmbi.2000.4099 PMID:10993727

Bogdanska J, Borg D, Sundström M, Bergström U, Halldin K, Abedi-Valugerdi M, et al. (2011). Tissue distribution of ^{35}S-labelled perfluorooctane sulfonate in adult mice after oral exposure to a low environmentally relevant dose or a high experimental dose. *Toxicology*. 284(1–3):54–62. doi:10.1016/j.tox.2011.03.014 PMID:21459123

Bonzo JA, Ferry CH, Matsubara T, Kim JH, Gonzalez FJ (2012). Suppression of hepatocyte proliferation by hepatocyte nuclear factor 4α in adult mice. *J Biol Chem*. 287(10):7345–56. doi:10.1074/jbc.M111.334599 PMID:22241473

Brede E, Wilhelm M, Göen T, Müller J, Rauchfuss K, Kraft M, et al. (2010). Two-year follow-up biomonitoring pilot study of residents' and controls' PFC plasma levels after PFOA reduction in public water system in Arnsberg, Germany. *Int J Hyg Environ Health*. 213(3):217–23. doi:10.1016/j.ijheh.2010.03.007 PMID:20488749

Brieger A, Bienefeld N, Hasan R, Goerlich R, Haase H (2011). Impact of perfluorooctanesulfonate and perfluorooctanoic acid on human peripheral leukocytes. *Toxicol In Vitro*. 25(4):960–8. doi:10.1016/j.tiv.2011.03.005 PMID:21397682

Broad Institute (2023). MSigDB. Molecular Signatures Database. USA: Broad Institute, Massachusetts Institute of Technology, and Regents of the University of California. Available from: https://www.gsea-msigdb.org/gsea/msigdb/index.jsp.

Broedbaek K, Siersma V, Henriksen T, Weimann A, Petersen M, Andersen JT, et al. (2015). Urinary markers of nucleic acid oxidation and cancer in type 2 diabetes. *Redox Biol*. 4:34–9. doi:10.1016/j.redox.2014.11.010 PMID:25498965

Buhrke T, Kibellus A, Lampen A (2013). In vitro toxicological characterization of perfluorinated carboxylic acids with different carbon chain lengths. *Toxicol Lett*. 218(2):97–104. doi:10.1016/j.toxlet.2013.01.025 PMID:23391484

Buhrke T, Krüger E, Pevny S, Rößler M, Bitter K, Lampen A (2015). Perfluorooctanoic acid (PFOA) affects distinct molecular signalling pathways in human primary

hepatocytes. *Toxicology*. 333:53–62. doi:10.1016/j.tox.2015.04.004 PMID:25868421

Butenhoff J, Costa G, Elcombe C, Farrar D, Hansen K, Iwai H, et al. (2002). Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. *Toxicol Sci*. 69(1):244–57. doi:10.1093/toxsci/69.1.244 PMID:12215680

Butenhoff JL, Chang SC, Olsen GW, Thomford PJ (2012b). Chronic dietary toxicity and carcinogenicity study with potassium perfluorooctanesulfonate in Sprague-Dawley rats. *Toxicology*. 293(1–3):1–15. doi:10.1016/j.tox.2012.01.003 PMID:22266392

Butenhoff JL, Kennedy GL Jr, Chang SC, Olsen GW (2012a). Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats. *Toxicology*. 298(1–3):1–13. doi:10.1016/j.tox.2012.04.001 PMID:22531602

Butenhoff JL, Kennedy GL Jr, Hinderliter PM, Lieder PH, Jung R, Hansen KJ, et al. (2004). Pharmacokinetics of perfluorooctanoate in cynomolgus monkeys. *Toxicol Sci*. 82(2):394–406. doi:10.1093/toxsci/kfh302 PMID:15470233

Butenhoff JL, Kennedy GL, Jung R, Chang SC (2014). Evaluation of perfluorooctanoate for potential genotoxicity. *Toxicol Rep*. 1:252–70. doi:10.1016/j.toxrep.2014.05.012 PMID:28962243

Byrne SC, Miller P, Seguinot-Medina S, Waghiyi V, Buck CL, von Hippel FA, et al. (2018). Exposure to perfluoroalkyl substances and associations with serum thyroid hormones in a remote population of Alaska natives. *Environ Res*. 166:537–43. doi:10.1016/j.envres.2018.06.014 PMID:29958161

Cadiou S, Bustamante M, Agier L, Andrusaityte S, Basagaña X, Carracedo A, et al. (2020). Using methylome data to inform exposome–health association studies: an application to the identification of environmental drivers of child body mass index. *Environ Int*. 138:105622. doi:10.1016/j.envint.2020.105622 PMID:32179316

Caron-Beaudoin É, Ayotte P, Laouan Sidi EA, Gros-Louis McHugh N, Lemire M; Community of Lac Simon; Community of Winneway–Long Point First Nation; CSSS Tshukuminu Kanani of Nutashkuan; Community of Unamen Shipu (2019). Exposure to perfluoroalkyl substances (PFAS) and associations with thyroid parameters in First Nation children and youth from Quebec. *Environ Int*. 128:13–23. doi:10.1016/j.envint.2019.04.029 PMID:31029975

Casals-Casas C, Desvergne B (2011). Endocrine disruptors: from endocrine to metabolic disruption. *Annu Rev Physiol*. 73(1):135–62. doi:10.1146/annurev-physiol-012110-142200 PMID:21054169

Caserta D, Ciardo F, Bordi G, Guerranti C, Fanello E, Perra G, et al. (2013). Correlation of endocrine disrupting chemicals serum levels and white blood cells gene expression of nuclear receptors in a population of infertile women. *Int J Endocrinol*. 2013:510703. doi:10.1155/2013/510703 PMID:23710174

Caverly Rae JM, Frame SR, Kennedy GL, Butenhoff JL, Chang SC (2014). Pathology review of proliferative lesions of the exocrine pancreas in two chronic feeding studies in rats with ammonium perfluorooctanoate. *Toxicol Rep*. 1:85–91. doi:10.1016/j.toxrep.2014.04.005 PMID:28962229

Çelik A, Eke D, Ekinci SY, Yıldırım S (2013). The protective role of curcumin on perfluorooctane sulfonate-induced genotoxicity: single cell gel electrophoresis and micronucleus test. *Food Chem Toxicol*. 53:249–55. doi:10.1016/j.fct.2012.11.054 PMID:23246701

Chang CJ, Barr DB, Ryan PB, Panuwet P, Smarr MM, Liu K, et al. (2022). Per- and polyfluoroalkyl substance (PFAS) exposure, maternal metabolomic perturbation, and fetal growth in African American women: a meet-in-the-middle approach. *Environ Int*. 158:106964. doi:10.1016/j.envint.2021.106964 PMID:34735953

Chang CJ, Barr DB, Zhang Q, Dunlop AL, Smarr MM, Kannan K, et al. (2021). Associations of single and multiple per- and polyfluoroalkyl substance (PFAS) exposure with vitamin D biomarkers in African American women during pregnancy. *Environ Res*. 202:111713. doi:10.1016/j.envres.2021.111713 PMID:34284018

Chang S, Allen BC, Andres KL, Ehresman DJ, Falvo R, Provencher A, et al. (2017). Evaluation of serum lipid, thyroid, and hepatic clinical chemistries in association with serum perfluorooctanesulfonate (PFOS) in cynomolgus monkeys after oral dosing with potassium PFOS. *Toxicol Sci*. 156(2):387–401. doi:10.1093/toxsci/kfw267 PMID:28115654

Chang S-C, Ehresman DJ, Bjork JA, Wallace KB, Parker GA, Stump DG, et al. (2009). Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+PFOS) in rats: toxicokinetics, thyroid hormone status, and related gene expression. *Reprod Toxicol*. 27(3–4):387–99. doi:10.1016/j.reprotox.2009.01.005 PMID:19429409

Chang S-C, Noker PE, Gorman GS, Gibson SJ, Hart JA, Ehresman DJ, et al. (2012). Comparative pharmacokinetics of perfluorooctanesulfonate (PFOS) in rats, mice, and monkeys. *Reprod Toxicol*. 33(4):428–40. doi:10.1016/j.reprotox.2011.07.002 PMID:21889587

Chang SC, Thibodeaux JR, Eastvold ML, Ehresman DJ, Bjork JA, Froehlich JW, et al. (2008). Thyroid hormone status and pituitary function in adult rats given oral doses of perfluorooctanesulfonate (PFOS). *Toxicology*. 243(3):330–9. doi:10.1016/j.tox.2007.10.014 PMID:18063289

Chao MR, Evans MD, Hu CW, Ji Y, Møller P, Rossner P, et al. (2021). Biomarkers of nucleic acid oxidation – a summary state-of-the-art. *Redox Biol*. 42:101872. doi:10.1016/j.redox.2021.101872 PMID:33579665

Charazac A, Hinault C, Dolfi B, Hautier S, Decondé Le Butor C, Bost F, et al. (2022). Low doses of PFOA promote prostate and breast cancer cells growth through different pathways. *Int J Mol Sci*. 23(14):7900. doi:10.3390/ijms23147900 PMID:35887249

Chen G, Xu LL, Huang YF, Wang Q, Wang BH, Yu ZH, et al. (2018). Prenatal exposure to perfluorooctane sulfonate impairs placental angiogenesis and induces aberrant expression of LncRNA xist. *Biomed Environ Sci*. 31(11):843–7. PMID:30558704

Chen J, Miao Y, Gao Q, Cui Z, Xiong B (2021). Exposure to perfluorooctane sulfonate in vitro perturbs the quality of porcine oocytes via induction of apoptosis. *Environ Pollut*. 284:117508. doi:10.1016/j.envpol.2021.117508 PMID:34261219

Chen JC, Baumert BO, Li Y, Li Y, Pan S, Robinson S, et al. (2023a). Associations of per- and polyfluoroalkyl substances, polychlorinated biphenyls, organochlorine pesticides, and polybrominated diphenyl ethers with oxidative stress markers: a systematic review and meta-analysis. *Environ Res*. 239(Pt 1):117308. doi:10.1016/j.envres.2023.117308 PMID:37813138

Chen L, Zhu M, Liu Y, Yang Z, Li H, Mu H, et al. (2023b). Perfluorobutanesulfonate exposure induces metabolic disturbances in different regions of mouse gut. *Sci Total Environ*. 866:161261. doi:10.1016/j.scitotenv.2022.161261 PMID:36587682

Chen Q, Huang R, Hua L, Guo Y, Huang L, Zhao Y, et al. (2018a). Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and childhood atopic dermatitis: a prospective birth cohort study. *Environ Health*. 17(1):8. doi:10.1186/s12940-018-0352-7 PMID:29343261

Chen T, Zhang L, Yue JQ, Lv ZQ, Xia W, Wan YJ, et al. (2012). Prenatal PFOS exposure induces oxidative stress and apoptosis in the lung of rat off-spring. *Reprod Toxicol*. 33(4):538–45. doi:10.1016/j.reprotox.2011.03.003 PMID:21440054

Chen X, Nie X, Mao J, Zhang Y, Yin K, Jiang S (2018b). Perfluorooctanesulfonate induces neuroinflammation through the secretion of TNF-α mediated by the JAK2/STAT3 pathway. *Neurotoxicology*. 66:32–42. doi:10.1016/j.neuro.2018.03.003 PMID:29526747

Chen Y, Hu W, Huang C, Hua S, Wei Q, Bai C, et al. (2016). Subchronic perfluorooctanesulfonate (PFOS) exposure induces elevated mutant frequency in an in vivo λ transgenic medaka mutation assay. *Sci Rep*. 6(1):38466. doi:10.1038/srep38466 PMID:27929129

Chen Y, Zhou L, Xu J, Zhang L, Li M, Xie X, et al. (2017). Maternal exposure to perfluorooctanoic acid inhibits luteal function via oxidative stress and apoptosis in pregnant mice. *Reprod Toxicol*. 69:159–66. doi:10.1016/j.reprotox.2017.02.010 PMID:28219760

Chen YM, Guo LH (2009). Fluorescence study on site-specific binding of perfluoroalkyl acids to human serum albumin. *Arch Toxicol*. 83(3):255–61. doi:10.1007/s00204-008-0359-x PMID:18854981

Cheng W, Yu Z, Feng L, Wang Y (2013). Perfluorooctane sulfonate (PFOS) induced embryotoxicity and disruption of cardiogenesis. *Toxicol In Vitro*. 27(5):1503–12. doi:10.1016/j.tiv.2013.03.014 PMID:23562911

Cheng X, Klaassen CD (2008). Perfluorocarboxylic acids induce cytochrome P450 enzymes in mouse liver through activation of PPAR-alpha and CAR transcription factors. *Toxicol Sci*. 106(1):29–36. doi:10.1093/toxsci/kfn147 PMID:18648086

Cheng X, Maher J, Lu H, Klaassen CD (2006). Endocrine regulation of gender-divergent mouse organic anion-transporting polypeptide (Oatp) expression. *Mol Pharmacol*. 70(4):1291–7. doi:10.1124/mol.106.025122 PMID:16807376

Cheng X, Wei Y, Zhang Z, Wang F, He J, Wang R, et al. (2022). Plasma PFOA and PFOS levels, DNA methylation, and blood lipid levels: a pilot study. *Environ Sci Technol*. 56(23):17039–51. doi:10.1021/acs.est.2c04107 PMID:36374530

Chiu WA, Lynch MT, Lay CR, Antezana A, Malek P, Sokolinski S, et al. (2022). Bayesian estimation of human population toxicokinetics of PFOA, PFOS, PFHxS, and PFNA from studies of contaminated drinking water. *Environ Health Perspect*. 130(12):127001. doi:10.1289/EHP10103 PMID:36454223

Choi EM, Suh KS, Rhee SY, Oh S, Woo JT, Kim SW, et al. (2016). Perfluorooctanoic acid induces mitochondrial dysfunction in MC3T3–E1 osteoblast cells. *J Environ Sci Health Part A Tox Hazard Subst Environ Eng*. 52(3):281–9. doi:10.1080/10934529.2016.1253402 PMID:27901621

Choi S-K, Kim J-H, Park J-K, Lee K-M, Kim E, Jeon WB (2013). Cytotoxicity and inhibition of intercellular interaction in N2a neurospheroids by perfluorooctanoic acid and perfluorooctanesulfonic acid. *Food Chem Toxicol*. 60(October):520–9. doi:10.1016/j.fct.2013.07.070 PMID:23948355

Chou HC, Wen LL, Chang CC, Lin CY, Jin L, Juan SH (2017). From the cover: l–carnitine via PPARγ- and sirt1-dependent mechanisms attenuates epithelial-mesenchymal transition and renal fibrosis caused by perfluorooctanesulfonate. *Toxicol Sci*. 160(2):217–29. doi:10.1093/toxsci/kfx183 PMID:28973641

Chou W-C, Lin Z (2019). Bayesian evaluation of a physiologically based pharmacokinetic (PBPK) model for perfluorooctane sulfonate (PFOS) to characterize the interspecies uncertainty between mice, rats, monkeys, and humans: development and performance verification. *Environ Int*. 129:408–22. doi:10.1016/j.envint.2019.03.058 PMID:31152982

Christensen BT, Calkins MM (2023). Occupational exposure to per- and polyfluoroalkyl substances: a scope review of the literature from 1980–2021. *J Expo Sci Environ Epidemiol*. 33(5):673–86. doi:10.1038/s41370-023-00536-y PMID:36977833

Clarity C, Trowbridge J, Gerona R, Ona K, McMaster M, Bessonneau V, et al. (2021). Associations between polyfluoroalkyl substance and organophosphate flame retardant exposures and telomere length in a cohort of women firefighters and office workers in San Francisco. *Environ Health*. 20(1):97. doi:10.1186/s12940-021-00778-z PMID:34454526

Clark KL, George JW, Hua G, Davis JS (2022). Perfluorooctanoic acid promotes proliferation of the human granulosa cell line HGrC1 and alters expression of cell cycle genes and Hippo pathway effector YAP1. *Reprod Toxicol*. 110(June):49–59. doi:10.1016/j.reprotox.2022.03.011 PMID:35346789

Conley JM, Lambright CS, Evans N, Medlock-Kakaley E, Hill D, McCord J, et al. (2022). Developmental toxicity of Nafion byproduct 2 (NBP2) in the Sprague-Dawley rat with comparisons to hexafluoropropylene oxide-dimer acid (HFPO-DA or GenX) and perfluorooctane sulfonate (PFOS). *Environ Int*. 160:107056. doi:10.1016/j.envint.2021.107056 PMID:34952357

Convertino M, Church TR, Olsen GW, Liu Y, Doyle E, Elcombe CR, et al. (2018). Stochastic pharmacokinetic-pharmacodynamic modeling for assessing the systemic health risk of perfluorooctanoate (PFOA). *Toxicol Sci*. 163(1):293–306. doi:10.1093/toxsci/kfy035 PMID:29462473

Coperchini F, Croce L, Pignatti P, Ricci G, Gangemi D, Magri F, et al. (2021b). The new generation PFAS C6O4 does not produce adverse effects on thyroid cells in vitro. *J Endocrinol Invest*. 44(8):1625–35. doi:10.1007/s40618-020-01466-4 PMID:33315184

Coperchini F, Croce L, Ricci G, Magri F, Rotondi M, Imbriani M, et al. (2021a). Thyroid disrupting effects of old and new generation PFAS. *Front Endocrinol (Lausanne)*. 11:612320. doi:10.3389/fendo.2020.612320 PMID:33542707

Coperchini F, Pignatti P, Lacerenza S, Negri S, Sideri R, Testoni C, et al. (2015). Exposure to perfluorinated compounds: in vitro study on thyroid cells. *Environ Sci Pollut Res Int*. 22(3):2287–94. doi:10.1007/s11356-014-3480-9 PMID:25182428

Corsini E, Avogadro A, Galbiati V, dell'Agli M, Marinovich M, Galli CL, et al. (2011). In vitro evaluation of the immunotoxic potential of perfluorinated compounds (PFCs). *Toxicol Appl Pharmacol*. 250(2):108–16. doi:10.1016/j.taap.2010.11.004 PMID:21075133

Corsini E, Sangiovanni E, Avogadro A, Galbiati V, Viviani B, Marinovich M, et al. (2012). In vitro characterization of the immunotoxic potential of several perfluorinated compounds (PFCs). *Toxicol Appl Pharmacol*. 258(2):248–55. doi:10.1016/j.taap.2011.11.004 PMID:22119708

Corton JC, Peters JM, Klaunig JE (2018). The PPARα-dependent rodent liver tumor response is not relevant to humans: addressing misconceptions. *Arch Toxicol*. 92(1):83–119. doi:10.1007/s00204-017-2094-7 PMID:29197930

Costa G, Sartori S, Consonni D (2009). Thirty years of medical surveillance in perfluorooctanoic acid production workers. *J Occup Environ Med*. 51(3):364–72. doi:10.1097/JOM.0b013e3181965d80 PMID:19225424

Costello E, Rock S, Stratakis N, Eckel SP, Walker DI, Valvi D, et al. (2022). Exposure to per- and polyfluoroalkyl substances and markers of liver injury: a systematic review and meta-analysis. *Environ Health Perspect*. 130(4):46001. doi:10.1289/EHP10092 PMID:35475652

Crawford L, Halperin SA, Dzierlenga MW, Skidmore B, Linakis MW, Nakagawa S, et al. (2023). Systematic review and meta-analysis of epidemiologic data on vaccine response in relation to exposure to five principal perfluoroalkyl substances. *Environ Int*. 172:107734. doi:10.1016/j.envint.2023.107734 PMID:36764183

Crawford NM, Fenton SE, Strynar M, Hines EP, Pritchard DA, Steiner AZ (2017). Effects of perfluorinated chemicals on thyroid function, markers of ovarian reserve, and natural fertility. *Reprod Toxicol*. 69:53–9. doi:10.1016/j.reprotox.2017.01.006 PMID:28111093

Crebelli R, Caiola S, Conti L, Cordelli E, De Luca G, Dellatte E, et al. (2019). Can sustained exposure to PFAS trigger a genotoxic response? A comprehensive genotoxicity assessment in mice after subacute oral administration of PFOA and PFBA. *Regul Toxicol Pharmacol*. 106:169–77. doi:10.1016/j.yrtph.2019.05.005 PMID:31071380

Croce L, Coperchini F, Tonacchera M, Imbriani M, Rotondi M, Chiovato L (2019). Effect of long- and short-chain perfluorinated compounds on cultured thyroid cells viability and response to TSH. *J Endocrinol Invest*. 42(11):1329–35. doi:10.1007/s40618-019-01062-1 PMID:31102255

Cui J, Wang P, Yan S, Liang Y, Liu D, Ren S (2022). Perfluorooctane sulfonate induces dysfunction of human umbilical vein endothelial cells via ferroptosis pathway. *Toxics*. 10(9):503. doi:10.3390/toxics10090503 PMID:36136468

Cui L, Liao CY, Zhou QF, Xia TM, Yun ZJ, Jiang GB (2010). Excretion of PFOA and PFOS in male rats during a subchronic exposure. *Arch Environ Contam Toxicol*. 58(1):205–13. doi:10.1007/s00244-009-9336-5 PMID:19468665

Cui L, Zhou QF, Liao CY, Fu JJ, Jiang GB (2009). Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis. *Arch Environ Contam Toxicol*. 56(2):338–49. doi:10.1007/s00244-008-9194-6 PMID:18661093

Cui Q, Pan Y, Wang J, Liu H, Yao B, Dai J (2020). Exposure to per- and polyfluoroalkyl substances (PFASs) in serum versus semen and their association with male reproductive hormones. *Environ Pollut*. 266(Pt 2):115330. doi:10.1016/j.envpol.2020.115330 PMID:32781340

Cui R, Li C, Wang J, Dai J (2019). Induction of hepatic miR-34a by perfluorooctanoic acid regulates metabolism-related genes in mice. *Environ Pollut*. 244:270–8. doi:10.1016/j.envpol.2018.10.061 PMID:30342367

D'Amico R, Gugliandolo E, Cordaro M, Fusco R, Genovese T, Peritore AF, et al. (2022). Toxic effects of endocrine disruptor exposure on collagen-induced arthritis. *Biomolecules*. 12(4):12. doi:10.3390/biom12040564 PMID:35454153

D'eon JC, Mabury SA (2011). Is indirect exposure a significant contributor to the burden of perfluorinated acids observed in humans? *Environ Sci Technol*. 45(19):7974–84. doi:10.1021/es200171y PMID:21630688

D'Eon JC, Simpson AJ, Kumar R, Baer AJ, Mabury SA (2010). Determining the molecular interactions of perfluorinated carboxylic acids with human sera and isolated human serum albumin using nuclear magnetic resonance spectroscopy. *Environ Toxicol Chem*. 29(8):1678–88. doi:10.1002/etc.204 PMID:20821619

Dallaire R, Dewailly E, Pereg D, Dery S, Ayotte P (2009). Thyroid function and plasma concentrations of polyhalogenated compounds in Inuit adults. *Environ Health Perspect*. 117(9):1380–6. doi:10.1289/ehp.0900633 PMID:19750101

Dalsager L, Christensen N, Halekoh U, Timmermann CAG, Nielsen F, Kyhl HB, et al. (2021). Exposure to perfluoroalkyl substances during fetal life and hospitalization for infectious disease in childhood: a study among 1503 children from the Odense Child Cohort. *Environ Int*. 149:106395. doi:10.1016/j.envint.2021.106395 PMID:33508532

Davidsen N, Ramhøj L, Lykkebo CA, Kugathas I, Poulsen R, Rosenmai AK, et al. (2022). PFOS-induced thyroid hormone system disrupted rats display organ-specific changes in their transcriptomes. *Environ Pollut*. 305:119340. doi:10.1016/j.envpol.2022.119340 PMID:35460815

de Cock M, de Boer MR, Lamoree M, Legler J, van de Bor M (2014). Prenatal exposure to endocrine disrupting chemicals in relation to thyroid hormone levels in infants – a Dutch prospective cohort study. *Environ Health*. 13(1):106. doi:10.1186/1476-069X-13-106 PMID:25495114

De Guise S, Levin M (2021). Suppression of Th2 cytokines as a potential mechanism for reduced antibody response following PFOA exposure in female B6C3F$_1$ mice. *Toxicol Lett*. 351:155–62. doi:10.1016/j.toxlet.2021.09.002 PMID:34517056

De Silva AO, Benskin JP, Martin LJ, Arsenault G, McCrindle R, Riddell N, et al. (2009). Disposition of perfluorinated acid isomers in Sprague-Dawley rats; part 2: subchronic dose. *Environ Toxicol Chem*. 28(3):555–67. doi:10.1897/08-254.1 PMID:18939893

De Toni L, Di Nisio A, Rocca MS, Pedrucci F, Garolla A, Dall'Acqua S, et al. (2022). Comparative evaluation of the effects of legacy and new generation perfluoralkyl substances (PFAS) on thyroid cells *in vitro*. *Front Endocrinol (Lausanne)*. 13:915096. doi:10.3389/fendo.2022.915096 PMID:35813651

Derakhshan A, Kortenkamp A, Shu H, Broeren MAC, Lindh CH, Peeters RP, et al. (2022). Association of per- and polyfluoroalkyl substances with thyroid homeostasis during pregnancy in the SELMA study. *Environ Int*. 167:107420. doi:10.1016/j.envint.2022.107420 PMID:35870378

Dewitt JC, Copeland CB, Strynar MJ, Luebke RW (2008). Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. *Environ Health Perspect*. 116(5):644–50. doi:10.1289/ehp.10896 PMID:18470313

DeWitt JC, Williams WC, Creech NJ, Luebke RW (2016). Suppression of antigen-specific antibody responses in mice exposed to perfluorooctanoic acid: role of PPARα and T- and B-cell targeting. *J Immunotoxicol*. 13(1):38–45. doi:10.3109/1547691X.2014.996682 PMID:25594567

Dhingra R, Winquist A, Darrow LA, Klein M, Steenland K (2017). A study of reverse causation: examining the associations of perfluorooctanoic acid serum levels with two outcomes. *Environ Health Perspect*. 125(3):416–21. doi:10.1289/EHP273 PMID:27529882

Di Nisio A, Pannella M, Vogiatzis S, Sut S, Dall'Acqua S, Rocca MS, et al. (2022). Impairment of human dopaminergic neurons at different developmental stages by perfluoro-octanoic acid (PFOA) and differential human brain areas accumulation of perfluoroalkyl chemicals. *Environ Int*. 158:106982. doi:10.1016/j.envint.2021.106982 PMID:34781208

Di Nisio A, Sabovic I, Valente U, Tescari S, Rocca MS, Guidolin D, et al. (2019). Endocrine disruption of androgenic activity by perfluoroalkyl substances: clinical and experimental evidence. *J Clin Endocrinol Metab*. 104(4):1259–71. doi:10.1210/jc.2018-01855 PMID:30403786

Ding J, Dai Y, Zhang J, Wang Z, Zhang L, Xu S, et al. (2023). Associations of perfluoroalkyl substances with adipocytokines in umbilical cord serum: a mixtures approach. *Environ Res*. 216(Pt 3):114654. doi:10.1016/j.envres.2022.114654 PMID:36309220

Dong GH, Liu MM, Wang D, Zheng L, Liang ZF, Jin YH (2011). Sub-chronic effect of perfluorooctanesulfonate (PFOS) on the balance of type 1 and type 2 cytokine in adult C57BL6 mice. *Arch Toxicol*. 85(10):1235–44. doi:10.1007/s00204-011-0661-x PMID:21327619

Dong GH, Wang J, Zhang YH, Liu MM, Wang D, Zheng L, et al. (2012b). Induction of p53-mediated apoptosis in splenocytes and thymocytes of C57BL/6 mice exposed to perfluorooctane sulfonate (PFOS). *Toxicol Appl Pharmacol*. 264(2):292–9. doi:10.1016/j.taap.2012.08.010 PMID:22959464

Dong G-H, Zhang Y-H, Zheng L, Liang Z-F, Jin Y-H, He Q-C (2012a). Subchronic effects of perfluorooctanesulfonate exposure on inflammation in adult

male C57BL/6 mice. *Environ Toxicol*. 27(5):285–96. doi:10.1002/tox.20642 PMID:20737580

Dong GH, Zhang YH, Zheng L, Liu W, Jin YH, He QC (2009). Chronic effects of perfluorooctanesulfonate exposure on immunotoxicity in adult male C57BL/6 mice. *Arch Toxicol*. 83(9):805–15. doi:10.1007/s00204-009-0424-0 PMID:19343326

Dong H, Curran I, Williams A, Bondy G, Yauk CL, Wade MG (2016). Hepatic miRNA profiles and thyroid hormone homeostasis in rats exposed to dietary potassium perfluorooctanesulfonate (PFOS). *Environ Toxicol Pharmacol*. 41:201–10. doi:10.1016/j.etap.2015.12.009 PMID:26724606

Dong Z, Wang J, Qiu T, Wu J, An Y, Shi X, et al. (2022). Perfluorooctane sulfonate induces mitochondrial calcium overload and early hepatic insulin resistance via autophagy/detyrosinated alpha-tubulin-regulated IP3R2–VDAC1–MICU1 interaction. *Sci Total Environ*. 825:153933. doi:10.1016/j.scitotenv.2022.153933 PMID:35192817

Dos Santos RS, Medina-Gali RM, Babiloni-Chust I, Marroqui L, Nadal A (2022). In vitro assays to identify metabolism-disrupting chemicals with diabetogenic activity in a human pancreatic β-cell model. *Int J Mol Sci*. 23(9):5040. doi:10.3390/ijms23095040 PMID:35563431

Dourson ML, Gadagbui B, Onyema C, McGinnis PM, York RG (2019). Data derived extrapolation factors for developmental toxicity: a preliminary research case study with perfluorooctanoate (PFOA). *Regul Toxicol Pharmacol*. 108:104446. doi:10.1016/j.yrtph.2019.104446 PMID:31425727

Dreyer AF, Jensen RC, Glintborg D, Schmedes AV, Brandslund I, Nielsen F, et al. (2020). Perfluoroalkyl substance exposure early in pregnancy was negatively associated with late pregnancy cortisone levels. *J Clin Endocrinol Metab*. 105(8):dgaa292. doi:10.1210/clinem/dgaa292 PMID:32436946

Du G, Hu J, Huang H, Qin Y, Han X, Wu D, et al. (2013). Perfluorooctane sulfonate (PFOS) affects hormone receptor activity, steroidogenesis, and expression of endocrine-related genes in vitro and in vivo. *Environ Toxicol Chem*. 32(2):353–60. doi:10.1002/etc.2034 PMID:23074026

Du J, Wang S, You H, Jiang R, Zhuang C, Zhang X (2016). Developmental toxicity and DNA damage to zebrafish induced by perfluorooctane sulfonate in the presence of ZnO nanoparticles. *Environ Toxicol*. 31(3):360–71. doi:10.1002/tox.22050 PMID:25258305

Du Y, Cai Z, Zhou G, Liang W, Man Q, Wang W (2022). Perfluorooctanoic acid exposure increases both proliferation and apoptosis of human placental trophoblast cells mediated by ER stress-induced ROS or UPR pathways. *Ecotoxicol Environ Saf*. 236(May):113508. doi:10.1016/j.ecoenv.2022.113508 PMID:35427876

Du Y, Xu T, Luo D, Wang Y, Yin H, Liu C, et al. (2023). Perfluorooctane sulfonate-induced apoptosis in kidney cells by triggering the NOX4/ROS/JNK axis and antagonism of cannabidiol. *Environ Toxicol*. 38(7):1651–64. doi:10.1002/tox.23794 PMID:36988283

Dusek J, Skoda J, Holas O, Horvatova A, Smutny T, Linhartova L, et al. (2019). Stilbene compound trans-3,4,5,4′-tetramethoxystilbene, a potential anti-cancer drug, regulates constitutive androstane receptor (Car) target genes, but does not possess proliferative activity in mouse liver. *Toxicol Lett*. 313:1–10. doi:10.1016/j.toxlet.2019.05.024 PMID:31170421

Dzierlenga AL, Robinson VG, Waidyanatha S, DeVito MJ, Eifrid MA, Gibbs ST, et al. (2020). Toxicokinetics of perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA) and perfluorodecanoic acid (PFDA) in male and female Hsd:Sprague-Dawley SD rats following intravenous or gavage administration. *Xenobiotica*. 50(6):722–32. doi:10.1080/00498254.2019.1683776 PMID:31680603

EFSA (2018). Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA Panel on Contaminants in the Food Chain (CONTAM). *EFSA J*. 16(12): e05194. https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5194 PMID:32625773

EFSA (2020). Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA Panel on Contaminants in the Food Chain. *EFSA J*. 18(9):6223. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2020.6223 PMID:32994824

Eick SM, Goin DE, Cushing L, DeMicco E, Park JS, Wang Y, et al. (2021). Mixture effects of prenatal exposure to per- and polyfluoroalkyl substances and polybrominated diphenyl ethers on maternal and newborn telomere length. *Environ Health*. 20(1):76. doi:10.1186/s12940-021-00765-4 PMID:34193151

Eke D, Çelik A (2016). Curcumin prevents perfluorooctane sulfonate-induced genotoxicity and oxidative DNA damage in rat peripheral blood. *Drug Chem Toxicol*. 39(1):97–103. doi:10.3109/01480545.2015.1041601 PMID:25950456

Eke D, Çelik A, Yilmaz MB, Aras N, Kocatürk Sel S, Alptekin D (2017). Apoptotic gene expression profiles and DNA damage levels in rat liver treated with perfluorooctane sulfonate and protective role of curcumin. *Int J Biol Macromol*. 104(Pt A):515–20. doi:10.1016/j.ijbiomac.2017.06.075 PMID:28634058

Elcombe CR, Elcombe BM, Foster JR, Chang SC, Ehresman DJ, Butenhoff JL (2012b). Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats from dietary exposure to potassium perfluorooctanesulfonate results from increased expression of xenosensor nuclear receptors PPARα and CAR/PXR. *Toxicology*. 293(1–3):16–29. doi:10.1016/j.tox.2011.12.014 PMID:22245121

Elcombe CR, Elcombe BM, Foster JR, Chang SC, Ehresman DJ, Butenhoff JL (2012c). Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats from dietary exposure to potassium perfluorooctanesulfonate results from increased expression of xenosensor nuclear receptors PPARγ and CAR/PXR. *Reprod Toxicol*. 33(4):626. doi:10.1016/j.reprotox.2011.11.108 PMID:22245121

Elcombe CR, Elcombe BM, Foster JR, Chang SC, Ehresman DJ, Noker PE, et al. (2012a). Evaluation of hepatic and thyroid responses in male Sprague-Dawley rats for up to eighty-four days following seven days of dietary exposure to potassium perfluorooctanesulfonate. *Toxicology*. 293(1–3):30–40. doi:10.1016/j.tox.2011.12.015 PMID:22239858

Elcombe CR, Elcombe BM, Foster JR, Farrar DG, Jung R, Chang SC, et al. (2010). Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats following dietary exposure to ammonium perfluorooctanoate occurs through increased activation of the xenosensor nuclear receptors PPARα and CAR/PXR. *Arch Toxicol*. 84(10):787–98. doi:10.1007/s00204-010-0572-2 PMID:20614104

Elcombe CR, Wolf CR, Westwood AL (2013). Compositions comprising perfluorooctanoic acid. Pub. No. US 2013/0029928. US Patent Application Publication. Available from: https://tera.org/Alliance%20for%20Risk/Workshop%20XII/Prelim%20Case%20Refs/Elcombe%20et%20al.,%202013+%20copy.pdf, accessed January 2025.

Elumalai S, Karunakaran U, Won KC, Chung SM, Moon JS (2023). Perfluorooctane sulfonate-induced oxidative stress contributes to pancreatic β-cell apoptosis by inhibiting cyclic adenosine monophosphate pathway: prevention by pentoxifylline. *Environ Pollut*. 320:120959. doi:10.1016/j.envpol.2022.120959 PMID:36621715

Emerce E, Çetin Ö (2018). Genotoxicity assessment of perfluoroalkyl substances on human sperm. *Toxicol Ind Health*. 34(12):884–90. doi:10.1177/0748233718799191 PMID:30326792

Endirlik BU, Eken A, Canpınar H, Öztürk F, Gürbay A (2022). Perfluorooctanoic acid affects mouse brain and liver tissue through oxidative stress. *Arh Hig Rada Toksikol*. 73(2):148–57. doi:10.2478/aiht-2022-73-3629 PMID:35792765

Eriksen KT, Raaschou-Nielsen O, Sørensen M, Roursgaard M, Loft S, Møller P (2010). Genotoxic potential of the perfluorinated chemicals PFOA, PFOS, PFBS, PFNA and PFHxA in human HepG2 cells. *Mutat Res*. 700(1–2):39–43. doi:10.1016/j.mrgentox.2010.04.024 PMID:20451658

Escher P, Wahli W (2000). Peroxisome proliferator-activated receptors: insight into multiple cellular functions. *Mutat Res*. 448(2):121–38. doi:10.1016/S0027-5107(99)00231-6 PMID:10725467

ESCODD (European Standards Committee on Oxidative DNA Damage) (2002). Comparative analysis of baseline 8-oxo-7,8-dihydroguanine in mammalian cell DNA, by different methods in different laboratories: an approach to consensus. *Carcinogenesis*. 23(12):2129–33. doi:10.1093/carcin/23.12.2129 PMID:12507938

Estefanía González-Alvarez M, Severin A, Sayadi M, Keating AF (2022). PFOA-induced ovotoxicity differs between lean and obese mice with impacts on ovarian reproductive and DNA damage sensing and repair proteins. *Toxicol Sci*. 190(2):173–88. doi:10.1093/toxsci/kfac104 PMID:36214631

Etzel TM, Braun JM, Buckley JP (2019). Associations of serum perfluoroalkyl substance and vitamin D biomarker concentrations in NHANES, 2003–2010. *Int J Hyg Environ Health*. 222(2):262–9. doi:10.1016/j.ijheh.2018.11.003 PMID:30503928

Evans N, Conley JM, Cardon M, Hartig P, Medlock-Kakaley E, Gray LEJ Jr (2022). In vitro activity of a panel of per- and polyfluoroalkyl substances (PFAS), fatty acids, and pharmaceuticals in peroxisome proliferator-activated receptor (PPAR) alpha, PPAR gamma, and estrogen receptor assays. *Toxicol Appl Pharmacol*. 449:116136. doi:10.1016/j.taap.2022.116136 PMID:35752307

Fàbrega F, Kumar V, Schuhmacher M, Domingo JL, Nadal M (2014). PBPK modeling for PFOS and PFOA: validation with human experimental data. *Toxicol Lett*. 230(2):244–51. doi:10.1016/j.toxlet.2014.01.007 PMID:24440341

Fair PA, Driscoll E, Mollenhauer MA, Bradshaw SG, Yun SH, Kannan K, et al. (2011). Effects of environmentally-relevant levels of perfluorooctane sulfonate on clinical parameters and immunological functions in B6C3F1 mice. *J Immunotoxicol*. 8(1):17–29. doi:10.3109/1547691X.2010.527868 PMID:21261439

Feldstein AE, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ (2009). Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. *Hepatology*. 50(4):1072–8. doi:10.1002/hep.23050 PMID:19585618

Feng X, Wang X, Cao X, Xia Y, Zhou R, Chen L (2015). Chronic exposure of female mice to an environmental level of perfluorooctane sulfonate suppresses estrogen synthesis through reduced histone H3K14 acetylation of the StAR promoter leading to deficits in follicular development and ovulation. *Toxicol Sci*. 148(2):368–79. doi:10.1093/toxsci/kfv197 PMID:26358002

Fenton SE, Reiner JL, Nakayama SF, Delinsky AD, Stanko JP, Hines EP, et al. (2009). Analysis of PFOA in dosed CD-1 mice. Part 2. Disposition of PFOA in tissues and fluids from pregnant and lactating mice and their pups. *Reprod Toxicol*. 27(3–4):365–72. doi:10.1016/j.reprotox.2009.02.012 PMID:19429407

Fernández Freire P, Pérez Martin JM, Herrero O, Peropadre A, de la Peña E, Hazen MJ (2008). In vitro assessment of the cytotoxic and mutagenic potential of perfluorooctanoic acid. *Toxicol In Vitro*. 22(5):1228–33. doi:10.1016/j.tiv.2008.04.004 PMID:18499391

Filgo AJ, Quist EM, Hoenerhoff MJ, Brix AE, Kissling GE, Fenton SE (2015). Perfluorooctanoic acid (PFOA)-induced liver lesions in two strains of mice following developmental exposures: PPARα is not required. *Toxicol Pathol*. 43(4):558–68. doi:10.1177/0192623314558463 PMID:25398757

Fletcher T, Galloway TS, Melzer D, Holcroft P, Cipelli R, Pilling LC, et al. (2013). Associations between PFOA, PFOS and changes in the expression of genes involved in cholesterol metabolism in humans. *Environ Int*. 57–58:2–10. doi:10.1016/j.envint.2013.03.008 PMID:23624243

Florentin A, Deblonde T, Diguio N, Hautemaniere A, Hartemann P (2011). Impacts of two perfluorinated compounds (PFOS and PFOA) on human hepatoma cells: cytotoxicity but no genotoxicity? *Int J Hyg Environ Health*. 214(6):493–9. doi:10.1016/j.ijheh.2011.05.010 PMID:21676652

Forsthuber M, Kaiser AM, Granitzer S, Hassl I, Hengstschläger M, Stangl H, et al. (2020). Albumin is the major carrier protein for PFOS, PFOA, PFHxS, PFNA and PFDA in human plasma. *Environ Int*. 137:105324. doi:10.1016/j.envint.2019.105324 PMID:32109724

Franco ME, Sutherland GE, Fernandez-Luna MT, Lavado R (2020). Altered expression and activity of phase I and II biotransformation enzymes in human liver cells by perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS). *Toxicology*. 430:152339. doi:10.1016/j.tox.2019.152339 PMID:31809754

Franken C, Koppen G, Lambrechts N, Govarts E, Bruckers L, Den Hond E, et al. (2017). Environmental exposure to human carcinogens in teenagers and the association with DNA damage. *Environ Res*. 152:165–74. doi:10.1016/j.envres.2016.10.012 PMID:27771571

Franko J, Meade BJ, Frasch HF, Barbero AM, Anderson SE (2012). Dermal penetration potential of perfluorooctanoic acid (PFOA) in human and mouse skin. *J Toxicol Environ Health A*. 75(1):50–62. doi:10.1080/15287394.2011.615108 PMID:22047163

Fromme H, Mosch C, Morovitz M, Alba-Alejandre I, Boehmer S, Kiranoglu M, et al. (2010). Pre- and postnatal exposure to perfluorinated compounds (PFCs). *Environ Sci Technol*. 44(18):7123–9. doi:10.1021/es101184f PMID:20722423

Fu J, Gao Y, Cui L, Wang T, Liang Y, Qu G, et al. (2016). Occurrence, temporal trends, and half-lives of perfluoroalkyl acids (PFAAs) in occupational workers in China. *Sci Rep*. 6(1):38039. doi:10.1038/srep38039 PMID:27905562

Fuentes S, Colomina MT, Rodriguez J, Vicens P, Domingo JL (2006). Interactions in developmental toxicology: concurrent exposure to perfluorooctane sulfonate (PFOS) and stress in pregnant mice. *Toxicol Lett*. 164(1):81–9. doi:10.1016/j.toxlet.2005.11.013 PMID:16384669

Fujii Y, Niisoe T, Harada KH, Uemoto S, Ogura Y, Takenaka K, et al. (2015). Toxicokinetics of perfluoroalkyl carboxylic acids with different carbon chain lengths in mice and humans. *J Occup Health*. 57(1):1–12. doi:10.1539/joh.14-0136-OA PMID:25422127

Gallo E, Barbiellini Amidei C, Barbieri G, Fabricio ASC, Gion M, Pitter G, et al. (2022). Perfluoroalkyl substances and thyroid stimulating hormone levels in a highly exposed population in the Veneto Region. *Environ Res*. 203:111794. doi:10.1016/j.envres.2021.111794 PMID:34358507

Gao B, He X, Liu W, Zhang H, Saito N, Tsuda S (2015b). Distribution of perfluoroalkyl compounds in rats: indication for using hair as bioindicator of exposure. *J Expo Sci Environ Epidemiol*. 25(6):632–8. doi:10.1038/jes.2014.54 PMID:25118134

Gao Y, Fu J, Cao H, Wang Y, Zhang A, Liang Y, et al. (2015a). Differential accumulation and elimination behavior of perfluoroalkyl acid isomers in occupational workers in a manufactory in China. *Environ Sci Technol*. 49(11):6953–62. doi:10.1021/acs.est.5b00778 PMID:25927957

Gao Y, Guo X, Wang S, Chen F, Ren X, Xiao H, et al. (2020). Perfluorooctane sulfonate enhances mRNA expression of PPARγ and ap2 in human mesenchymal stem cells monitored by long-retained intracellular nanosensor. *Environ Pollut*. 263(Pt B):114571. doi:10.1016/j.envpol.2020.114571 PMID:32320904

Garoche C, Boulahtouf A, Grimaldi M, Chiavarina B, Toporova L, den Broeder MJ, et al. (2021). Interspecies differences in activation of peroxisome proliferator-activated receptor γ by pharmaceutical and environmental chemicals. *Environ Sci Technol*. 55(24):16489–501. doi:10.1021/acs.est.1c04318 PMID:34843233

Gebbink WA, Berger U, Cousins IT (2015). Estimating human exposure to PFOS isomers and PFCA homologues: the relative importance of direct and indirect (precursor) exposure. *Environ Int*. 74:160–9. doi:10.1016/j.envint.2014.10.013 PMID:25454233

Genuis SJ, Birkholz D, Ralitsch M, Thibault N (2010). Human detoxification of perfluorinated compounds. *Public Health*. 124(7):367–75. doi:10.1016/j.puhe.2010.03.002 PMID:20621793

Giménez-Bastida JA, Surma M, Zieliński H (2015). In vitro evaluation of the cytotoxicity and modulation of mechanisms associated with inflammation induced by perfluorooctanesulfonate and perfluorooctanoic acid in human colon myofibroblasts CCD-18Co. *Toxicol In Vitro*. 29(7):1683–91. doi:10.1016/j.tiv.2015.07.001 PMID:26142696

Gingrich J, Pu Y, Upham BL, Hulse M, Pearl S, Martin D, et al. (2021). Bisphenol S enhances gap junction intercellular communication in ovarian theca cells. *Chemosphere*. 263(January):128304. doi:10.1016/j.chemosphere.2020.128304 PMID:33155548

Glynn A, Berger U, Bignert A, Ullah S, Aune M, Lignell S, et al. (2012). Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996–2010. *Environ Sci Technol*. 46(16):9071–9. doi:10.1021/es301168c PMID:22770559

Goecke CM, Jarnot BM, Reo NV (1992). A comparative toxicological investigation of perfluorocarboxylic acids in rats by fluorine-19 NMR spectroscopy. *Chem Res Toxicol*. 5(4):512–9. doi:10.1021/tx00028a009 PMID:1391617

Goeden HM, Greene CW, Jacobus JA (2019). A transgenerational toxicokinetic model and its use in derivation of Minnesota PFOA water guidance. *J Expo Sci Environ Epidemiol*. 29(2):183–95. doi:10.1038/s41370-018-0110-5 PMID:30631142

Gogola J, Hoffmann M, Ptak A (2019). Persistent endocrine-disrupting chemicals found in human follicular fluid stimulate the proliferation of granulosa tumor spheroids via GPR30 and IGF1R but not via the classic estrogen receptors. *Chemosphere*. 217(February):100–10. doi:10.1016/j.chemosphere.2018.11.018 PMID:30414542

Goines S, Dick JE (2022). Investigating the cytotoxic redox mechanism of PFOS within Hep G2 by hyperspectral-assisted scanning electrochemical microscopy. *Analyst (Lond)*. 147(19):4356–64. doi:10.1039/D2AN00904H PMID:36043461

Gomis MI, Vestergren R, MacLeod M, Mueller JF, Cousins IT (2017). Historical human exposure to perfluoroalkyl acids in the United States and Australia reconstructed from biomonitoring data using population-based pharmacokinetic modelling. *Environ Int*. 108:92–102. doi:10.1016/j.envint.2017.08.002 PMID:28818713

Gomis MI, Vestergren R, Nilsson H, Cousins IT (2016). Contribution of direct and indirect exposure to human serum concentrations of perfluorooctanoic acid in an occupationally exposed group of ski waxers. *Environ Sci Technol*. 50(13):7037–46. doi:10.1021/acs.est.6b01477 PMID:27304840

Gong X, Yang C, Hong Y, Chung ACK, Cai Z (2019). PFOA and PFOS promote diabetic renal injury in vitro by impairing the metabolisms of amino acids and purines. *Sci Total Environ*. 676:72–86. doi:10.1016/j.scitotenv.2019.04.208 PMID:31029902

Gonzalez FJ, Shah YM (2008). PPARalpha: mechanism of species differences and hepatocarcinogenesis of peroxisome proliferators. *Toxicology*. 246(1 August):2–8. doi:10.1016/j.tox.2007.09.030 PMID:18006136

Goodrich JM, Calkins MM, Caban-Martinez AJ, Stueckle T, Grant C, Calafat AM, et al. (2021). Per- and polyfluoroalkyl substances, epigenetic age and DNA methylation: a cross-sectional study of firefighters. *Epigenomics*. 13(20):1619–36. doi:10.2217/epi-2021-0225 PMID:34670402

Gorrochategui E, Pérez-Albaladejo E, Casas J, Lacorte S, Porte C (2014). Perfluorinated chemicals: differential toxicity, inhibition of aromatase activity and alteration of cellular lipids in human placental cells. *Toxicol Appl Pharmacol*. 277(2):124–30. doi:10.1016/j.taap.2014.03.012 PMID:24680846

Goudarzi H, Araki A, Itoh S, Sasaki S, Miyashita C, Mitsui T, et al. (2017b). The association of prenatal exposure to perfluorinated chemicals with glucocorticoid and androgenic hormones in cord blood samples: the Hokkaido Study. *Environ Health Perspect*. 125(1):111–8. doi:10.1289/EHP142 PMID:27219028

Goudarzi H, Miyashita C, Okada E, Kashino I, Chen CJ, Ito S, et al. (2017a). Prenatal exposure to perfluoroalkyl acids and prevalence of infectious diseases up to 4 years of age. *Environ Int*. 104:132–8. doi:10.1016/j.envint.2017.01.024 PMID:28392064

Grandjean P, Andersen EW, Budtz-Jørgensen E, Nielsen F, Mølbak K, Weihe P, et al. (2012). Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA*. 307(4):391–7. doi:10.1001/jama.2011.2034 PMID:22274686

Grandjean P, Heilmann C, Weihe P, Nielsen F, Mogensen UB, Timmermann A, et al. (2017). Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5 years. *J Immunotoxicol*. 14(1):188–95. doi:10.1080/1547691X.2017.1360968 PMID:28805477

Granum B, Haug LS, Namork E, Stølevik SB, Thomsen C, Aaberge IS, et al. (2013). Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. *J Immunotoxicol*. 10(4):373–9. doi:10.3109/1547691X.2012.755580 PMID:23350954

Griesbacher T, Pommer V, Schuligoi R, Tiran B, Peskar BA (2008). Anti-inflammatory actions of perfluorooctanoic acid and peroxisome proliferator-activated receptors (PPAR) alpha and gamma in experimental acute pancreatitis. *Int Immunopharmacol*. 8(2):325–9. doi:10.1016/j.intimp.2007.08.005 PMID:18182248

Griffith FD, Long JE (1980). Animal toxicity studies with ammonium perfluorooctanoate. *Am Ind Hyg Assoc J*. 41(8):576–83. doi:10.1080/15298668091425301 PMID:6773404

Guerrero-Preston R, Goldman LR, Brebi-Mieville P, Ili-Gangas C, Lebron C, Witter FR, et al. (2010). Global DNA hypomethylation is associated with in utero exposure to cotinine and perfluorinated alkyl compounds. *Epigenetics*. 5(6):539–46. doi:10.4161/epi.5.6.12378 PMID:20523118

Guo C, Zhao Z, Zhao K, Huang J, Ding L, Huang X, et al. (2021a). Perfluorooctanoic acid inhibits the maturation rate of mouse oocytes cultured in vitro by triggering mitochondrial and DNA damage. *Birth Defects Res*. 113(14):1074–83. doi:10.1002/bdr2.1899 PMID:33871176

Guo H, Zhang H, Sheng N, Wang J, Chen J, Dai J (2021b). Perfluorooctanoic acid (PFOA) exposure induces splenic atrophy via overactivation of macrophages in male mice. *J Hazard Mater*. 407:124862. doi:10.1016/j.jhazmat.2020.124862 PMID:33360190

Guo J, Zhang J, Wang Z, Zhang L, Qi X, Zhang Y, et al. (2021c). Umbilical cord serum perfluoroalkyl substance mixtures in relation to thyroid function of newborns: findings from Sheyang Mini Birth Cohort Study. *Chemosphere*. 273:129664. doi:10.1016/j.chemosphere.2021.129664 PMID:33493812

Guo P, Furnary T, Vasiliou V, Yan Q, Nyhan K, Jones DP, et al. (2022b). Non-targeted metabolomics and associations with per- and polyfluoroalkyl substances (PFAS) exposure in humans: a scoping review. *Environ Int*. 162:107159. doi:10.1016/j.envint.2022.107159 PMID:35231839

Guo XX, He QZ, Li W, Long DX, Pan XY, Chen C, et al. (2017). Brain-derived neurotrophic factor mediated perfluorooctane sulfonate induced-neurotoxicity via epigenetics regulation in SK-N-SH cells. *Int J Mol Sci*. 18(4):893. doi:10.3390/ijms18040893 PMID:28441774

Guo Y, Yuan J, Ni H, Ji J, Zhong S, Zheng Y, et al. (2022a). Perfluorooctanoic acid-induced developmental cardiotoxicity in chicken embryo: roles of miR-490-5p. *Environ Pollut*. 312:120022. doi:10.1016/j.envpol.2022.120022 PMID:36028080

Guruge KS, Hikono H, Shimada N, Murakami K, Hasegawa J, Yeung LW, et al. (2009). Effect of perfluorooctane sulfonate (PFOS) on influenza A virus-induced mortality in female B6C3F$_1$ mice. *J Toxicol Sci*. 34(6):687–91. doi:10.2131/jts.34.687 PMID:19952504

Guruge KS, Yeung LW, Yamanaka N, Miyazaki S, Lam PK, Giesy JP, et al. (2006). Gene expression profiles in rat liver treated with perfluorooctanoic acid (PFOA). *Toxicol Sci*. 89(1):93–107. doi:10.1093/toxsci/kfj011 PMID:16221955

Gyllenhammar I, Benskin JP, Sandblom O, Berger U, Ahrens L, Lignell S, et al. (2018). Perfluoroalkyl acids (PFAAs) in serum from 2–4-month-old infants: influence of maternal serum concentration, gestational age, breast-feeding, and contaminated drinking water. *Environ Sci Technol*. 52(12):7101–10. doi:10.1021/acs.est.8b00770 PMID:29758986

Hallberg I, Persson S, Olovsson M, Sirard MA, Damdimopoulou P, Rüegg J, et al. (2021). Perfluorooctane sulfonate (PFOS) exposure of bovine oocytes affects early embryonic development at human-relevant levels in an in vitro model. *Toxicology*. 464:153028. doi:10.1016/j.tox.2021.153028 PMID:34762985

Hamm MP, Cherry NM, Chan E, Martin JW, Burstyn I (2010). Maternal exposure to perfluorinated acids and fetal growth. *J Expo Sci Environ Epidemiol*. 20(7):589–97. doi:10.1038/jes.2009.57 PMID:19865074

Han JS, Jang S, Son HY, Kim YB, Kim Y, Noh JH, et al. (2020). Subacute dermal toxicity of perfluoroalkyl carboxylic acids: comparison with different carbon-chain lengths in human skin equivalents and systemic effects of perfluoroheptanoic acid in Sprague-Dawley rats. *Arch Toxicol*. 94(2):523–39. doi:10.1007/s00204-019-02634-z PMID:31797001

Han R, Hu M, Zhong Q, Wan C, Liu L, Li F, et al. (2018a). Perfluorooctane sulphonate induces oxidative hepatic damage via mitochondria-dependent and NF-κB/TNF-α-mediated pathway. *Chemosphere*. 191:1056–64. doi:10.1016/j.chemosphere.2017.08.070 PMID:28939271

Han R, Zhang F, Wan C, Liu L, Zhong Q, Ding W (2018b). Effect of perfluorooctane sulphonate-induced Kupffer cell activation on hepatocyte proliferation through the NF-κB/TNF-α/IL-6-dependent pathway. *Chemosphere*. 200:283–94. doi:10.1016/j.chemosphere.2018.02.137 PMID:29494909

Han X, Alam MN, Cao M, Wang X, Cen M, Tian M, et al. (2022). Low levels of perfluorooctanoic acid exposure activates steroid hormone biosynthesis through repressing histone methylation in rats. *Environ Sci Technol*. 56(9):5664–72. doi:10.1021/acs.est.1c08885 PMID:35438966

Han X, Hinderliter PM, Snow TA, Jepson GW (2004). Binding of perfluorooctanoic acid to rat liver-form and kidney-form alpha2u-globulins. *Drug Chem Toxicol*. 27(4):341–60. doi:10.1081/DCT-200039725 PMID:15573471

Han X, Kemper RA, Jepson GW (2005). Subcellular distribution and protein binding of perfluorooctanoic acid in rat liver and kidney. *Drug Chem Toxicol*. 28(2):197–209. doi:10.1081/DCT-52547 PMID:15865261

Han X, Snow TA, Kemper RA, Jepson GW (2003). Binding of perfluorooctanoic acid to rat and human plasma proteins. *Chem Res Toxicol*. 16(6):775–81. doi:10.1021/tx034005w PMID:12807361

Harada K, Inoue K, Morikawa A, Yoshinaga T, Saito N, Koizumi A (2005). Renal clearance of perfluorooctane sulfonate and perfluorooctanoate in humans and their species-specific excretion. *Environ Res*. 99(2):253–61. doi:10.1016/j.envres.2004.12.003 PMID:16194675

Harada KH, Hashida S, Kaneko T, Takenaka K, Minata M, Inoue K, et al. (2007). Biliary excretion and cerebrospinal fluid partition of perfluorooctanoate and perfluorooctane sulfonate in humans. *Environ Toxicol Pharmacol*. 24(2):134–9. doi:10.1016/j.etap.2007.04.003 PMID:21783801

Harlow SD, Hood MM, Ding N, Mukherjee B, Calafat AM, Randolph JF Jr, et al. (2021). Per- and polyfluoroalkyl substances and hormone levels during the menopausal

transition. *J Clin Endocrinol Metab*. 106(11):e4427–37. doi:10.1210/clinem/dgab476 PMID:34181018

Hayhurst GP, Lee YH, Lambert G, Ward JM, Gonzalez FJ (2001). Hepatocyte nuclear factor 4alpha (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. *Mol Cell Biol*. 21(4):1393–403. doi:10.1128/MCB.21.4.1393-1403.2001 PMID:11158324

Heffernan AL, Cunningham TK, Drage DS, Aylward LL, Thompson K, Vijayasarathy S, et al. (2018). Perfluorinated alkyl acids in the serum and follicular fluid of UK women with and without polycystic ovarian syndrome undergoing fertility treatment and associations with hormonal and metabolic parameters. *Int J Hyg Environ Health*. 221(7):1068–75. doi:10.1016/j.ijheh.2018.07.009 PMID:30037723

Hinderliter PM, DeLorme MP, Kennedy GL (2006). Perfluorooctanoic acid: relationship between repeated inhalation exposures and plasma PFOA concentration in the rat. *Toxicology*. 222(1–2):80–5. doi:10.1016/j.tox.2006.01.029 PMID:16513238

Hines EP, White SS, Stanko JP, Gibbs-Flournoy EA, Lau C, Fenton SE (2009). Phenotypic dichotomy following developmental exposure to perfluorooctanoic acid (PFOA) in female CD-1 mice: low doses induce elevated serum leptin and insulin, and overweight in mid-life. *Mol Cell Endocrinol*. 304(1–2):97–105. doi:10.1016/j.mce.2009.02.021 PMID:19433254

Højsager FD, Andersen M, Juul A, Nielsen F, Möller S, Christensen HT, et al. (2022). Prenatal and early postnatal exposure to perfluoroalkyl substances and bone mineral content and density in the Odense child cohort. *Environ Int*. 167:107417. doi:10.1016/j.envint.2022.107417 PMID:35914335

Houck KA, Friedman KP, Feshuk M, Patlewicz G, Smeltz M, Clifton MS, et al. (2023). Evaluation of 147 perfluoroalkyl substances for immunotoxic and other (patho) physiological activities through phenotypic screening of human primary cells. *ALTEX*. 40(2):248–70. doi:10.14573/altex.2203041 PMID:36129398

Houck KA, Patlewicz G, Richard AM, Williams AJ, Shobair MA, Smeltz M, et al. (2021). Bioactivity profiling of per- and polyfluoroalkyl substances (PFAS) identifies potential toxicity pathways related to molecular structure. *Toxicology*. 457:152789. doi:10.1016/j.tox.2021.152789 PMID:33887376

Hu Q, Franklin JN, Bryan I, Morris E, Wood A, DeWitt JC (2012). Does developmental exposure to perfluorooctanoic acid (PFOA) induce immunopathologies commonly observed in neurodevelopmental disorders? *Neurotoxicology*. 33(6):1491–8. doi:10.1016/j.neuro.2012.10.016 PMID:23137609

Hu Q, Strynar MJ, DeWitt JC (2010). Are developmentally exposed C57BL/6 mice insensitive to suppression of TDAR by PFOA? *J Immunotoxicol*. 7(4):344–9. doi:10.3109/1547691X.2010.520045 PMID:20954796

Hu W, Jones PD, Upham BL, Trosko JE, Lau C, Giesy JP (2002). Inhibition of gap junctional intercellular communication by perfluorinated compounds in rat liver and dolphin kidney epithelial cell lines in vitro and Sprague-Dawley rats in vivo. *Toxicol Sci*. 68(2):429–36. doi:10.1093/toxsci/68.2.429 PMID:12151638

Hu WY, Lu R, Hu DP, Imir OB, Zuo Q, Moline D, et al. (2022). Per- and polyfluoroalkyl substances target and alter human prostate stem-progenitor cells. *Biochem Pharmacol*. 197:114902. doi:10.1016/j.bcp.2021.114902 PMID:34968493

Hu X, Li S, Cirillo PM, Krigbaum NY, Tran V, Jones DP, et al. (2019). Metabolome wide association study of serum poly and perfluoroalkyl substances (PFASs) in pregnancy and early postpartum. *Reprod Toxicol*. 87:70–8. doi:10.1016/j.reprotox.2019.05.058 PMID:31121237

Hu XZ, Hu DC (2009). Effects of perfluorooctanoate and perfluorooctane sulfonate exposure on hepatoma Hep G2 cells. *Arch Toxicol*. 83(9):851–61. doi:10.1007/s00204-009-0441-z PMID:19468714

Huang H, Wang Q, He X, Wu Y, Xu C (2019b). Association between polyfluoroalkyl chemical concentrations and leucocyte telomere length in US adults. *Sci Total Environ*.653:547–53.doi:10.1016/j.scitotenv.2018.10.400 PMID:30414584

Huang J, Ren H, Chen A, Li T, Wang H, Jiang L, et al. (2022a). Perfluorooctane sulfonate induces suppression of testosterone biosynthesis via Sertoli cell-derived exosomal/miR-9–3p downregulating StAR expression in Leydig cells. *Environ Pollut*. 301:118960. doi:10.1016/j.envpol.2022.118960 PMID:35150797

Huang MC, Dzierlenga AL, Robinson VG, Waidyanatha S, DeVito MJ, Eifrid MA, et al. (2019a). Toxicokinetics of perfluorobutane sulfonate (PFBS), perfluorohexane-1-sulphonic acid (PFHxS), and perfluorooctane sulfonic acid (PFOS) in male and female Hsd:Sprague-Dawley SD rats after intravenous and gavage administration. *Toxicol Rep*. 6:645–55. doi:10.1016/j.toxrep.2019.06.016 PMID:31334035

Huang MC, Dzierlenga AL, Robinson VG, Waidyanatha S, DeVito MJ, Eifrid MA, et al. (2021). Corrigendum to "Toxicokinetics of perfluorobutane sulfonate (PFBS), perfluorohexane-1-sulphonic acid (PFHxS), and perfluorooctane sulfonic acid (PFOS) in male and female Hsd:Sprague-Dawley SD rats after intravenous and gavage administration" [Toxicol. Rep. (2019) 6:645–655]. *Toxicol Rep*. 8:365. doi:10.1016/j.toxrep.2021.02.001 PMID:33665134

Huang Q, Luo L, Han X, Li F, Zhang X, Tian M (2022b). Low-dose perfluorooctanoic acid stimulates steroid hormone synthesis in Leydig cells: integrated proteomics and metabolomics evidence. *J Hazard Mater*. 424(Pt D):127656. doi:10.1016/j.jhazmat.2021.127656 PMID:34774353

Huang Q, Zhang J, Martin FL, Peng S, Tian M, Mu X, et al. (2013). Perfluorooctanoic acid induces apoptosis through the p53-dependent mitochondrial pathway in human hepatic cells: a proteomic study. *Toxicol Lett*. 223(2):211–20. doi:10.1016/j.toxlet.2013.09.002 PMID:24035753

Huang Q, Zhang J, Peng S, Du M, Ow S, Pu H, et al. (2014). Proteomic analysis of perfluorooctane sulfonate-induced apoptosis in human hepatic cells using the iTRAQ technique. *J Appl Toxicol*. 34(12):1342–51. doi:10.1002/jat.2963 PMID:24301089

Huang T, Zhang Y, Zhang W, Lin T, Chen L, Yang B, et al. (2020). Attenuation of perfluorooctane sulfonate-induced steatohepatitis by grape seed proanthocyanidin extract in mice. *BioMed Res Int*. 2020:8818160. doi:10.1155/2020/8818160 PMID:33457418

Hundley SG, Sarrif AM, Kennedy GL Jr (2006). Absorption, distribution, and excretion of ammonium perfluorooctanoate (APFO) after oral administration to various species. *Drug Chem Toxicol*. 29(2):137–45. doi:10.1080/01480540600561361 PMID:16707323

Husøy T, Caspersen IH, Thépaut E, Knutsen H, Haug LS, Andreassen M, et al. (2023). Comparison of aggregated exposure to perfluorooctanoic acid (PFOA) from diet and personal care products with concentrations in blood using a PBPK model – results from the Norwegian biomonitoring study in EuroMix. *Environ Res*. 239(Pt 2):117341. doi:10.1016/j.envres.2023.117341 PMID:37839534

Imir OB, Kaminsky AZ, Zuo QY, Liu YJ, Singh R, Spinella MJ, et al. (2021). Per- and polyfluoroalkyl substance exposure combined with high-fat diet supports prostate cancer progression. *Nutrients*. 13(11):3902. doi:10.3390/nu13113902 PMID:34836157

Impinen A, Longnecker MP, Nygaard UC, London SJ, Ferguson KK, Haug LS, et al. (2019). Maternal levels of perfluoroalkyl substances (PFASs) during pregnancy and childhood allergy and asthma related outcomes and infections in the Norwegian Mother and Child (MoBa) cohort. *Environ Int*. 124:462–72. doi:10.1016/j.envint.2018.12.041 PMID:30684804

Impinen A, Nygaard UC, Lødrup Carlsen KC, Mowinckel P, Carlsen KH, Haug LS, et al. (2018). Prenatal exposure to perfluoralkyl substances (PFASs) associated with respiratory tract infections but not allergy- and asthma-related health outcomes in childhood. *Environ Res*. 160:518–23. doi:10.1016/j.envres.2017.10.012 PMID:29106950

Inoue K, Ritz B, Andersen SL, Ramlau-Hansen CH, Høyer BB, Bech BH, et al. (2019). Perfluoroalkyl substances and maternal thyroid hormones in early pregnancy; findings in the Danish National Birth Cohort. *Environ Health Perspect*. 127(11):117002. doi:10.1289/EHP5482 PMID:31714155

Ishibashi H, Hirano M, Kim EY, Iwata H (2019). In vitro and in silico evaluations of binding affinities of perfluoroalkyl substances to Baikal seal and human peroxisome proliferator-activated receptor α. *Environ Sci Technol*. 53(4):2181–8. doi:10.1021/acs.est.8b07273 PMID:30649875

Ishida K, Tsuyama Y, Sanoh S, Ohta S, Kotake Y (2017). Perfluorooctane sulfonate induces neuronal vulnerability by decreasing GluR2 expression. *Arch Toxicol*. 91(2):885–95. doi:10.1007/s00204-016-1731-x PMID:27155986

Itoh S, Araki A, Mitsui T, Miyashita C, Goudarzi H, Sasaki S, et al. (2016). Association of perfluoroalkyl substances exposure in utero with reproductive hormone levels in cord blood in the Hokkaido Study on Environment and Children's Health. *Environ Int*. 94:51–9. doi:10.1016/j.envint.2016.05.011 PMID:27209000

Itoh S, Araki A, Miyashita C, Yamazaki K, Goudarzi H, Minatoya M, et al. (2019). Association between perfluoroalkyl substance exposure and thyroid hormone/thyroid antibody levels in maternal and cord blood: the Hokkaido Study. *Environ Int*. 133(Pt A):105139. doi:10.1016/j.envint.2019.105139 PMID:31518930

Iwabuchi K, Senzaki N, Mazawa D, Sato I, Hara M, Ueda F, et al. (2017). Tissue toxicokinetics of perfluoro compounds with single and chronic low doses in male rats. *J Toxicol Sci*. 42(3):301–17. doi:10.2131/jts.42.301 PMID:28496036

Iwai H, Yamashita K (2006). A fourteen-day repeated dose oral toxicity study of APFO in rats. *Drug Chem Toxicol*. 29(3):323–32. doi:10.1080/01480540600653143 PMID:16777710

Jabeen M, Fayyaz M, Irudayaraj J (2020). Epigenetic modifications, and alterations in cell cycle and apoptosis pathway in A549 lung carcinoma cell line upon exposure to perfluoroalkyl substances. *Toxics*. 8(4):112. doi:10.3390/toxics8040112 PMID:33238432

Jacquet N, Maire MA, Landkocz Y, Vasseur P (2012b). Carcinogenic potency of perfluorooctane sulfonate (PFOS) on Syrian hamster embryo (SHE) cells. *Arch Toxicol*. 86(2):305–14. doi:10.1007/s00204-011-0752-8 PMID:22057587

Jacquet N, Maire MA, Rast C, Bonnard M, Vasseur P (2012a). Perfluorooctanoic acid (PFOA) acts as a tumor promoter on Syrian hamster embryo (SHE) cells. *Environ Sci Pollut Res Int*. 19(7):2537–49. doi:10.1007/s11356-012-0968-z PMID:22828883

Jain RB (2013). Association between thyroid profile and perfluoroalkyl acids: data from NHANES 2007–2008. *Environ Res*. 126:51–9. doi:10.1016/j.envres.2013.08.006 PMID:24053974

Jain RB, Ducatman A (2022). Associations between the concentrations of α-klotho and selected perfluoroalkyl substances in the presence of eGFR based kidney function and albuminuria: data for US adults aged 40–79

years. *Sci Total Environ*. 838(Pt 1):155994. doi:10.1016/j.scitotenv.2022.155994 PMID:35595139

Jandacek RJ, Rider T, Keller ER, Tso P (2010). The effect of olestra on the absorption, excretion and storage of 2,2′,5,5′ tetrachlorobiphenyl; 3,3′,4,4′ tetrachlorobiphenyl; and perfluorooctanoic acid. *Environ Int*. 36(8):880–3. doi:10.1016/j.envint.2009.06.010 PMID:19616301

Janssen AWF, Louisse J, Rijkers D, Pinckaers NET, Hoekstra SA, Hoogenboom RLAP, et al. (2022). Perfluoroalkyl substances (PFASs) decrease the expression of recombination-activating genes (RAG1 and RAG2) in human B lymphoma Namalwa cells. *Arch Toxicol*. 97(2):457–68. doi:10.1007/s00204-022-03405-z PMID:36326898

Jensen RC, Glintborg D, Gade Timmermann CA, Nielsen F, Kyhl HB, Frederiksen H, et al.; The Odense Child Cohort (2020). Prenatal exposure to perfluorodecanoic acid is associated with lower circulating concentration of adrenal steroid metabolites during mini puberty in human female infants. *Environ Res*. 182:109101. doi:10.1016/j.envres.2019.109101 PMID:32069767

Jensen RC, Glintborg D, Timmermann CAG, Nielsen F, Boye H, Madsen JB, et al.; The Odense Child Cohort (2022). Higher free thyroxine associated with PFAS exposure in first trimester. *Environ Res*. 212(Pt D):113492. doi:10.1016/j.envres.2022.113492 PMID:35597289

Jernbro S, Rocha PS, Keiter S, Skutlarek D, Färber H, Jones PD, et al. (2007). Perfluorooctane sulfonate increases the genotoxicity of cyclophosphamide in the micronucleus assay with V79 cells. Further proof of alterations in cell membrane properties caused by PFOS. *Environ Sci Pollut Res Int*. 14(2):85–7. doi:10.1065/espr2007.01.384 PMID:17455816

Ji K, Kim S, Kho Y, Paek D, Sakong J, Ha J, et al. (2012). Serum concentrations of major perfluorinated compounds among the general population in Korea: dietary sources and potential impact on thyroid hormones. *Environ Int*. 45:78–85. doi:10.1016/j.envint.2012.03.007 PMID:22580293

Jin H, Zhang Y, Jiang W, Zhu L, Martin JW (2016). Isomer-specific distribution of perfluoroalkyl substances in blood. *Environ Sci Technol*. 50(14):7808–15. doi:10.1021/acs.est.6b01698 PMID:27295125

Joensen UN, Bossi R, Leffers H, Jensen AA, Skakkebaek NE, Jørgensen N (2009). Do perfluoroalkyl compounds impair human semen quality? *Environ Health Perspect*. 117(6):923–7. doi:10.1289/ehp.0800517 PMID:19590684

Joensen UN, Veyrand B, Antignac J-P, Blomberg Jensen M, Petersen JH, Marchand P, et al. (2013). PFOS (perfluorooctanesulfonate) in serum is negatively associated with testosterone levels, but not with semen quality, in healthy men. *Hum Reprod*. 28(3):599–608. doi:10.1093/humrep/des425 PMID:23250927

Johnson JD, Gibson SJ, Ober RE (1984). Cholestyramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [^{14}C]perfluorooctanoate or potassium [^{14}C]perfluorooctanesulfonate. *Fundam Appl Toxicol*. 4(6):972–6. doi:10.1016/0272-0590(84)90235-5 PMID:6519377

Jones LE, Ghassabian A, Lawrence DA, Sundaram R, Yeung E, Kannan K, et al. (2022). Exposure to perfluoroalkyl substances and neonatal immunoglobulin profiles in the upstate KIDS study (2008–2010). *Environ Pollut*. 308:119656. doi:10.1016/j.envpol.2022.119656 PMID:35787426

Kamei Y, Suganami T, Kohda T, Ishino F, Yasuda K, Miura S, et al. (2007). Peg1/Mest in obese adipose tissue is expressed from the paternal allele in an isoform-specific manner. *FEBS Lett*. 581(1):91–6. doi:10.1016/j.febslet.2006.12.002 PMID:17182038

Kamendulis LM, Hocevar JM, Stephens M, Sandusky GE, Hocevar BA (2022). Exposure to perfluorooctanoic acid leads to promotion of pancreatic cancer. *Carcinogenesis*. 43(5):469–78. doi:10.1093/carcin/bgac005 PMID:35022659

Kamendulis LM, Wu Q, Sandusky GE, Hocevar BA (2014). Perfluorooctanoic acid exposure triggers oxidative stress in the mouse pancreas. *Toxicol Rep*. 1:513–21. doi:10.1016/j.toxrep.2014.07.015 PMID:28962265

Kang H, Lee HK, Moon HB, Kim S, Lee J, Ha M, et al. (2018). Perfluoroalkyl acids in serum of Korean children: occurrences, related sources, and associated health outcomes. *Sci Total Environ*. 645:958–65. doi:10.1016/j.scitotenv.2018.07.177 PMID:30032090

Kang JS, Choi JS, Park JW (2016). Transcriptional changes in steroidogenesis by perfluoroalkyl acids (PFOA and PFOS) regulate the synthesis of sex hormones in H295R cells. *Chemosphere*. 155:436–43. doi:10.1016/j.chemosphere.2016.04.070 PMID:27139122

Kasten-Jolly J, Lawrence DA (2022). Perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) modify *in vitro* mitogen- and antigen-induced human peripheral blood mononuclear cell (PBMC) responses. *J Toxicol Environ Health A*. 85(17):715–37. doi:10.1080/15287394.2022.2075816 PMID:35611390

Katakura M, Kudo N, Tsuda T, Hibino Y, Mitsumoto A, Kawashima Y (2007). Rat organic anion transporter 3 and organic anion transporting polypeptide 1 mediate perfluorooctanoic acid transport. *J Health Sci*. 53(1):77–83. doi:10.1248/jhs.53.77

Kato K, Wong LY, Chen A, Dunbar C, Webster GM, Lanphear BP, et al. (2014). Changes in serum concentrations of maternal poly- and perfluoroalkyl substances over the course of pregnancy and predictors of exposure in a multiethnic cohort of Cincinnati, Ohio pregnant women during 2003–2006. *Environ Sci Technol*. 48(16):9600–8. doi:10.1021/es501811k PMID:25026485

Kato S, Itoh S, Yuasa M, Baba T, Miyashita C, Sasaki S, et al. (2016). Association of perfluorinated chemical exposure in utero with maternal and infant thyroid hormone levels in the Sapporo cohort of Hokkaido Study on the Environment and Children's Health. *Environ Health Prev Med*. 21(5):334–44. doi:10.1007/s12199-016-0534-2 PMID:27137816

Kaur K, Lesseur C, Chen L, Andra SS, Narasimhan S, Pulivarthi D, et al. (2023). Cross-sectional associations of maternal PFAS exposure on SARS-CoV-2 IgG antibody levels during pregnancy. *Environ Res*. 219:115067. doi:10.1016/j.envres.2022.115067 PMID:36528042

Kawamoto K, Oashi T, Oami K, Liu W, Jin Y, Saito N, et al. (2010). Perfluorooctanoic acid (PFOA) but not perfluorooctane sulfonate (PFOS) showed DNA damage in comet assay on *Paramecium caudatum*. *J Toxicol Sci*. 35(6):835–41. doi:10.2131/jts.35.835 PMID:21139333

Kawashima Y, Suzuki S, Kozuka H, Sato M, Suzuki Y (1994). Effects of prolonged administration of perfluorooctanoic acid on hepatic activities of enzymes which detoxify peroxide and xenobiotic in the rat. *Toxicology*. 93(2–3):85–97. doi:10.1016/0300-483X(94)90070-1 PMID:7974521

Keil DE, Mehlmann T, Butterworth L, Peden-Adams MM (2008). Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F$_1$ mice. *Toxicol Sci*. 103(1):77–85. doi:10.1093/toxsci/kfn015 PMID:18252804

Kennedy GL Jr (1985). Dermal toxicity of ammonium perfluorooctanoate. *Toxicol Appl Pharmacol*. 81(2):348–55. doi:10.1016/0041-008X(85)90172-3 PMID:4060160

Khalil N, Ebert JR, Honda M, Lee M, Nahhas RW, Koskela A, et al. (2018). Perfluoroalkyl substances, bone density, and cardio-metabolic risk factors in obese 8–12 year old children: a pilot study. *Environ Res*. 160:314–21. doi:10.1016/j.envres.2017.10.014 PMID:29040951

Khansari MR, Yousefsani BS, Kobarfard F, Faizi M, Pourahmad J (2017). In vitro toxicity of perfluorooctane sulfonate on rat liver hepatocytes: probability of distructive binding to CYP 2E1 and involvement of cellular proteolysis. *Environ Sci Pollut Res Int*. 24(29):23382–8. doi:10.1007/s11356-017-9908-2 PMID:28842823

Kim H, Hong MW, Bae YH, Lee SJ (2021). Epigenetic toxicity and cytotoxicity of perfluorooctanoic acid and its effects on gene expression in embryonic mouse hypothalamus cells. *Arh Hig Rada Toksikol*. 72(3):182–90. doi:10.2478/aiht-2021-72-3555 PMID:34587672

Kim HY, Kim K-N, Shin CH, Lim Y-H, Kim JI, Kim B-N, et al. (2020). The relationship between perfluoroalkyl substances concentrations and thyroid function in early childhood: a prospective cohort study. *Thyroid*. 30(11):1556–65. doi:10.1089/thy.2019.0436 PMID:32368952

Kim JH, Park HY, Jeon JD, Kho Y, Kim SK, Park MS, et al. (2016b). The modifying effect of vitamin C on the association between perfluorinated compounds and insulin resistance in the Korean elderly: a double-blind, randomized, placebo-controlled crossover trial. *Eur J Nutr*. 55(3):1011–20. doi:10.1007/s00394-015-0915-0 PMID:25939797

Kim MJ, Moon S, Oh BC, Jung D, Ji K, Choi K, et al. (2018). Association between perfluoroalkyl substances exposure and thyroid function in adults: a meta-analysis. *PLoS One*. 13(5):e0197244. doi:10.1371/journal.pone.0197244 PMID:29746532

Kim S, Choi K, Ji K, Seo J, Kho Y, Park J, et al. (2011). Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. *Environ Sci Technol*. 45(17):7465–72. doi:10.1021/es202408a PMID:21805959

Kim SJ, Heo SH, Lee DS, Hwang IG, Lee YB, Cho HY (2016a). Gender differences in pharmacokinetics and tissue distribution of 3 perfluoroalkyl and polyfluoroalkyl substances in rats. *Food Chem Toxicol*. 97:243–55. doi:10.1016/j.fct.2016.09.017 PMID:27637925

Kim WK, Lee SK, Jung J (2010). Integrated assessment of biomarker responses in common carp (*Cyprinus carpio*) exposed to perfluorinated organic compounds. *J Hazard Mater*. 180(1–3):395–400. doi:10.1016/j.jhazmat.2010.04.044 PMID:20452121

Kimura O, Fujii Y, Haraguchi K, Kato Y, Ohta C, Koga N, et al. (2017). Uptake of perfluorooctanoic acid by Caco-2 cells: involvement of organic anion transporting polypeptides. *Toxicol Lett*. 277:18–23. doi:10.1016/j.toxlet.2017.05.012 PMID:28552774

Kimura O, Fujii Y, Haraguchi K, Kato Y, Ohta C, Koga N, et al. (2020). Effects of perfluoroalkyl carboxylic acids on the uptake of sulfobromophthalein via organic anion transporting polypeptides in human intestinal Caco-2 cells. *Biochem Biophys Rep*. 24:100807. doi:10.1016/j.bbrep.2020.100807 PMID:32964147

Kingsley SL, Kelsey KT, Butler R, Chen A, Eliot MN, Romano ME, et al. (2017). Maternal serum PFOA concentration and DNA methylation in cord blood: a pilot study. *Environ Res*. 158:174–8. doi:10.1016/j.envres.2017.06.013 PMID:28645023

Kingsley SL, Walker DI, Calafat AM, Chen A, Papandonatos GD, Xu Y, et al. (2019). Metabolomics of childhood exposure to perfluoroalkyl substances: a cross-sectional study. *Metabolomics*. 15(7):95. doi:10.1007/s11306-019-1560-z PMID:31227916

Kjeldsen LS, Bonefeld-Jørgensen EC (2013). Perfluorinated compounds affect the function of sex hormone receptors. *Environ Sci Pollut Res Int*. 20(11):8031–44. doi:10.1007/s11356-013-1753-3 PMID:23764977

Klaunig JE, Babich MA, Baetcke KP, Cook JC, Corton JC, David RM, et al. (2003). PPARalpha agonist-induced rodent tumors: modes of action and human relevance. *Crit Rev Toxicol*. 33(6):655–780. doi:10.1080/713608372 PMID:14727734

Knox SS, Jackson T, Javins B, Frisbee SJ, Shankar A, Ducatman AM (2011). Implications of early menopause in women exposed to perfluorocarbons. *J Clin Endocrinol Metab*. 96(6):1747–53. doi:10.1210/jc.2010-2401 PMID:21411548

Kobayashi S, Azumi K, Goudarzi H, Araki A, Miyashita C, Kobayashi S, et al. (2017). Effects of prenatal perfluoroalkyl acid exposure on cord blood IGF2/H19 methylation and ponderal index: the Hokkaido Study. *J Expo Sci Environ Epidemiol*. 27(3):251–9. doi:10.1038/jes.2016.50 PMID:27553991

Kobayashi S, Sata F, Ikeda-Araki A, Miyashita C, Itoh S, Goudarzi H, et al. (2021). Associations among maternal perfluoroalkyl substance levels, fetal sex-hormone enzymatic gene polymorphisms, and fetal sex hormone levels in the Hokkaido study. *Reprod Toxicol*. 105:221–31. doi:10.1016/j.reprotox.2021.09.003 PMID:34536543

Kraugerud M, Zimmer KE, Ropstad E, Verhaegen S (2011). Perfluorinated compounds differentially affect steroidogenesis and viability in the human adrenocortical carcinoma (H295R) in vitro cell assay. *Toxicol Lett*. 205(1):62–8. doi:10.1016/j.toxlet.2011.05.230 PMID:21641976

Kristensen SL, Ramlau-Hansen CH, Ernst E, Olsen SF, Bonde JP, Vested A, et al. (2013). Long-term effects of prenatal exposure to perfluoroalkyl substances on female reproduction. *Hum Reprod*. 28(12):3337–48. doi:10.1093/humrep/det382 PMID:24129614

Ku MS, Pan WC, Huang YT, Hsieh WS, Hsu YH, Chen PC, et al. (2022). Associations between prenatal exposure to perfluoroalkyl substances, hypomethylation of MEST imprinted gene and birth outcomes. *Environ Pollut*. 304:119183. doi:10.1016/j.envpol.2022.119183 PMID:35331797

Kudo N, Katakura M, Sato Y, Kawashima Y (2002). Sex hormone-regulated renal transport of perfluorooctanoic acid. *Chem Biol Interact*. 139(3):301–16. doi:10.1016/S0009-2797(02)00006-6 PMID:11879818

Kudo N, Sakai A, Mitsumoto A, Hibino Y, Tsuda T, Kawashima Y (2007). Tissue distribution and hepatic subcellular distribution of perfluorooctanoic acid at low dose are different from those at high dose in rats. *Biol Pharm Bull*. 30(8):1535–40. doi:10.1248/bpb.30.1535 PMID:17666816

Kummu M, Sieppi E, Koponen J, Laatio L, Vähäkangas K, Kiviranta H, et al. (2015). Organic anion transporter 4 (OAT 4) modifies placental transfer of perfluorinated alkyl acids PFOS and PFOA in human placental ex vivo perfusion system. *Placenta*. 36(10):1185–91. doi:10.1016/j.placenta.2015.07.119 PMID:26303760

Kung Y-P, Lin C-C, Chen M-H, Tsai M-S, Hsieh W-S, Chen P-C (2021). Intrauterine exposure to per- and polyfluoroalkyl substances may harm children's lung function development. *Environ Res*. 192:110178. doi:10.1016/j.envres.2020.110178 PMID:32991923

Kuslikis BI, Vanden Heuvel JP, Peterson RE (1992). Lack of evidence for perfluorodecanoyl- or perfluorooctanoyl-coenzyme A formation in male and female rats. *J Biochem Toxicol*. 7(1):25–9. doi:10.1002/jbt.2570070106 PMID:1588571

Kvalem HE, Nygaard UC, Lødrup Carlsen KC, Carlsen KH, Haug LS, Granum B (2020). Perfluoroalkyl substances, airways infections, allergy and asthma related health outcomes – implications of gender, exposure period and study design. *Environ Int*. 134:105259. doi:10.1016/j.envint.2019.105259 PMID:31733527

La Rocca C, Tait S, Guerranti C, Busani L, Ciardo F, Bergamasco B, et al. (2015). Exposure to endocrine disruptors and nuclear receptors gene expression in infertile and fertile men from Italian areas with different environmental features. *Int J Environ Res Public Health*. 12(10):12426–45. doi:10.3390/ijerph121012426 PMID:26445054

Lai KP, Lee JC, Wan HT, Li JW, Wong AY, Chan TF, et al. (2017a). Effects of in utero PFOS exposure on transcriptome, lipidome, and function of mouse testis. *Environ Sci Technol*. 51(15):8782–94. doi:10.1021/acs.est.7b02102 PMID:28654245

Lai KP, Li JW, Cheung A, Li R, Billah MB, Chan TF, et al. (2017b). Transcriptome sequencing reveals prenatal PFOS exposure on liver disorders. *Environ Pollut*. 223:416–25. doi:10.1016/j.envpol.2017.01.041 PMID:28131474

Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, et al. (2003). Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. *Toxicol Sci*. 74(2):382–92. doi:10.1093/toxsci/kfg122 PMID:12773772

Lebeaux RM, Doherty BT, Gallagher LG, Zoeller RT, Hoofnagle AN, Calafat AM, et al. (2020). Maternal serum perfluoroalkyl substance mixtures and thyroid hormone concentrations in maternal and cord sera: the HOME Study. *Environ Res*. 185:109395. doi:10.1016/j.envres.2020.109395 PMID:32222633

Lee HG, Lee YJ, Yang JH (2012). Perfluorooctane sulfonate induces apoptosis of cerebellar granule cells via a ROS-dependent protein kinase C signalling pathway. *Neurotoxicology*. 33(3):314–20. doi:10.1016/j.neuro.2012.01.017 PMID:22326494

Lee J-K, Lee S, Baek M-C, Lee B-H, Lee H-S, Kwon TK, et al. (2017). Association between perfluorooctanoic acid exposure and degranulation of mast cells in allergic inflammation. *J Appl Toxicol*. 37(5):554–62. doi:10.1002/jat.3389 PMID:27682001

Lee YY, Wong CK, Oger C, Durand T, Galano JM, Lee JC (2015). Prenatal exposure to the contaminant perfluorooctane sulfonate elevates lipid peroxidation during mouse fetal development but not in the pregnant dam. *Free Radic Res*. 49(8):1015–25. doi:10.3109/10715762.2015.1027199 PMID:25787935

Lefebvre DE, Curran I, Armstrong C, Coady L, Parenteau M, Liston V, et al. (2008). Immunomodulatory effects of dietary potassium perfluorooctane sulfonate (PFOS) exposure in adult Sprague-Dawley rats. *J Toxicol Environ Health A*. 71(23):1516–25. doi:10.1080/15287390802391943 PMID:18923994

Leter G, Consales C, Eleuteri P, Uccelli R, Specht IO, Toft G, et al. (2014). Exposure to perfluoroalkyl substances and sperm DNA global methylation in Arctic and European populations. *Environ Mol Mutagen*. 55(7):591–600. doi:10.1002/em.21874 PMID:24889506

Lewis RC, Johns LE, Meeker JD (2015). Serum biomarkers of exposure to perfluoroalkyl substances in relation to serum testosterone and measures of thyroid function among adults and adolescents from NHANES 2011–2012. *Int J Environ Res Public Health*. 12(6):6098–114. doi:10.3390/ijerph120606098 PMID:26035660

Li A, Koch Z, Ideker T (2022b). Epigenetic aging: biological age prediction and informing a mechanistic theory of aging. *J Intern Med*. 292(5):733–44. doi:10.1111/joim.13533 PMID:35726002

Li C, Jiang L, Qi Y, Zhang D, Liu X, Han W, et al. (2023b). Integration of metabolomics and proteomics reveals the underlying hepatotoxic mechanism of perfluorooctane sulfonate (PFOS) and 6:2 chlorinated polyfluoroalkyl ether sulfonic acid (6:2 Cl-PFESA) in primary human hepatocytes. *Ecotoxicol Environ Saf*. 249:114361. doi:10.1016/j.ecoenv.2022.114361 PMID:36508832

Li C, Liu X, Liu Q, Li S, Li Y, Hu H, et al. (2017b). Protection of taurine against PFOS-induced neurotoxicity in PC12 Cells. *Adv Exp Med Biol*. 975(Pt 2):907–16. doi:10.1007/978-94-024-1079-2_72 PMID:28849510

Li C-H, Ren X-M, Guo L-H (2019b). Adipogenic activity of oligomeric hexafluoropropylene oxide (perfluorooctanoic acid alternative) through peroxisome proliferator-activated receptor γ pathway. *Environ Sci Technol*. 53(6):3287–95. doi:10.1021/acs.est.8b06978 PMID:30785727

Li C-H, Ren X-M, Ruan T, Cao L-Y, Xin Y, Guo L-H, et al. (2018b). Chlorinated polyfluorinated ether sulfonates exhibit higher activity toward peroxisome proliferator-activated receptors signalling pathways than perfluorooctanesulfonate. *Environ Sci Technol*. 52(5):3232–9. doi:10.1021/acs.est.7b06327 PMID:29389105

Li D, Zhang L, Zhang Y, Guan S, Gong X, Wang X (2019a). Maternal exposure to perfluorooctanoic acid (PFOA) causes liver toxicity through PPAR-α pathway and lowered histone acetylation in female offspring mice. *Environ Sci Pollut Res Int*. 26(18):18866–75. doi:10.1007/s11356-019-05258-z PMID:31062244

Li J, Cao H, Feng H, Xue Q, Zhang A, Fu J (2020e). Evaluation of the estrogenic/antiestrogenic activities of perfluoroalkyl substances and their interactions with the human estrogen receptor by combining in vitro assays and in silico modeling. *Environ Sci Technol*. 54(22):14514–24. doi:10.1021/acs.est.0c03468 PMID:33111528

Li J, Quan X, Lei S, Chen G, Hong J, Huang Z, et al. (2022c). LncRNA MEG3 alleviates PFOS induced placental cell growth inhibition through its derived miR-770 targeting PTX3. *Environ Pollut*. 293:118542. doi:10.1016/j.envpol.2021.118542 PMID:34801623

Li J, Quan X, Lei S, Huang Z, Wang Q, Xu P (2021a). PFOS inhibited normal functional development of placenta cells via *PPARγ* signalling. *Biomedicines*. 9(6):677. doi:10.3390/biomedicines9060677 PMID:34203907

Li J, Quan XJ, Chen G, Hong JW, Wang Q, Xu LL, et al. (2020b). PFOS-induced placental cell growth inhibition is partially mediated by lncRNA H19 through interacting with miR-19a and miR-19b. *Chemosphere*. 261:127640. doi:10.1016/j.chemosphere.2020.127640 PMID:32738709

Li K, Sun J, Yang J, Roberts SM, Zhang X, Cui X, et al. (2017a). Molecular mechanisms of perfluorooctanoate-induced hepatocyte apoptosis in mice using proteomic techniques. *Environ Sci Technol*. 51(19):11380–9. doi:10.1021/acs.est.7b02690 PMID:28885018

Li L, Guo Y, Ma S, Wen H, Li Y, Qiao J (2024). Association between exposure to per- and perfluoroalkyl substances (PFAS) and reproductive hormones in human: a systematic review and meta-analysis. *Environ Res*. 241:117553. doi:10.1016/j.envres.2023.117553 PMID:37931739

Li L, Xu ZS, Song GW (2009). Study on the Langmuir aggregation of fluorinated surfactants on protein. *J Fluor Chem*. 130(2):225–30. doi:10.1016/j.jfluchem.2008.10.007

Li Q-Q, Liu J-J, Su F, Zhang Y-T, Wu L-Y, Chu C, et al. (2022d). Chlorinated polyfluorinated ether sulfonates and thyroid hormone levels in adults: isomers of C8 Health Project in China. *Environ Sci Technol*. 56(10):6152–61. doi:10.1021/acs.est.1c03757 PMID:35380809

Li R, Guo C, Lin X, Chan TF, Lai KP, Chen J (2020c). Integrative omics analyses uncover the mechanism underlying the immunotoxicity of perfluorooctanesulfonate in human lymphocytes. *Chemosphere*. 256:127062. doi:10.1016/j.chemosphere.2020.127062 PMID:32434090

Li R, Guo C, Lin X, Chan TF, Su M, Zhang Z, et al. (2022g). Integrative omics analysis reveals the protective role of vitamin C on perfluorooctanoic acid-induced hepatoxicity. *J Adv Res*. 35:279–94. doi:10.1016/j.jare.2021.04.003 PMID:35024202

Li R, Guo C, Tse WKF, Su M, Zhang X, Lai KP (2020d). Metabolomic analysis reveals metabolic alterations of human peripheral blood lymphocytes by perfluorooctanoic acid. *Chemosphere*. 239:124810. doi:10.1016/j.chemosphere.2019.124810 PMID:31520980

Li W, He QZ, Wu CQ, Pan XY, Wang J, Tan Y, et al. (2015). PFOS disturbs BDNF-ERK-CREB signalling in association with increased microRNA-22 in SH-SY5Y cells.

BioMed Res Int. 2015:302653. doi:10.1155/2015/302653 PMID:26649298

Li X, Bao C, Ma Z, Xu B, Ying X, Liu X, et al. (2018c). Perfluorooctanoic acid stimulates ovarian cancer cell migration, invasion via ERK/NF-κB/MMP-2/-9 pathway. *Toxicol Lett*. 294(15 September):44–50. doi:10.1016/j.toxlet.2018.05.009 PMID:29753068

Li X, Chen L, Zhou H, Wang J, Zhao C, Pang X (2022f). PFOA regulate adenosine receptors and downstream concentration-response cAMP–PKA pathway revealed by integrated omics and molecular dynamics analyses. *Sci Total Environ*. 803:149910. doi:10.1016/j.scitotenv.2021.149910 PMID:34500266

Li X, Liu H, Wan H, Li Y, Xu S, Xiao H, et al. (2023a). Sex-specific associations between legacy and novel per- and polyfluoroalkyl substances and telomere length in newborns in Wuhan, China: mixture and single pollutant associations. *Sci Total Environ*. 857(Pt 3):159676. doi:10.1016/j.scitotenv.2022.159676 PMID:36283531

Li X, Wang Z, Klaunig JE (2019c). The effects of perfluorooctanoate on high fat diet induced non-alcoholic fatty liver disease in mice. *Toxicology*. 416:1–14. doi:10.1016/j.tox.2019.01.017 PMID:30711707

Li Y, Andersson A, Xu Y, Pineda D, Nilsson CA, Lindh CH, et al. (2022a). Determinants of serum half-lives for linear and branched perfluoroalkyl substances after long-term high exposure – a study in Ronneby, Sweden. *Environ Int*. 163:107198. doi:10.1016/j.envint.2022.107198 PMID:35447437

Li Y, Barregard L, Xu Y, Scott K, Pineda D, Lindh CH, et al. (2020a). Associations between perfluoroalkyl substances and serum lipids in a Swedish adult population with contaminated drinking water. *Environ Health*. 19(1):33. doi:10.1186/s12940-020-00588-9 PMID:32169067

Li Y, Cheng Y, Xie Z, Zeng F (2017c). Perfluorinated alkyl substances in serum of the southern Chinese general population and potential impact on thyroid hormones. *Sci Rep*. 7(1):43380. doi:10.1038/srep43380 PMID:28240244

Li Y, Fletcher T, Mucs D, Scott K, Lindh CH, Tallving P, et al. (2018a). Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. *Occup Environ Med*. 75(1):46–51. doi:10.1136/oemed-2017-104651 PMID:29133598

Li Y, Ramdhan DH, Naito H, Yamagishi N, Ito Y, Hayashi Y, et al. (2011). Ammonium perfluorooctanoate may cause testosterone reduction by adversely affecting testis in relation to PPARα. *Toxicol Lett*. 205(3):265–72. doi:10.1016/j.toxlet.2011.06.015 PMID:21712084

Li Y, Xu Y, Fletcher T, Scott K, Nielsen C, Pineda D, et al. (2021b). Associations between perfluoroalkyl substances and thyroid hormones after high exposure through drinking water. *Environ Res*. 194:110647. doi:10.1016/j.envres.2020.110647 PMID:33358873

Li Z, Lin Z, Ji S, Lai KP, Wan HT, Wong CKC, et al. (2022e). Perfluorooctanesulfonic acid exposure altered hypothalamic metabolism and disturbed male fecundity. *Sci Total Environ*. 844:156881. doi:10.1016/j.scitotenv.2022.156881 PMID:35753445

Liang H, Wang Z, Miao M, Tian Y, Zhou Y, Wen S, et al. (2020). Prenatal exposure to perfluoroalkyl substances and thyroid hormone concentrations in cord plasma in a Chinese birth cohort. *Environ Health*. 19(1):127. doi:10.1186/s12940-020-00679-7 PMID:33243245

Liao Y, Dong S, Kiyama R, Cai P, Liu L, Shen H (2013). *Flos lonicerae* extracts and chlorogenic acid protect human umbilical vein endothelial cells from the toxic damage of perfluorooctane sulphonate. *Inflammation*. 36(3):767–79.doi:10.1007/s10753-013-9603-5PMID:23392856

Liao Y, Wang J, Huang QS, Fang C, Kiyama R, Shen H, et al. (2012). Evaluation of cellular response to perfluorooctane sulfonate in human umbilical vein endothelial cells. *Toxicol In Vitro*. 26(3):421–8. doi:10.1016/j.tiv.2012.01.011 PMID:22269386

Lin CY, Chen PC, Lo SC, Torng PL, Sung FC, Su TC (2016). The association of carotid intima–media thickness with serum level of perfluorinated chemicals and endothelium-platelet microparticles in adolescents and young adults. *Environ Int*. 94:292–9. doi:10.1016/j.envint.2016.06.004 PMID:27288966

Lin CY, Lee HL, Chen CW, Wang C, Sung FC, Su TC (2022). Global DNA methylation mediates the association between serum perfluorooctane sulfonate and carotid intima–media thickness in young and middle-aged Taiwanese populations. *Ecotoxicol Environ Saf*. 241:113782. doi:10.1016/j.ecoenv.2022.113782 PMID:35753273

Lin CY, Lee HL, Hwang YT, Su TC (2020b). The association between total serum isomers of per- and polyfluoroalkyl substances, lipid profiles, and the DNA oxidative/nitrative stress biomarkers in middle-aged Taiwanese adults. *Environ Res*. 182:109064. doi:10.1016/j.envres.2019.109064 PMID:31884197

Lin C-Y, Wen LL, Lin L-Y, Wen T-W, Lien G-W, Chen C-Y, et al. (2011). Associations between levels of serum perfluorinated chemicals and adiponectin in a young hypertension cohort in Taiwan. *Environ Sci Technol*. 45(24):10691–8. doi:10.1021/es201964x PMID:22047537

Lin CY, Wen LL, Lin LY, Wen TW, Lien GW, Hsu SH, et al. (2013). The associations between serum perfluorinated chemicals and thyroid function in adolescents and young adults. *J Hazard Mater*. 244–245:637–44. doi:10.1016/j.jhazmat.2012.10.049 PMID:23177245

Lin T, Zhang Y, Ding X, Huang T, Zhang W, Zou W, et al. (2020a). Perfluorooctanoic acid induces cytotoxicity in spermatogonial GC-1 cells. *Chemosphere*. 260:127545. doi:10.1016/j.chemosphere.2020.127545 PMID:32653749

Lindeman B, Maass C, Duale N, Gützkow KB, Brunborg G, Andreassen Å (2012). Effects of per- and polyfluorinated compounds on adult rat testicular cells following in vitro exposure. *Reprod Toxicol*. 33(4):531–7. doi:10.1016/j.reprotox.2011.04.001 PMID:21530637

Liu C, Chang VW, Gin KY (2014a). Oxidative toxicity of perfluorinated chemicals in green mussel and bioaccumulation factor dependent quantitative structure–activity relationship. *Environ Toxicol Chem*. 33(10):2323–32. doi:10.1002/etc.2679 PMID:24995545

Liu C, Chang VW, Gin KY, Nguyen VT (2014b). Genotoxicity of perfluorinated chemicals (PFCs) to the green mussel (*Perna viridis*). *Sci Total Environ*. 487:117–22. doi:10.1016/j.scitotenv.2014.04.017 PMID:24784736

Liu CY, Chen PC, Lien PC, Liao YP (2018a). Prenatal perfluorooctyl sulfonate exposure and Alu DNA hypomethylation in cord blood. *Int J Environ Res Public Health*. 15(6):1066. doi:10.3390/ijerph15061066 PMID:29795014

Liu D, Tang B, Nie S, Zhao N, He L, Cui J, et al. (2023a). Distribution of per- and poly-fluoroalkyl substances and their precursors in human blood. *J Hazard Mater*. 441:129908. doi:10.1016/j.jhazmat.2022.129908 PMID:36115093

Liu H, Chen Q, Lei L, Zhou W, Huang L, Zhang J, et al. (2018b). Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances affects leukocyte telomere length in female newborns. *Environ Pollut*. 235:446–52. doi:10.1016/j.envpol.2017.12.095 PMID:29310088

Liu H, Huang Y, Pan Y, Cheng R, Li X, Li Y, et al. (2023c). Associations between per and polyfluoroalkyl ether sulfonic acids and vitamin D biomarker levels in Chinese newborns. *Sci Total Environ*. 866:161410. doi:10.1016/j.scitotenv.2023.161410 PMID:36621489

Liu H, Pan Y, Jin S, Li Y, Zhao L, Sun X, et al. (2020b). Associations of per-/polyfluoroalkyl substances with glucocorticoids and progestogens in newborns. *Environ Int*. 140:105636. doi:10.1016/j.envint.2020.105636 PMID:32474218

Liu H, Pan Y, Jin S, Sun X, Jiang Y, Wang Y, et al. (2021). Associations between six common per- and polyfluoroalkyl substances and estrogens in neonates of China. *J Hazard Mater*. 407:124378. doi:10.1016/j.jhazmat.2020.124378 PMID:33139105

Liu L, Liu W, Song J, Yu H, Jin Y, Oami K, et al. (2009). A comparative study on oxidative damage and distributions of perfluorooctane sulfonate (PFOS) in mice at different postnatal developmental stages. *J Toxicol Sci*. 34(3):245–54. doi:10.2131/jts.34.245 PMID:19483379

Liu M, Zhang G, Meng L, Han X, Li Y, Shi Y, et al. (2022b). Associations between novel and legacy per- and polyfluoroalkyl substances in human serum and thyroid cancer: a case and healthy population in Shandong Province, East China. *Environ Sci Technol*. 56(10):6144–51. doi:10.1021/acs.est.1c02850 PMID:34618433

Liu RC, Hurtt ME, Cook JC, Biegel LB (1996). Effect of the peroxisome proliferator, ammonium perfluorooctanoate (C8), on hepatic aromatase activity in adult male Crl:CD BR (CD) rats. *Fundam Appl Toxicol*. 30(2):220–8. doi:10.1006/faat.1996.0059 PMID:8812269

Liu W, Irudayaraj J (2020). Perfluorooctanoic acid (PFOA) exposure inhibits DNA methyltransferase activities and alters constitutive heterochromatin organization. *Food Chem Toxicol*. 141:111358. doi:10.1016/j.fct.2020.111358 PMID:32315686

Liu W, Qin H, Pan Y, Luo F, Zhang Z (2019). Low concentrations of perfluorooctane sulfonate repress osteogenic and enhance adipogenic differentiation of human mesenchymal stem cells. *Toxicol Appl Pharmacol*. 367:82–91. doi:10.1016/j.taap.2019.02.001 PMID:30738844

Liu W, Xu C, Sun X, Kuang H, Kuang X, Zou W, et al. (2015b). Grape seed proanthocyanidin extract protects against perfluorooctanoic acid-induced hepatotoxicity by attenuating inflammatory response, oxidative stress and apoptosis in mice. *Toxicol Res (Camb)*. 5(1):224–34. doi:10.1039/C5TX00260E PMID:30090339

Liu W, Yang B, Wu L, Zou W, Pan X, Zou T, et al. (2015a). Involvement of NRF2 in perfluorooctanoic acid-induced testicular damage in male mice. *Biol Reprod*. 93(2):41. doi:10.1095/biolreprod.115.128819 PMID:26108789

Liu X, Chen D, Wang B, Xu F, Pang Y, Zhang L, et al. (2020a). Does low maternal exposure to per- and polyfluoroalkyl substances elevate the risk of spontaneous preterm birth? A nested case–control study in China. *Environ Sci Technol*. 54(13):8259–68. doi:10.1021/acs.est.0c01930 PMID:32510220

Liu Y, Eliot MN, Papandonatos GD, Kelsey KT, Fore R, Langevin S, et al. (2022a). Gestational perfluoroalkyl substance exposure and DNA methylation at birth and 12 years of age: a longitudinal epigenome-wide association study. *Environ Health Perspect*. 130(3):37005. doi:10.1289/EHP10118 PMID:35266797

Liu Y, Li J, Ding H, Ge D, Wang J, Xu C (2022c). Perfluorooctane sulfonate (PFOS) triggers migration and invasion of esophageal squamous cell carcinoma cells via regulation of Zeb1. *Drug Chem Toxicol*. 45(6):2804–13. doi:10.1080/01480545.2021.1991775 PMID:34732098

Liu Y, Wang J, Wei Y, Zhang H, Liu Y, Dai J (2008). Molecular characterization of cytochrome P450 1A and 3A and the effects of perfluorooctanoic acid on their mRNA levels in rare minnow (*Gobiocypris rarus*) gills. *Aquat Toxicol*. 88(3):183–90. doi:10.1016/j.aquatox.2008.04.008 PMID:18508136

Liu Y, Yu G, Zhang R, Feng L, Zhang J (2023b). Early life exposure to low-dose perfluorooctane sulfonate disturbs gut barrier homeostasis and increases the risk of intestinal inflammation in offspring. *Environ*

Pollut. 329:121708. doi:10.1016/j.envpol.2023.121708 PMID:37100370

Loccisano AE, Campbell JL Jr, Andersen ME, Clewell HJ 3rd (2011). Evaluation and prediction of pharmacokinetics of PFOA and PFOS in the monkey and human using a PBPK model. *Regul Toxicol Pharmacol*. 59(1):157–75. doi:10.1016/j.yrtph.2010.12.004 PMID:21168463

Loccisano AE, Longnecker MP, Campbell JL Jr, Andersen ME, Clewell HJ 3rd (2013). Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages. *J Toxicol Environ Health A*. 76(1):25–57. doi:10.1080/15287394.2012.722523 PMID:23151209

Loft S, Olsen A, Møller P, Poulsen HE, Tjønneland A (2013). Association between 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion and risk of postmenopausal breast cancer: nested case–control study. *Cancer Epidemiol Biomarkers Prev*. 22(7):1289–96. doi:10.1158/1055-9965.EPI-13-0229 PMID:23658396

Loft S, Svoboda P, Kasai H, Tjønneland A, Vogel U, Møller P, et al. (2006). Prospective study of 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion and the risk of lung cancer. *Carcinogenesis*. 27(6):1245–50. doi:10.1093/carcin/bgi313 PMID:16364924

Loft S, Svoboda P, Kawai K, Kasai H, Sørensen M, Tjønneland A, et al. (2012). Association between 8-oxo-7,8-dihydroguanine excretion and risk of lung cancer in a prospective study. *Free Radic Biol Med*. 52(1):167–72. doi:10.1016/j.freeradbiomed.2011.10.439 PMID:22044660

Logeshwaran P, Sivaram AK, Surapaneni A, Kannan K, Naidu R, Megharaj M (2021). Exposure to perfluorooctanesulfonate (PFOS) but not perfluorooctanoic acid (PFOA) at ppb concentration induces chronic toxicity in *Daphnia carinata*. *Sci Total Environ*. 769:144577. doi:10.1016/j.scitotenv.2020.144577 PMID:33482550

Long M, Ghisari M, Bonefeld-Jørgensen EC (2013). Effects of perfluoroalkyl acids on the function of the thyroid hormone and the aryl hydrocarbon receptor. *Environ Sci Pollut Res Int*. 20(11):8045–56. doi:10.1007/s11356-013-1628-7 PMID:23539207

Looker C, Luster MI, Calafat AM, Johnson VJ, Burleson GR, Burleson FG, et al. (2014). Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. *Toxicol Sci*. 138(1):76–88. doi:10.1093/toxsci/kft269 PMID:24284791

López-Arellano P, López-Arellano K, Luna J, Flores D, Jiménez-Salazar J, Gavia G, et al. (2019). Perfluorooctanoic acid disrupts gap junction intercellular communication and induces reactive oxygen species formation and apoptosis in mouse ovaries. *Environ Toxicol*. 34(1):92–8. doi:10.1002/tox.22661 PMID:30277307

López-Doval S, Salgado R, Fernández-Pérez B, Lafuente A (2015). Possible role of serotonin and neuropeptide Y on the disruption of the reproductive axis activity by perfluorooctane sulfonate. *Toxicol Lett*. 233(2):138–47. doi:10.1016/j.toxlet.2015.01.012 PMID:25623392

Lopez-Espinosa MJ, Carrizosa C, Luster MI, Margolick JB, Costa O, Leonardi GS, et al. (2021). Perfluoroalkyl substances and immune cell counts in adults from the Mid-Ohio Valley (USA). *Environ Int*. 156:106599. doi:10.1016/j.envint.2021.106599 PMID:33993002

Lopez-Espinosa M-J, Mondal D, Armstrong B, Bloom MS, Fletcher T (2012). Thyroid function and perfluoroalkyl acids in children living near a chemical plant. *Environ Health Perspect*. 120(7):1036–41. doi:10.1289/ehp.1104370 PMID:22453676

Lopez-Espinosa M-J, Mondal D, Armstrong BG, Eskenazi B, Fletcher T (2016). Perfluoroalkyl substances, sex hormones, and insulin-like growth factor-1 at 6–9 years of age: a cross-sectional analysis within the C8 Health Project. *Environ Health Perspect*. 124(8):1269–75. doi:10.1289/ehp.1509869 PMID:26794451

Lou I, Wambaugh JF, Lau C, Hanson RG, Lindstrom AB, Strynar MJ, et al. (2009). Modeling single and repeated dose pharmacokinetics of PFOA in mice. *Toxicol Sci*. 107(2):331–41. doi:10.1093/toxsci/kfn234 PMID:19005225

Louisse J, Fragki S, Rijkers D, Janssen A, van Dijk B, Leenders L, et al. (2023). Determination of in vitro hepatotoxic potencies of a series of perfluoroalkyl substances (PFASs) based on gene expression changes in HepaRG liver cells. *Arch Toxicol*. 97(4):1113–31. doi:10.1007/s00204-023-03450-2 PMID:36864359

Louisse J, Rijkers D, Stoopen G, Janssen A, Staats M, Hoogenboom R, et al. (2020). Perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), and perfluorononanoic acid (PFNA) increase triglyceride levels and decrease cholesterogenic gene expression in human HepaRG liver cells. *Arch Toxicol*. 94(9):3137–55. doi:10.1007/s00204-020-02808-0 PMID:32588087

Loveless SE, Finlay C, Everds NE, Frame SR, Gillies PJ, O'Connor JC, et al. (2006). Comparative responses of rats and mice exposed to linear/branched, linear, or branched ammonium perfluorooctanoate (APFO). *Toxicology*. 220(2–3):203–17. doi:10.1016/j.tox.2006.01.003 PMID:16448737

Loveless SE, Hoban D, Sykes G, Frame SR, Everds NE (2008). Evaluation of the immune system in rats and mice administered linear ammonium perfluorooctanoate. *Toxicol Sci*. 105(1):86–96. doi:10.1093/toxsci/kfn113 PMID:18559402

Lu H, Zhang H, Gao J, Li Z, Bao S, Chen X, et al. (2019). Effects of perfluorooctanoic acid on stem Leydig cell functions in the rat. *Environ Pollut*. 250:206–15. doi:10.1016/j.envpol.2019.03.120 PMID:30999198

Lu L, Guo L, Li M, Kang T, Cheng S, Miao W (2016a). Investigation of perfluorooctanoic acid induced DNA damage using electrogenerated chemiluminescence associated with charge transfer in DNA. *Anal Bioanal*

Chem. 408(25):7137–45. doi:10.1007/s00216-016-9559-z PMID:27108285

Lu L, Xu L, Kang T, Cheng S (2012). Investigation of DNA damage treated with perfluorooctane sulfonate (PFOS) on ZrO2/DDAB active nano-order film. *Biosens Bioelectron*. 35(1):180–5. doi:10.1016/j.bios.2012.02.043 PMID:22459586

Lu Y, Luo B, Li J, Dai J (2016c). Perfluorooctanoic acid disrupts the blood–testis barrier and activates the TNFα/p38 MAPK signalling pathway in vivo and in vitro. *Arch Toxicol*. 90(4):971–83. doi:10.1007/s00204-015-1492-y PMID:25743374

Lu Y, Pan Y, Sheng N, Zhao AZ, Dai J (2016b). Perfluorooctanoic acid exposure alters polyunsaturated fatty acid composition, induces oxidative stress and activates the AKT/AMPK pathway in mouse epididymis. *Chemosphere*. 158:143–53. doi:10.1016/j.chemosphere.2016.05.071 PMID:27262104

Lu Y, Wang J, Guo X, Yan S, Dai J (2017). Perfluorooctanoic acid affects endocytosis involving clathrin light chain A and microRNA-133b-3p in mouse testes. *Toxicol Appl Pharmacol*. 318:41–8. doi:10.1016/j.taap.2017.01.014 PMID:28126411

Luebker DJ, Hansen KJ, Bass NM, Butenhoff JL, Seacat AM (2002). Interactions of fluorochemicals with rat liver fatty acid-binding protein. *Toxicology*. 176(3):175–85. doi:10.1016/S0300-483X(02)00081-1 PMID:12093614

Luebker DJ, York RG, Hansen KJ, Moore JA, Butenhoff JL (2005). Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters. *Toxicology*. 215(1–2):149–69. doi:10.1016/j.tox.2005.07.019 PMID:16129535

Luo K, Liu X, Nian M, Wang Y, Qiu J, Yu H, et al.; Shanghai Birth Cohort (2021). Environmental exposure to per- and polyfluoroalkyl substances mixture and male reproductive hormones. *Environ Int*. 152:106496. doi:10.1016/j.envint.2021.106496 PMID:33744484

Luo Z, Shi X, Hu Q, Zhao B, Huang M (2012). Structural evidence of perfluorooctane sulfonate transport by human serum albumin. *Chem Res Toxicol*. 25(5):990–2. doi:10.1021/tx300112p PMID:22482699

Lv QY, Wan B, Guo LH, Yang Y, Ren XM, Zhang H (2015). In vivo immunotoxicity of perfluorooctane sulfonate in BALB/c mice: identification of T-cell receptor and calcium-mediated signalling pathway disruption through gene expression profiling of the spleen. *Chem Biol Interact*. 240:84–93. doi:10.1016/j.cbi.2015.07.015 PMID:26300304

Lv Z, Li G, Li Y, Ying C, Chen J, Chen T, et al. (2013). Glucose and lipid homeostasis in adult rat is impaired by early-life exposure to perfluorooctane sulfonate. *Environ Toxicol*. 28(9):532–42. doi:10.1002/tox.20747 PMID:23983163

Lv Z, Wu W, Ge S, Jia R, Lin T, Yuan Y, et al. (2018). Naringin protects against perfluorooctane sulfonate-induced liver injury by modulating NRF2 and NF-κB in mice. *Int Immunopharmacol*. 65:140–7. doi:10.1016/j.intimp.2018.09.019 PMID:30316072

Ma S, Sun Y, Zheng X, Yang Y (2021). Gastrodin attenuates perfluorooctanoic acid-induced liver injury by regulating gut microbiota composition in mice. *Bioengineered*. 12(2):11546–56. doi:10.1080/21655979.2021.2009966 PMID:34866532

Ma X, Ren X, Zhang X, Griffin N, Liu H, Wang L (2023). Rutin ameliorates perfluorooctanoic acid-induced testicular injury in mice by reducing oxidative stress and improving lipid metabolism. *Drug Chem Toxicol*. 46(6):1223-34. https://www.tandfonline.com/doi/full/10.1080/01480545.2022.2145483?scroll=top&needAccess=truedoi:10.1080/01480545.2022.2145483 PMID:36373176

Ma Y, Yang J, Wan Y, Peng Y, Ding S, Li Y, et al. (2018). Low-level perfluorooctanoic acid enhances 3T3–L1 preadipocyte differentiation via altering peroxisome proliferator activated receptor gamma expression and its promoter DNA methylation. *J Appl Toxicol*. 38(3):398–407. doi:10.1002/jat.3549 PMID:29094436

Ma Z, Liu X, Li F, Wang Y, Xu Y, Zhang M, et al. (2016). Perfluorooctanoic acid induces human Ishikawa endometrial cancer cell migration and invasion through activation of ERK/mTOR signalling. *Oncotarget*. 7(41):66558–68. doi:10.18632/oncotarget.11684 PMID:27589685

Macon MB, Villanueva LR, Tatum-Gibbs K, Zehr RD, Strynar MJ, Stanko JP, et al. (2011). Prenatal perfluorooctanoic acid exposure in CD-1 mice: low-dose developmental effects and internal dosimetry. *Toxicol Sci*. 122(1):134–45. doi:10.1093/toxsci/kfr076 PMID:21482639

Maddalon A, Cari L, Iulini M, Alhosseini MN, Galbiati V, Marinovich M, et al. (2023a). Impact of endocrine disruptors on peripheral blood mononuclear cells in vitro: role of gender. *Arch Toxicol*. 97(12):3129–50. doi:10.1007/s00204-023-03592-3 PMID:37676302

Maddalon A, Pierzchalski A, Kretschmer T, Bauer M, Zenclussen AC, Marinovich M, et al. (2023b). Mixtures of per- and poly-fluoroalkyl substances (PFAS) reduce the in vitro activation of human T cells and basophils. *Chemosphere*. 336:139204. doi:10.1016/j.chemosphere.2023.139204 PMID:37315852

Maestri L, Negri S, Ferrari M, Ghittori S, Fabris F, Danesino P, et al. (2006). Determination of perfluorooctanoic acid and perfluorooctanesulfonate in human tissues by liquid chromatography/single quadrupole mass spectrometry. *Rapid Commun Mass Spectrom*. 20(18):2728–34. doi:10.1002/rcm.2661 PMID:16915561

Magnifico MC, Xhani M, Sprovera B, Buttari B, Abballe G, Desideri F, et al. (2022). Nitro-oxidative stress and mitochondrial dysfunction in human cell lines exposed

to the environmental contaminants PFOA and BPA. *Front Biosci (Landmark Ed)*. 27(10):292. doi:10.31083/j.fbl2710292 PMID:36336871

Maisonet M, Calafat AM, Marcus M, Jaakkola JJK, Lashen H (2015). Prenatal exposure to perfluoroalkyl acids and serum testosterone concentrations at 15 years of age in female ALSPAC study participants. *Environ Health Perspect*. 123(12):1325–30. doi:10.1289/ehp.1408847 PMID:26034840

Maloney EK, Waxman DJ (1999). *trans*-Activation of PPARα and PPARγ by structurally diverse environmental chemicals. *Toxicol Appl Pharmacol*. 161(2):209–18. doi:10.1006/taap.1999.8809 PMID:10581215

Mamsen LS, Björvang RD, Mucs D, Vinnars M-T, Papadogiannakis N, Lindh CH, et al. (2019). Concentrations of perfluoroalkyl substances (PFASs) in human embryonic and fetal organs from first, second, and third trimester pregnancies. *Environ Int*. 124:482–92. doi:10.1016/j.envint.2019.01.010 PMID:30684806

Mandard S, Müller M, Kersten S (2004). Peroxisome proliferator-activated receptor alpha target genes. *Cell Mol Life Sci*. 61(4):393–416. doi:10.1007/s00018-003-3216-3 PMID:14999402

Manzano-Salgado CB, Granum B, Lopez-Espinosa MJ, Ballester F, Iñiguez C, Gascón M, et al. (2019). Prenatal exposure to perfluoroalkyl substances, immune-related outcomes, and lung function in children from a Spanish birth cohort study. *Int J Hyg Environ Health*. 222(6):945–54. doi:10.1016/j.ijheh.2019.06.005 PMID:31262703

Mao B, Li C, Wen Z, Li H, Wang Y, Chen L, et al. (2021). Short-term perfluorooctane sulfonate exposure impairs Leydig cell regeneration in the adult rat testis via targeting hedgehog signalling. *Ecotoxicol Environ Saf*. 214:112121. doi:10.1016/j.ecoenv.2021.112121 PMID:33721578

Mao Z, Xia W, Wang J, Chen T, Zeng Q, Xu B, et al. (2013). Perfluorooctane sulfonate induces apoptosis in lung cancer A549 cells through reactive oxygen species-mediated mitochondrion-dependent pathway. *J Appl Toxicol*. 33(11):1268–76. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/epdf/10.1002/jat.2785 PMID:22976841

Mario T, Yvonne D, Veronica S, Alejandro D, Juan RM, Diana F, et al. (2022). Effects of perfluorooctanoic acid in oxidative stress generation, DNA damage in cumulus cells, and its impact on in vitro maturation of porcine oocytes. *Environ Toxicol*. 37(6):1394–403. doi:10.1002/tox.23492 PMID:35187785

Marques E, Pfohl M, Wei W, Tarantola G, Ford L, Amaeze O, et al. (2022). Replacement per- and polyfluoroalkyl substances (PFAS) are potent modulators of lipogenic and drug metabolizing gene expression signatures in primary human hepatocytes. *Toxicol Appl Pharmacol*. 442:115991. doi:10.1016/j.taap.2022.115991 PMID:35337807

Martin MT, Brennan RJ, Hu W, Ayanoglu E, Lau C, Ren H, et al. (2007). Toxicogenomic study of triazole fungicides and perfluoroalkyl acids in rat livers predicts toxicity and categorizes chemicals based on mechanisms of toxicity. *Toxicol Sci*. 97(2):595–613. doi:10.1093/toxsci/kfm065 PMID:17383973

Mashayekhi V, Tehrani KH, Hashemzaei M, Tabrizian K, Shahraki J, Hosseini MJ (2015). Mechanistic approach for the toxic effects of perfluorooctanoic acid on isolated rat liver and brain mitochondria. *Hum Exp Toxicol*. 34(10):985–96. doi:10.1177/0960327114565492 PMID:25586001

Masi M, Maddalon A, Iulini M, Linciano P, Galbiati V, Marinovich M, et al. (2022). Effects of endocrine disrupting chemicals on the expression of RACK1 and LPS-induced THP-1 cell activation. *Toxicology*. 480:153321. doi:10.1016/j.tox.2022.153321 PMID:36113621

Maso L, Trande M, Liberi S, Moro G, Daems E, Linciano S, et al. (2021). Unveiling the binding mode of perfluorooctanoic acid to human serum albumin. *Protein Sci*. 30(4):830–41. doi:10.1002/pro.4036 PMID:33550662

Matkowskyj KA, Bai H, Liao J, Zhang W, Li H, Rao S, et al. (2014). Aldoketoreductase family 1B10 (AKR1B10) as a biomarker to distinguish hepatocellular carcinoma from benign liver lesions. *Hum Pathol*. 45(4):834–43. doi:10.1016/j.humpath.2013.12.002 PMID:24656094

Matrix Science (2023). Mascot search engine. Boston (MA), USA: Matrix Science. Available from: http://www.matrixscience.com.

Max Planck Institute of Biochemistry (2023). MaxQuant software v1.6.10.43. Martinsried, Germany: Max Planck Institute of Biochemistry. Available from: http://maxquant.org/.

Mazumder N-U-S, Hossain MT, Jahura FT, Girase A, Hall AS, Lu J, et al. (2023). Firefighters' exposure to per-and polyfluoroalkyl substances (PFAS) as an occupational hazard: a review. *Front Mater*. 10:1143411. doi:10.3389/fmats.2023.1143411 PMID:38074949

McCoy JA, Bangma JT, Reiner JL, Bowden JA, Schnorr J, Slowey M, et al. (2017). Associations between perfluorinated alkyl acids in blood and ovarian follicular fluid and ovarian function in women undergoing assisted reproductive treatment. *Sci Total Environ*. 605–606:9–17. doi:10.1016/j.scitotenv.2017.06.137 PMID:28651210

McDonough CA, Ward C, Hu Q, Vance S, Higgins CP, DeWitt JC (2020). Immunotoxicity of an electrochemically fluorinated aqueous film-forming foam. *Toxicol Sci*. 178(1):104–14. doi:10.1093/toxsci/kfaa138 PMID:32898269

Messina P, Prieto G, Dodero V, Ruso JM, Schulz P, Sarmiento F (2005a). Ultraviolet-circular dichroism spectroscopy and potentiometric study of the interaction between human serum albumin and sodium perfluorooctanoate. *Biopolymers*. 79(6):300–9. doi:10.1002/bip.20353 PMID:16113999

Messina PV, Prieto G, Ruso JM, Sarmiento F (2005b). Conformational changes in human serum albumin induced by sodium perfluorooctanoate in aqueous solutions. *J Phys Chem B*. 109(32):15566–73. doi:10.1021/jp051655v PMID:16852974

Midgett K, Peden-Adams MM, Gilkeson GS, Kamen DL (2015). In vitro evaluation of the effects of perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) on IL-2 production in human T-cells. *J Appl Toxicol*. 35(5):459–65. doi:10.1002/jat.3037 PMID:25056757

Midic U, Goheen B, Vincent KA, VandeVoort CA, Latham KE (2018). Changes in gene expression following long-term in vitro exposure of *Macaca mulatta* trophoblast stem cells to biologically relevant levels of endocrine disruptors. *Reprod Toxicol*. 77:154–65. doi:10.1016/j.reprotox.2018.02.012 PMID:29505797

Minata M, Harada KH, Kärrman A, Hitomi T, Hirosawa M, Murata M, et al. (2010). Role of peroxisome proliferator-activated receptor-alpha in hepatobiliary injury induced by ammonium perfluorooctanoate in mouse liver. *Ind Health*. 48(1):96–107. doi:10.2486/indhealth.48.96 PMID:20160413

Mitro SD, Sagiv SK, Fleisch AF, Jaacks LM, Williams PL, Rifas-Shiman SL, et al. (2020). Pregnancy per- and polyfluoroalkyl substance concentrations and postpartum health in Project Viva: a prospective cohort. *J Clin Endocrinol Metab*. 105(9):e3415–26. doi:10.1210/clinem/dgaa431 PMID:32620010

Miura R, Araki A, Miyashita C, Kobayashi S, Kobayashi S, Wang SL, et al. (2018). An epigenome-wide study of cord blood DNA methylations in relation to prenatal perfluoroalkyl substance exposure: the Hokkaido study. *Environ Int*. 115:21–8. doi:10.1016/j.envint.2018.03.004 PMID:29544137

Modaresi SMS, Wei W, Emily M, DaSilva NA, Slitt AL (2022). Per- and polyfluoroalkyl substances (PFAS) augment adipogenesis and shift the proteome in murine 3T3–L1 adipocytes. *Toxicology*. 465:153044. doi:10.1016/j.tox.2021.153044 PMID:34800597

Mogensen UB, Grandjean P, Heilmann C, Nielsen F, Weihe P, Budtz-Jørgensen E (2015b). Structural equation modeling of immunotoxicity associated with exposure to perfluorinated alkylates. *Environ Health*. 14(1):47. doi:10.1186/s12940-015-0032-9 PMID:26041029

Mogensen UB, Grandjean P, Nielsen F, Weihe P, Budtz-Jørgensen E (2015a). Breastfeeding as an exposure pathway for perfluorinated alkylates. *Environ Sci Technol*. 49(17):10466–73. doi:10.1021/acs.est.5b02237 PMID:26291735

Mollenhauer MA, Bradshaw SG, Fair PA, McGuinn WD, Peden-Adams MM (2011). Effects of perfluorooctane sulfonate (PFOS) exposure on markers of inflammation in female B6C3F$_1$ mice. *J Environ Sci Health Part A Tox Hazard Subst Environ Eng*. 46(2):97–108. doi:10.1080/10934529.2011.532418 PMID:21170772

Mondal D, Weldon RH, Armstrong BG, Gibson LJ, Lopez-Espinosa M-J, Shin H-M, et al. (2014). Breastfeeding: a potential excretion route for mothers and implications for infant exposure to perfluoroalkyl acids. *Environ Health Perspect*. 122(2):187–92. doi:10.1289/ehp.1306613 PMID:24280536

Morimura K, Cheung C, Ward JM, Reddy JK, Gonzalez FJ (2006). Differential susceptibility of mice humanized for peroxisome proliferator-activated receptor alpha to Wy-14,643-induced liver tumorigenesis. *Carcinogenesis*. 27(5):1074–80. doi:10.1093/carcin/bgi329 PMID:16377806

Mortensen AS, Letcher RJ, Cangialosi MV, Chu S, Arukwe A (2011). Tissue bioaccumulation patterns, xenobiotic biotransformation and steroid hormone levels in Atlantic salmon (*Salmo salar*) fed a diet containing perfluoroactane sulfonic or perfluorooctane carboxylic acids. *Chemosphere*. 83(8):1035–44. doi:10.1016/j.chemosphere.2011.01.067 PMID:21354591

Murase W, Kubota A, Ikeda-Araki A, Terasaki M, Nakagawa K, Shizu R, et al. (2023). Effects of perfluorooctanoic acid (PFOA) on gene expression profiles via nuclear receptors in HepaRG cells: comparative study with in vitro transactivation assays. *Toxicology*. 494:153577. doi:10.1016/j.tox.2023.153577 PMID:37302725

Naderi M, Seyedabadi M, Amiri FT, Mohammadi E, Akbari S, Shaki F (2023). Taurine protects against perfluorooctanoic acid-induced hepatotoxicity via inhibition of oxidative stress, inflammatory, and apoptotic pathways. *Toxicol Res (Camb)*. 12(1):124–32. doi:10.1093/toxres/tfad005 PMID:36866213

Nakagawa H, Hirata T, Terada T, Jutabha P, Miura D, Harada KH, et al. (2008). Roles of organic anion transporters in the renal excretion of perfluorooctanoic acid. *Basic Clin Pharmacol Toxicol*. 103(1):1–8. doi:10.1111/j.1742-7843.2007.00155.x PMID:18373647

Nakagawa H, Terada T, Harada KH, Hitomi T, Inoue K, Inui K, et al. (2009). Human organic anion transporter hOAT4 is a transporter of perfluorooctanoic acid. *Basic Clin Pharmacol Toxicol*. 105(2):136–8. doi:10.1111/j.1742-7843.2009.00409.x PMID:19371258

Nakagawa T, Ramdhan DH, Tanaka N, Naito H, Tamada H, Ito Y, et al. (2012). Modulation of ammonium perfluorooctanoate-induced hepatic damage by genetically different PPARα in mice. *Arch Toxicol*. 86(1):63–74. doi:10.1007/s00204-011-0704-3 PMID:21499893

Nakamura R, Takeuchi R, Kuramochi K, Mizushina Y, Ishimaru C, Takakusagi Y, et al. (2007). Chemical properties of fatty acid derivatives as inhibitors of DNA polymerases. *Org Biomol Chem*. 5(24):3912–21. doi:10.1039/b710944j PMID:18043794

Nakamura T, Ito Y, Yanagiba Y, Ramdhan DH, Kono Y, Naito H, et al. (2009). Microgram-order ammonium perfluorooctanoate may activate mouse peroxisome proliferator-activated receptor α, but not human

PPARα. *Toxicology*. 265(1–2):27–33. doi:10.1016/j.tox.2009.09.004 PMID:19751795

National Center for Health Statistics (2023). NHANES 2007–2008 Laboratory Data Overview. USA: National Center for Health Statistics. Available from: https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/overviewlab.aspx?BeginYear=2007.

NCBI (2023). ACOX1 acyl-CoA oxidase 1 [*Homo sapiens* (human)]. Bethesda (MD), USA: National Library of Medicine, National Center for Biotechnology Information. Available from: https://www.ncbi.nlm.nih.gov/gene/51.

Nielsen G, Heiger-Bernays WJ, Schlezinger JJ, Webster TF (2022). Predicting the effects of per- and polyfluoroalkyl substance mixtures on peroxisome proliferator-activated receptor alpha activity in vitro. *Toxicology*. 465:153024. doi:10.1016/j.tox.2021.153024 PMID:34743024

Nilsson S, Thompson J, Mueller JF, Bräunig J (2022). Apparent half-lives of chlorinated-perfluorooctane sulfonate and perfluorooctane sulfonate isomers in aviation firefighters. *Environ Sci Technol*. 56(23):17052–60. doi:10.1021/acs.est.2c04637 PMID:36367310

Nishimura Y, Moriya K, Kobayashi S, Ikeda-Araki A, Sata F, Mitsui T, et al. (2022). Association of exposure to prenatal perfluoroalkyl substances and estrogen receptor 1 polymorphisms with the second to fourth digit ratio in school-aged children: the Hokkaido study. *Reprod Toxicol*. 109:10–8. doi:10.1016/j.reprotox.2022.02.002 PMID:35217143

NIST (2023). Automated mass spectral deconvolution and identification system for GC/MS Mass Spectrometry Data Center. AMDIS 2.71 database. Gaithersburg (MD), USA: National Institute of Standards and Technology. Available from: https://chemdata.nist.gov/.

Nøst TH, Vestergren R, Berg V, Nieboer E, Odland JØ, Sandanger TM (2014). Repeated measurements of per- and polyfluoroalkyl substances (PFASs) from 1979 to 2007 in males from northern Norway: assessing time trends, compound correlations and relations to age/birth cohort. *Environ Int*. 67:43–53. doi:10.1016/j.envint.2014.02.011 PMID:24657493

NTP (2019). Toxicity studies of perfluoroalkyl sulfonates administered by gavage to Sprague Dawley (Hsd:Sprague Dawley SD) rats (revised). *Toxic Rep Ser*. (96):NTP-TOX-96. PMID:33533754

NTP (2020). NTP technical report on the toxicology and carcinogenesis studies of perfluorooctanoic acid (CASRN 335-67-1) administered in feed to Sprague-Dawley (Hsd:Sprague-Dawley® SD®) rats (revised). *Natl Toxicol Program Tech Rep Ser*. (598). Revised February 2023. https://www.ncbi.nlm.nih.gov/books/NBK560147/

Oda Y, Nakayama S, Harada KH, Koizumi A (2007). Negative results of *umu* genotoxicity test of fluorotelomer alcohols and perfluorinated alkyl acids. *Environ Health Prev Med*. 12(5):217–9. doi:10.1265/ehpm.12.217 PMID:21432084

OECD (2002). Co-operation on existing chemicals hazard of perfluorooctane sulfonate (PFOS) and its salts. Report ENV/JM/RD(2002)17/FINAL. JT00135607. Paris, France: Organisation for Economic Co-operation and Development. Available from: https://www.regulations.gov/document/EPA-HQ-OPPT-2005-0015-0016, accessed January 2025.

Ojo AF, Peng C, Ng JC (2022a). Genotoxicity assessment of per- and polyfluoroalkyl substances mixtures in human liver cells (HepG2). *Toxicology*. 482:153359. doi:10.1016/j.tox.2022.153359 PMID:36341878

Ojo AF, Peng C, Ng JC (2022b). Combined effects of mixed per- and polyfluoroalkyl substances on the Nrf2–ARE pathway in ARE reporter–HepG2 cells. *J Hazard Mater*. 421:126827. doi:10.1016/j.jhazmat.2021.126827 PMID:34388917

Ojo AF, Xia Q, Peng C, Ng JC (2021). Evaluation of the individual and combined toxicity of perfluoroalkyl substances to human liver cells using biomarkers of oxidative stress. *Chemosphere*. 281:130808. doi:10.1016/j.chemosphere.2021.130808 PMID:34022600

Okada E, Sasaki S, Saijo Y, Washino N, Miyashita C, Kobayashi S, et al. (2012). Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. *Environ Res*. 112:118–25. doi:10.1016/j.envres.2011.10.003 PMID:22030285

Olsen GW, Burris JM, Burlew MM, Mandel JH (2003b). Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations. *J Occup Environ Med*. 45(3):260–70. doi:10.1097/01.jom.0000052958.59271.10 PMID:12661183

Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL, et al. (2007). Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ Health Perspect*. 115(9):1298–305. doi:10.1289/ehp.10009 PMID:17805419

Olsen GW, Hansen KJ, Stevenson LA, Burris JM, Mandel JH (2003a). Human donor liver and serum concentrations of perfluorooctanesulfonate and other perfluorochemicals. *Environ Sci Technol*. 37(5):888–91. doi:10.1021/es020955c PMID:12666917

Olsen GW, Lange CC, Ellefson ME, Mair DC, Church TR, Goldberg CL, et al. (2012). Temporal trends of perfluoroalkyl concentrations in American Red Cross adult blood donors, 2000–2010. *Environ Sci Technol*. 46(11):6330–8. doi:10.1021/es300604p PMID:22554481

Olsen GW, Zobel LR (2007). Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentrations in fluorochemical production workers. *Int Arch Occup Environ Health*. 81(2):231–46. doi:10.1007/s00420-007-0213-0 PMID:17605032

Omoike OE, Pack RP, Mamudu HM, Liu Y, Strasser S, Zheng S, et al. (2021). Association between per and polyfluoroalkyl substances and markers of inflammation and oxidative stress. *Environ Res*. 196:110361. doi:10.1016/j.envres.2020.110361 PMID:33131681

Ophaug RH, Singer L (1980). Metabolic handling of perfluorooctanoic acid in rats. *Proc Soc Exp Biol Med*. 163(1):19–23. doi:10.3181/00379727-163-40715 PMID:7352143

Orbach SM, Ehrich MF, Rajagopalan P (2018). High-throughput toxicity testing of chemicals and mixtures in organotypic multi-cellular cultures of primary human hepatic cells. *Toxicol In Vitro*. 51:83–94. doi:10.1016/j.tiv.2018.05.006 PMID:29751030

Oseguera-López I, Pérez-Cerezales S, Ortiz-Sánchez PB, Mondragon-Payne O, Sánchez-Sánchez R, Jiménez-Morales I, et al. (2020). Perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonate (PFHxS) alters protein phosphorylation, increase ROS levels and DNA fragmentation during in vitro capacitation of boar spermatozoa. *Animals (Basel)*. 10(10):1934. doi:10.3390/ani10101934 PMID:33096732

Osemwegie O, Butler L, Subbiah S, Smith E (2021). Effects of in vitro exposure of perfluorooctanoic acid and monocrotophos on astroglia SVG p12 cells. *J Appl Toxicol*. 41(9):1380–9. doi:10.1002/jat.4129 PMID:33569802

Ouidir M, Mendola P, Buck Louis GM, Kannan K, Zhang C, Tekola-Ayele F (2020). Concentrations of persistent organic pollutants in maternal plasma and epigenome-wide placental DNA methylation. *Clin Epigenetics*. 12(1):103. doi:10.1186/s13148-020-00894-6 PMID:32653021

Owumi S, Bello T, Oyelere AK (2021a). *N*-acetyl cysteine abates hepatorenal toxicities induced by perfluorooctanoic acid exposure in male rats. *Environ Toxicol Pharmacol*. 86:103667. doi:10.1016/j.etap.2021.103667 PMID:33933708

Owumi SE, Akomolafe AP, Imosemi IO, Odunola OA, Oyelere AK (2021b). *N*-acetyl cysteine co-treatment abates perfluorooctanoic acid-induced reproductive toxicity in male rats. *Andrologia*. 53(5):e14037. doi:10.1111/and.14037 PMID:33724529

Pan D, Shao Y, Song Y, Huang D, Liu S, Zeng X, et al. (2022). Association between maternal per- and polyfluoroalkyl substance exposure and newborn telomere length: effect modification by birth seasons. *Environ Int*. 161:107125. doi:10.1016/j.envint.2022.107125 PMID:35183942

Pan X, Qin P, Liu R, Yu W, Dong X (2018). Effects of carbon chain length on the perfluoroalkyl acids-induced oxidative stress of erythrocytes in vitro. *J Agric Food Chem*. 66(25):6414–20. doi:10.1021/acs.jafc.8b02197 PMID:29860827

Pan Y, Cui Q, Wang J, Sheng N, Jing J, Yao B, et al. (2019). Profiles of emerging and legacy per-/polyfluoroalkyl substances in matched serum and semen samples: new implications for human semen quality. *Environ Health Perspect*. 127(12):127005. doi:10.1289/EHP4431 PMID:31841032

Panaretakis T, Shabalina IG, Grandér D, Shoshan MC, DePierre JW (2001). Reactive oxygen species and mitochondria mediate the induction of apoptosis in human hepatoma HepG2 cells by the rodent peroxisome proliferator and hepatocarcinogen, perfluorooctanoic acid. *Toxicol Appl Pharmacol*. 173(1):56–64. doi:10.1006/taap.2001.9159 PMID:11350215

Papadopoulou E, Stratakis N, Basagaña X, Brantsæter AL, Casas M, Fossati S, et al. (2021). Prenatal and postnatal exposure to PFAS and cardiometabolic factors and inflammation status in children from six European cohorts. *Environ Int*. 157:106853. doi:10.1016/j.envint.2021.106853 PMID:34500361

Park MH, Gutiérrez-García AK, Choudhury M (2019). Mono-(2-ethylhexyl) phthalate aggravates inflammatory response via sirtuin regulation and inflammasome activation in RAW 264.7 cells. *Chem Res Toxicol*. 32(5):935–42. doi:10.1021/acs.chemrestox.9b00101 PMID:31016965

Park SJ, Sim KH, Shrestha P, Yang JH, Lee YJ (2021). Perfluorooctane sulfonate and bisphenol A induce a similar level of mast cell activation via a common signalling pathway, Fyn-Lyn-Syk activation. *Food Chem Toxicol*. 156:112478. doi:10.1016/j.fct.2021.112478 PMID:34363875

Parolini M, Colombo G, Valsecchi S, Mazzoni M, Possenti CD, Caprioli M, et al. (2016). Potential toxicity of environmentally relevant perfluorooctane sulfonate (PFOS) concentrations to yellow-legged gull *Larus michahellis* embryos. *Environ Sci Pollut Res Int*. 23(1):426–37. doi:10.1007/s11356-015-5248-2 PMID:26310703

Peden-Adams MM, Keller JM, Eudaly JG, Berger J, Gilkeson GS, Keil DE (2008). Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. *Toxicol Sci*. 104(1):144–54. doi:10.1093/toxsci/kfn059 PMID:18359764

Perera NLD, Betancourt J, Miksovska J, O'Shea KE (2023). Detail study on the interaction between perfluorooctanoic acid (PFOA) with human hemoglobin (Hb). *Curr Res Toxicol*. 5:100130. doi:10.1016/j.crtox.2023.100130 PMID:37822784

Pérez F, Nadal M, Navarro-Ortega A, Fàbrega F, Domingo JL, Barceló D, et al. (2013). Accumulation of perfluoroalkyl substances in human tissues. *Environ Int*. 59:354–62. doi:10.1016/j.envint.2013.06.004 PMID:23892228

Perkins RG, Butenhoff JL, Kennedy GL Jr, Palazzolo MJ (2004). 13-week dietary toxicity study of ammonium perfluorooctanoate (APFO) in male rats. *Drug Chem Toxicol*. 27(4):361–78. doi:10.1081/DCT-200039773 PMID:15573472

Peropadre A, Fernández Freire P, Hazen MJ (2018). A moderate exposure to perfluorooctanoic acid causes persistent DNA damage and senescence in human

epidermal HaCaT keratinocytes. *Food Chem Toxicol*. 121:351–9. doi:10.1016/j.fct.2018.09.020 PMID:30217483

Petersen KU, Hærvig KK, Flachs EM, Bonde JP, Lindh C, Hougaard KS, et al. (2022). Per- and polyfluoroalkyl substances (PFAS) and male reproductive function in young adulthood; a cross-sectional study. *Environ Res*. 212(Pt A):113157. doi:10.1016/j.envres.2022.113157 PMID:35318009

Petersen KU, Larsen JR, Deen L, Flachs EM, Hærvig KK, Hull SD, et al. (2020). Per- and polyfluoroalkyl substances and male reproductive health: a systematic review of the epidemiological evidence. *J Toxicol Environ Health B Crit Rev*. 23(6):276–91. doi:10.1080/10937404.2020.1798315 PMID:32741292

Petersen MS, Halling J, Jørgensen N, Nielsen F, Grandjean P, Jensen TK, et al. (2018). Reproductive function in a population of young Faroese men with elevated exposure to polychlorinated biphenyls (PCBs) and perfluorinated alkylate substances (PFAS). *Int J Environ Res Public Health*. 15(9):1880. doi:10.3390/ijerph15091880 PMID:30200252

Petro EML, D'Hollander W, Covaci A, Bervoets L, Fransen E, De Neubourg D, et al. (2014). Perfluoroalkyl acid contamination of follicular fluid and its consequence for in vitro oocyte developmental competence. *Sci Total Environ*. 496:282–8. doi:10.1016/j.scitotenv.2014.07.028 PMID:25089690

Pierozan P, Cattani D, Karlsson O (2020). Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) induce epigenetic alterations and promote human breast cell carcinogenesis in vitro. *Arch Toxicol*. 94(11):3893–906. doi:10.1007/s00204-020-02848-6 PMID:32700164

Pierozan P, Karlsson O (2018). PFOS induces proliferation, cell-cycle progression, and malignant phenotype in human breast epithelial cells. *Arch Toxicol*. 92(2):705–16. doi:10.1007/s00204-017-2077-8 PMID:29063134

Pierpont TM, Limper C, Elmore J, Redko A, Anannya O, Imbiakha B, et al. (2023). Effects of PFOS and cyclophosphamide exposure on immune homeostasis in mice. *Immunobiology*. 228(3):152356. doi:10.1016/j.imbio.2023.152356 PMID:36857909

Pilkerton CS, Hobbs GR, Lilly C, Knox SS (2018). Rubella immunity and serum perfluoroalkyl substances: sex and analytic strategy. *PLoS One*. 13(9):e0203330. doi:10.1371/journal.pone.0203330 PMID:30248109

Pinney SM, Fassler CS, Windham GC, Herrick RL, Xie C, Kushi LH, et al. (2023). Exposure to perfluoroalkyl substances and associations with pubertal onset and serum reproductive hormones in a longitudinal study of young girls in Greater Cincinnati and the San Francisco Bay Area. *Environ Health Perspect*. 131(9):97009. doi:10.1289/EHP11811 PMID:37751325

Pirali B, Negri S, Chytiris S, Perissi A, Villani L, La Manna L, et al. (2009). Perfluorooctane sulfonate and perfluorooctanoic acid in surgical thyroid specimens of patients with thyroid diseases. *Thyroid*. 19(12):1407–12. doi:10.1089/thy.2009.0174 PMID:20001722

Pizzurro DM, Seeley M, Kerper LE, Beck BD (2019). Interspecies differences in perfluoroalkyl substances (PFAS) toxicokinetics and application to health-based criteria. *Regul Toxicol Pharmacol*. 106:239–50. doi:10.1016/j.yrtph.2019.05.008 PMID:31078680

Poothong S, Thomsen C, Padilla-Sanchez JA, Papadopoulou E, Haug LS (2017). Distribution of novel and well-known poly-and perfluoroalkyl substances (PFASs) in human serum, plasma, and whole blood. *Environ Sci Technol*. 51(22):13388–96. doi:10.1021/acs.est.7b03299 PMID:29056041

Porter AK, Kleinschmidt SE, Andres KL, Reusch CN, Krisko RM, Taiwo OA, et al. (2022). Antibody response to COVID-19 vaccines among workers with a wide range of exposure to per- and polyfluoroalkyl substances. *Environ Int*. 169:107537. doi:10.1016/j.envint.2022.107537 PMID:36183490

Poteser M, Hutter H-P, Moshammer H, Weitensfelder L (2020). Perfluoroctanoic acid (PFOA) enhances NOTCH-signalling in an angiogenesis model of placental trophoblast cells. *Int J Hyg Environ Health*. 229(August):113566. doi:10.1016/j.ijheh.2020.113566 PMID:32485599

Preston EV, Webster TF, Oken E, Claus Henn B, McClean MD, Rifas-Shiman SL, et al. (2018). Maternal plasma per- and polyfluoroalkyl substance concentrations in early pregnancy and maternal and neonatal thyroid function in a prospective birth cohort: Project Viva (USA). *Environ Health Perspect*. 126(2):027013. doi:10.1289/EHP2534 PMID:29488882

Qazi MR, Hassan M, Nelson BD, Depierre JW, Abedi-Valugerdi M (2013). Sub-acute, moderate-dose, but not short-term, low-dose dietary pre-exposure of mice to perfluorooctanoate aggravates concanavalin A-induced hepatitis. *Toxicol Lett*. 219(1):1–7. doi:10.1016/j.toxlet.2013.02.017 PMID:23458897

Qazi MR, Nelson BD, Depierre JW, Abedi-Valugerdi M (2010). 28-Day dietary exposure of mice to a low total dose (7 mg/kg) of perfluorooctanesulfonate (PFOS) alters neither the cellular compositions of the thymus and spleen nor humoral immune responses: does the route of administration play a pivotal role in PFOS-induced immunotoxicity? *Toxicology*. 267(1–3):132–9. doi:10.1016/j.tox.2009.10.035 PMID:19900501

Qazi MR, Xia Z, Bogdanska J, Chang SC, Ehresman DJ, Butenhoff JL, et al. (2009). The atrophy and changes in the cellular compositions of the thymus and spleen observed in mice subjected to short-term exposure to perfluorooctanesulfonate are high-dose phenomena mediated in part by peroxisome proliferator-activated receptor-alpha (PPARalpha). *Toxicology*. 260(1–3):68–76. doi:10.1016/j.tox.2009.03.009 PMID:19464571

Qi Q, Niture S, Gadi S, Arthur E, Moore J, Levine KE, et al. (2023). Per- and polyfluoroalkyl substances activate UPR pathway, induce steatosis and fibrosis in liver cells. *Environ Toxicol*. 38(1):225–42. doi:10.1002/tox.23680 PMID:36251517

Qian Y, Ducatman A, Ward R, Leonard S, Bukowski V, Lan Guo N, et al. (2010). Perfluorooctane sulfonate (PFOS) induces reactive oxygen species (ROS) production in human microvascular endothelial cells: role in endothelial permeability. *J Toxicol Environ Health A*. 73(12):819–36. doi:10.1080/15287391003689317 PMID:20391123

Qin C, Lu YX, Borch T, Yang LL, Li YW, Zhao HM, et al. (2022a). Interactions between extracellular DNA and perfluoroalkyl acids (PFAAs) decrease the bioavailability of PFAAs in pakchoi (*Brassica chinensis* L.). *J Agric Food Chem*. 70(46):14622–32. doi:10.1021/acs.jafc.2c04597 PMID:36375011

Qin H, Niu Y, Luan H, Li M, Zheng L, Pan Y, et al. (2022c). Effects of legacy and emerging per- and polyfluoroalkyl substances on PPARα/β/γ regulation and osteogenic/adipogenic differentiation. *Environ Int*. 170:107584. doi:10.1016/j.envint.2022.107584 PMID:36265359

Qin X, Xie G, Wu X, Xu X, Su M, Yang B (2018). Prenatal exposure to perfluorooctanoic acid induces nerve growth factor expression in cerebral cortex cells of mouse offspring. *Environ Sci Pollut Res Int*. 25(19):18914–20. doi:10.1007/s11356-018-2117-9 PMID:29717428

Qin Y, Gu T, Ling J, Luo J, Zhao J, Hu B, et al. (2022b). PFOS facilitates liver inflammation and steatosis: an involvement of NLRP3 inflammasome-mediated hepatocyte pyroptosis. *J Appl Toxicol*. 42(5):806–17. doi:10.1002/jat.4258 PMID:34687223

Qiu L, Wang H, Dong T, Huang J, Li T, Ren H, et al. (2021). Perfluorooctane sulfonate (PFOS) disrupts testosterone biosynthesis via CREB/CRTC2/StAR signalling pathway in Leydig cells. *Toxicology*. 449:152663. doi:10.1016/j.tox.2020.152663 PMID:33359577

Qiu Z, Qu K, Luan F, Liu Y, Zhu Y, Yuan Y, et al. (2020). Binding specificities of estrogen receptor with perfluorinated compounds: a cross species comparison. *Environ Int*. 134:105284. doi:10.1016/j.envint.2019.105284 PMID:31707300

Qu JH, Lu CC, Xu C, Chen G, Qiu LL, Jiang JK, et al. (2016). Perfluorooctane sulfonate-induced testicular toxicity and differential testicular expression of estrogen receptor in male mice. *Environ Toxicol Pharmacol*. 45:150–7. doi:10.1016/j.etap.2016.05.025 PMID:27310206

Qu W, Yan Y, Gerrish K, Scappini E, Tucker CJ, Dixon D, et al. (2023). Chronic PFOA exposure in vitro causes acquisition of multiple tumor cell characteristics in rat liver cells. *Toxicol In Vitro*. 89:105577. doi:10.1016/j.tiv.2023.105577 PMID:36849026

Quist EM, Filgo AJ, Cummings CA, Kissling GE, Hoenerhoff MJ, Fenton SE (2015). Hepatic mitochondrial alteration in CD-1 mice associated with prenatal exposures to low doses of perfluorooctanoic acid (PFOA). *Toxicol Pathol*. 43(4):546–57. doi:10.1177/0192623314551841 PMID:25326589

Rakhshandehroo M, Sanderson LM, Matilainen M, Stienstra R, Carlberg C, de Groot PJ, et al. (2007). Comprehensive analysis of PPARalpha-dependent regulation of hepatic lipid metabolism by expression profiling. *PPAR Res*. 2007:26839. doi:10.1155/2007/26839 PMID:18288265

Rashid F, Ahmad S, Irudayaraj JMK (2020b). Effect of perfluorooctanoic acid on the epigenetic and tight junction genes of the mouse intestine. *Toxics*. 8(3):64. doi:10.3390/toxics8030064 PMID:32872178

Rashid F, Ramakrishnan A, Fields C, Irudayaraj J (2020a). Acute PFOA exposure promotes epigenomic alterations in mouse kidney tissues. *Toxicol Rep*. 7:125–32. doi:10.1016/j.toxrep.2019.12.010 PMID:31938689

Raymer JH, Michael LC, Studabaker WB, Olsen GW, Sloan CS, Wilcosky T, et al. (2012). Concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) and their associations with human semen quality measurements. *Reprod Toxicol*. 33(4):419–27. doi:10.1016/j.reprotox.2011.05.024 PMID:21736937

Reardon AJF, Khodayari Moez E, Dinu I, Goruk S, Field CJ, Kinniburgh DW, et al.; APrON Study (2019). Longitudinal analysis reveals early-pregnancy associations between perfluoroalkyl sulfonates and thyroid hormone status in a Canadian prospective birth cohort. *Environ Int*. 129:389–99. doi:10.1016/j.envint.2019.04.023 PMID:31150980

Reisfeld B, de Conti A, El Ghissassi F, Benbrahim-Tallaa L, Gwinn W, Grosse Y, et al. (2022). kc-hits: a tool to aid in the evaluation and classification of chemical carcinogens. *Bioinformatics*. 38(10):2961–2. doi:10.1093/bioinformatics/btac189 PMID:35561175

Reistad T, Fonnum F, Mariussen E (2013). Perfluoroalkylated compounds induce cell death and formation of reactive oxygen species in cultured cerebellar granule cells. *Toxicol Lett*. 218(1):56–60. doi:10.1016/j.toxlet.2013.01.006 PMID:23340305

Remy S, Govarts E, Wens B, De Boever P, Den Hond E, Croes K, et al. (2016). Metabolic targets of endocrine disrupting chemicals assessed by cord blood transcriptome profiling. *Reprod Toxicol*. 65:307–20. doi:10.1016/j.reprotox.2016.08.018 PMID:27589886

Ren XM, Qin WP, Cao LY, Zhang J, Yang Y, Wan B, et al. (2016). Binding interactions of perfluoroalkyl substances with thyroid hormone transport proteins and potential toxicological implications. *Toxicology*. 366–367(367):32–42. doi:10.1016/j.tox.2016.08.011 PMID:27528273

Ren XM, Zhang YF, Guo LH, Qin ZF, Lv QY, Zhang LY (2015). Structure–activity relations in binding of perfluoroalkyl compounds to human thyroid hormone T3 receptor. *Arch Toxicol*. 89(2):233–42. doi:10.1007/s00204-014-1258-y PMID:24819616

Rhee J, Loftfield E, Albanes D, Layne TM, Stolzenberg-Solomon R, Liao LM, et al. (2023). A metabolomic investigation of serum perfluorooctane sulfonate and perfluorooctanoate. *Environ Int*. 180:108198. doi:10.1002/jat.3207 PMID:26178269

Rigden M, Pelletier G, Poon R, Zhu J, Auray-Blais C, Gagnon R, et al. (2015). Assessment of urinary metabolite excretion after rat acute exposure to perfluorooctanoic acid and other peroxisomal proliferators. *Arch Environ Contam Toxicol*. 68(1):148–58. doi:10.1007/s00244-014-0058-y PMID:25015730

Robinson SL, Zeng X, Guan W, Sundaram R, Mendola P, Putnick DL, et al. (2021). Perfluorooctanoic acid (PFOA) or perfluorooctane sulfonate (PFOS) and DNA methylation in newborn dried blood spots in the Upstate KIDS cohort. *Environ Res*. 194:110668. doi:10.1016/j.envres.2020.110668 PMID:33387539

Rosen MB, Abbott BD, Wolf DC, Corton JC, Wood CR, Schmid JE, et al. (2008a). Gene profiling in the livers of wild-type and PPARalpha-null mice exposed to perfluorooctanoic acid. *Toxicol Pathol*. 36(4):592–607. doi:10.1177/0192623308318208 PMID:18467677

Rosen MB, Das KP, Rooney J, Abbott B, Lau C, Corton JC (2017). PPARα-independent transcriptional targets of perfluoroalkyl acids revealed by transcript profiling. *Toxicology*. 387:95–107. doi:10.1016/j.tox.2017.05.013 PMID:28558994

Rosen MB, Das KP, Wood CR, Wolf CJ, Abbott BD, Lau C (2013). Evaluation of perfluoroalkyl acid activity using primary mouse and human hepatocytes. *Toxicology*. 308:129–37. doi:10.1016/j.tox.2013.03.011 PMID:23567314

Rosen MB, Lee JS, Ren H, Vallanat B, Liu J, Waalkes MP, et al. (2008b). Toxicogenomic dissection of the perfluorooctanoic acid transcript profile in mouse liver: evidence for the involvement of nuclear receptors PPAR alpha and CAR. *Toxicol Sci*. 103(1):46–56. doi:10.1093/toxsci/kfn025 PMID:18281256

Rosen MB, Schmid JE, Das KP, Wood CR, Zehr RD, Lau C (2009). Gene expression profiling in the liver and lung of perfluorooctane sulfonate-exposed mouse fetuses: comparison to changes induced by exposure to perfluorooctanoic acid. *Reprod Toxicol*. 27(3–4):278–88. doi:10.1016/j.reprotox.2009.01.007 PMID:19429403

Rosen MB, Schmid JR, Corton JC, Zehr RD, Das KP, Abbott BD, et al. (2010). Gene expression profiling in wild-type and PPARα-null mice exposed to perfluorooctane sulfonate reveals PPARα-independent effects. *PPAR Res*. 2010:794739. doi:10.1155/2010/794739 PMID:20936131

Rosen MB, Thibodeaux JR, Wood CR, Zehr RD, Schmid JE, Lau C (2007). Gene expression profiling in the lung and liver of PFOA-exposed mouse fetuses. *Toxicology*. 239(1–2):15–33. doi:10.1016/j.tox.2007.06.095 PMID:17681415

Rosen Vollmar AK, Lin EZ, Nason SL, Santiago K, Johnson CH, Ma X, et al. (2023). Per- and polyfluoroalkyl substances (PFAS) and thyroid hormone measurements in dried blood spots and neonatal characteristics: a pilot study. *J Expo Sci Environ Epidemiol*. 33(5):737–47. doi:10.1038/s41370-023-00603-4 PMID:37730931

Rosenmai AK, Ahrens L, le Godec T, Lundqvist J, Oskarsson A (2018). Relationship between peroxisome proliferator-activated receptor alpha activity and cellular concentration of 14 perfluoroalkyl substances in HepG2 cells. *J Appl Toxicol*. 38(2):219–26. doi:10.1002/jat.3515 PMID:28857218

Rosenmai AK, Nielsen FK, Pedersen M, Hadrup N, Trier X, Christensen JH, et al. (2013). Fluorochemicals used in food packaging inhibit male sex hormone synthesis. *Toxicol Appl Pharmacol*. 266(1):132–42. doi:10.1016/j.taap.2012.10.022 PMID:23142464

Rosenmai AK, Taxvig C, Svingen T, Trier X, van Vugt-Lussenburg BM, Pedersen M, et al. (2016). Fluorinated alkyl substances and technical mixtures used in food paper-packaging exhibit endocrine-related activity in vitro. *Andrology*. 4(4):662–72. doi:10.1111/andr.12190 PMID:27152447

Rowan-Carroll A, Reardon A, Leingartner K, Gagné R, Williams A, Meier MJ, et al. (2021). High-throughput transcriptomic analysis of human primary hepatocyte spheroids exposed to per- and polyfluoroalkyl substances (PFAS) as a platform for relative potency characterization. *Toxicol Sci*. 181(2):199–214. doi:10.1093/toxsci/kfab039 PMID:33772556

Ruggiero MJ, Miller H, Idowu JY, Zitzow JD, Chang SC, Hagenbuch B (2021). Perfluoroalkyl carboxylic acids interact with the human bile acid transporter NTCP. *Livers*. 1(4):221–9. doi:10.3390/livers1040017 PMID:34738093

Russell MH, Waterland RL, Wong F (2015). Calculation of chemical elimination half-life from blood with an ongoing exposure source: the example of perfluorooctanoic acid (PFOA). *Chemosphere*. 129:210–6. doi:10.1016/j.chemosphere.2014.07.061 PMID:25149361

Rylander C, Dumeaux V, Olsen KS, Waaseth M, Sandanger TM, Lund E (2011). Using blood gene signatures for assessing effects of exposure to perfluoroalkyl acids (PFAAs) in humans: the NOWAC postgenome study. *Int J Mol Epidemiol Genet*. 2(3):207–16. https://pmc.ncbi.nlm.nih.gov/articles/PMC3166148/ PMID:21915359

Saejia P, Lirdprapamongkol K, Svasti J, Paricharttanakul NM (2019). Perfluorooctanoic acid enhances invasion of follicular thyroid carcinoma cells through NF-κB and matrix metalloproteinase-2 activation. *Anticancer*

Res. 39(5):2429–35. doi:10.21873/anticanres.13360 PMID:31092435

Sakai G, Hirao-Suzuki M, Koga T, Kobayashi T, Kamishikiryo J, Tanaka M, et al. (2022). Perfluorooctanoic acid (PFOA) as a stimulator of estrogen receptor-negative breast cancer MDA-MB-231 cell aggressiveness: evidence for involvement of fatty acid 2-hydroxylase (FA2H) in the stimulated cell migration. *J Toxicol Sci*. 47(4):159–68. doi:10.2131/jts.47.159 PMID:35370244

Salgado R, Pereiro N, López-Doval S, Lafuente A (2015). Initial study on the possible mechanisms involved in the effects of high doses of perfluorooctane sulfonate (PFOS) on prolactin secretion. *Food Chem Toxicol*. 83:10–6. doi:10.1016/j.fct.2015.05.013 PMID:26032630

Salihović S, Dickens AM, Schoultz I, Fart F, Sinisalu L, Lindeman T, et al. (2020a). Simultaneous determination of perfluoroalkyl substances and bile acids in human serum using ultra-high-performance liquid chromatography–tandem mass spectrometry. *Anal Bioanal Chem*. 412(10):2251–9. doi:10.1007/s00216-019-02263-6 PMID:31760452

Salihović S, Kärrman A, Lind L, Lind PM, Lindström G, van Bavel B (2015). Perfluoroalkyl substances (PFAS) including structural PFOS isomers in plasma from elderly men and women from Sweden: results from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS). *Environ Int*. 82:21–7. doi:10.1016/j.envint.2015.05.003 PMID:26001496

Salihović S, Lind L, Larsson A, Lind PM (2020b). Plasma perfluoroalkyls are associated with decreased levels of proteomic inflammatory markers in a cross-sectional study of an elderly population. *Environ Int*. 145:106099. doi:10.1016/j.envint.2020.106099 PMID:32916415

Salimi A, Nikoosiar Jahromi M, Pourahmad J (2019). Maternal exposure causes mitochondrial dysfunction in brain, liver, and heart of mouse fetus: an explanation for perfluorooctanoic acid induced abortion and developmental toxicity. *Environ Toxicol*. 34(7):878–85. doi:10.1002/tox.22760 PMID:31037826

Sarzo B, Ballesteros V, Iñiguez C, Manzano-Salgado CB, Casas M, Llop S, et al. (2021). Maternal perfluoroalkyl substances, thyroid hormones, and *DIO* genes: a Spanish cross-sectional study. *Environ Sci Technol*. 55(16):11144–54. doi:10.1021/acs.est.1c01452 PMID:34314170

Satija Lab (2023). Seurat Package. R toolkit for single cell genomics. New York, USA: Satija Lab, New York Genome Center. Available from: https://satijalab.org/seurat/.

Savitz DA, Stein CR, Bartell SM, Elston B, Gong J, Shin H-M, et al. (2012). Perfluorooctanoic acid exposure and pregnancy outcome in a highly exposed community. *Epidemiology*. 23(3):386–92. doi:10.1097/EDE.0b013e31824cb93b PMID:22370857

Scharmach E, Buhrke T, Lichtenstein D, Lampen A (2012). Perfluorooctanoic acid affects the activity of the hepatocyte nuclear factor 4 alpha (HNF4α). *Toxicol Lett*. 212(2):106–12. doi:10.1016/j.toxlet.2012.05.007 PMID:22609092

Schlezinger JJ, Hyötyläinen T, Sinioja T, Boston C, Puckett H, Oliver J, et al. (2021). Perfluorooctanoic acid induces liver and serum dyslipidemia in humanized PPARα mice fed an American diet. *Toxicol Appl Pharmacol*. 426:115644. doi:10.1016/j.taap.2021.115644 PMID:34252412

Schlezinger JJ, Puckett H, Oliver J, Nielsen G, Heiger-Bernays W, Webster TF (2020). Perfluorooctanoic acid activates multiple nuclear receptor pathways and skews expression of genes regulating cholesterol homeostasis in liver of humanized PPARα mice fed an American diet. *Toxicol Appl Pharmacol*. 405:115204. doi:10.1016/j.taap.2020.115204 PMID:32822737

Schnellmann RG (1990). The cellular effects of a unique pesticide sulfluramid (*N*-ethylperfluorooctane sulphonamide) on rabbit renal proximal tubules. *Toxicol In Vitro*. 4(1):71–4. doi:10.1016/0887-2333(90)90012-I PMID:20702287

Scinicariello F, Buser MC, Balluz L, Gehle K, Murray HE, Abadin HG, et al. (2020). Perfluoroalkyl acids, hyperuricemia and gout in adults: analyses of NHANES 2009–2014. *Chemosphere*. 259:127446. doi:10.1016/j.chemosphere.2020.127446 PMID:32590180

Seacat AM, Thomford PJ, Hansen KJ, Clemen LA, Eldridge SR, Elcombe CR, et al. (2003). Sub-chronic dietary toxicity of potassium perfluorooctanesulfonate in rats. *Toxicology*. 183(1–3):117–31. doi:10.1016/S0300-483X(02)00511-5 PMID:12504346

Seacat AM, Thomford PJ, Hansen KJ, Olsen GW, Case MT, Butenhoff JL (2002). Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys. *Toxicol Sci*. 68(1):249–64. doi:10.1093/toxsci/68.1.249 PMID:12075127

Seals R, Bartell SM, Steenland K (2011). Accumulation and clearance of perfluorooctanoic acid (PFOA) in current and former residents of an exposed community. *Environ Health Perspect*. 119(1):119–24. doi:10.1289/ehp.1002346 PMID:20870569

Selano J, Richardson V, Washington J, Mazur C (2019). Characterization of non-radiolabeled thyroxine (T_4) uptake in cryopreserved rat hepatocyte suspensions: pharmacokinetic implications for PFOA and PFOS chemical exposure. *Toxicol In Vitro*. 58:230–8. doi:10.1016/j.tiv.2019.03.022 PMID:30930230

Shabalina IG, Panaretakis T, Bergstrand A, DePierre JW (1999). Effects of the rodent peroxisome proliferator and hepatocarcinogen, perfluorooctanoic acid, on apoptosis in human hepatoma HepG2 cells. *Carcinogenesis*. 20(12):2237–46. doi:10.1093/carcin/20.12.2237 PMID:10590214

Shalaby AM, Albakkosh AM, Shalaby RH, Alabiad MA, Elshamy AM, Alorini M, et al. (2023). Lemongrass essential oil attenuates perfluorooctane sulfonate-induced jejunal mucosal injury in rat: a histological, immunohistochemical, and biochemical study. *Microsc Microanal*. 29(2):841–57. doi:10.1093/micmic/ozad009 PMID:37749735

Shan G, Wang Z, Zhou L, Du P, Luo X, Wu Q, et al. (2016). Impacts of daily intakes on the isomeric profiles of perfluoroalkyl substances (PFASs) in human serum. *Environ Int*. 89–90:62–70. doi:10.1016/j.envint.2016.01.002 PMID:26826363

Shan G, Ye M, Zhu B, Zhu L (2013). Enhanced cytotoxicity of pentachlorophenol by perfluorooctane sulfonate or perfluorooctanoic acid in HepG2 cells. *Chemosphere*. 93(9):2101–7. doi:10.1016/j.chemosphere.2013.07.054 PMID:23972907

Shane HL, Baur R, Lukomska E, Weatherly L, Anderson SE (2020). Immunotoxicity and allergenic potential induced by topical application of perfluorooctanoic acid (PFOA) in a murine model. *Food Chem Toxicol*. 136:111114. doi:10.1016/j.fct.2020.111114 PMID:31904477

Shao X, Ji F, Wang Y, Zhu L, Zhang Z, Du X, et al. (2018). Integrative chemical proteomics–metabolomics approach reveals acaca/acacb as direct molecular targets of PFOA. *Anal Chem*. 90(18):11092–8. doi:10.1021/acs.analchem.8b02995 PMID:30134650

Shao X, Zhao B, Wang B, Zhao B, Zhu Y, Yuan Z, et al. (2019). Neuroprotective effects of blueberry anthocyanins against perfluorooctanoic sulfonate on planarian *Dugesia japonica*. *Ecotoxicol Environ Saf*. 175:39–47. doi:10.1016/j.ecoenv.2019.03.023 PMID:30884343

Sharma S, Kelly TK, Jones PA (2010). Epigenetics in cancer. *Carcinogenesis*. 31(1):27–36. doi:10.1093/carcin/bgp220 PMID:19752007

Sheng N, Li J, Liu H, Zhang A, Dai J (2016). Interaction of perfluoroalkyl acids with human liver fatty acid-binding protein. *Arch Toxicol*. 90(1):217–27. doi:10.1007/s00204-014-1391-7 PMID:25370009

Shi L, Zheng J, Yan S, Li Y, Wang Y, Liu X, et al. (2020). Exposure to perfluorooctanoic acid induces cognitive deficits via altering gut microbiota composition, impairing intestinal barrier integrity, and causing inflammation in gut and brain. *J Agric Food Chem*. 68(47):13916–28. doi:10.1021/acs.jafc.0c05834 PMID:33151664

Shin H-M, Vieira VM, Ryan PB, Steenland K, Bartell SM (2011). Retrospective exposure estimation and predicted versus observed serum perfluorooctanoic acid concentrations for participants in the C8 Health Project. *Environ Health Perspect*. 119(12):1760–5. doi:10.1289/ehp.1103729 PMID:21813367

Shipley JM, Hurst CH, Tanaka SS, DeRoos FL, Butenhoff JL, Seacat AM, et al. (2004). *trans*-Activation of PPARalpha and induction of PPARalpha target genes by perfluorooctane-based chemicals. *Toxicol Sci*. 80(1):151–60. doi:10.1093/toxsci/kfh130 PMID:15071170

Shrestha S, Bloom MS, Yucel R, Seegal RF, Wu Q, Kannan K, et al. (2015). Perfluoroalkyl substances and thyroid function in older adults. *Environ Int*. 75:206–14. doi:10.1016/j.envint.2014.11.018 PMID:25483837

SIB (2024). Cellosaurus L-02 (CVCL_6926). The Cellosaurus [online database]. Geneva, Switzerland: Swiss Institute of Bioinformatics. Available from: https://www.cellosaurus.org/CVCL_6926, accessed January 2025.

Simmon VF, Marx N (1978). In-vitro microbiological mutagenicity assays of 3M Company compounds T-2247 CoC and T-2248 CoC. Final report. SRI Project LSC-442-016. Prepared by Simmon VF for SRI International, USA. EPA AR 226-0134. US Environmental Protection Agency.

Singh TS, Lee S, Kim HH, Choi JK, Kim SH (2012). Perfluorooctanoic acid induces mast cell-mediated allergic inflammation by the release of histamine and inflammatory mediators. *Toxicol Lett*. 210(1):64–70. doi:10.1016/j.toxlet.2012.01.014 PMID:22322153

Sivaram AK, Logeshwaran P, Surapaneni A, Shah K, Crosbie N, Rogers Z, et al. (2021). Evaluation of cyto-genotoxicity of perfluorooctane sulfonate (PFOS) to *Allium cepa*. *Environ Toxicol Chem*. 40(3):792–8. doi:10.1002/etc.4905 PMID:33074584

Slotkin TA, MacKillop EA, Melnick RL, Thayer KA, Seidler FJ (2008). Developmental neurotoxicity of perfluorinated chemicals modeled in vitro. *Environ Health Perspect*. 116(6):716–22. doi:10.1289/ehp.11253 PMID:18560525

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect*. 124(6):713–21. doi:10.1289/ehp.1509912 PMID:26600562

Smutny T, Bernhauerova V, Smutna L, Tebbens JD, Pavek P (2022). Expression dynamics of pregnane X receptor-controlled genes in 3D primary human hepatocyte spheroids. *Arch Toxicol*. 96(1):195–210. doi:10.1007/s00204-021-03177-y PMID:34689256

Soltani M, Pourfarzam M, Sharifabad AH, Neisiani AK, Mousavi MK, Aliomrani M (2023). Effect of pretreatment with a synbiotic on perfluorooctanoic acid-induced liver damage after sub-acute oral exposure in C57BL/6J mice. *Toxicol Appl Pharmacol*. 459:116360. doi:10.1016/j.taap.2022.116360 PMID:36572227

Son H-Y, Lee S, Tak E-N, Cho H-S, Shin H-I, Kim S-H, et al. (2009). Perfluorooctanoic acid alters T lymphocyte phenotypes and cytokine expression in mice. *Environ Toxicol*. 24(6):580–8. doi:10.1002/tox.20459 PMID:19051282

Song M, Kim Y-J, Park Y-K, Ryu J-C (2012). Changes in thyroid peroxidase activity in response to various chemicals. *J Environ Monit*. 14(8):2121–6. doi:10.1039/c2em30106g PMID:22699773

Song P, Li D, Wang X, Zhong X (2018). Effects of perfluorooctanoic acid exposure during pregnancy on the reproduction and development of male offspring mice. *Andrologia*. 50(8):e13059. doi:10.1111/and.13059 PMID:29862542

Sonkar R, Kay MK, Choudhury M (2019). PFOS modulates interactive epigenetic regulation in first-trimester human trophoblast cell line HTR-8/SV_{neo}. *Chem Res Toxicol*. 32(10):2016–27. doi:10.1021/acs.chemrestox.9b00198 PMID:31508952

Sonthithai P, Suriyo T, Thiantanawat A, Watcharasit P, Ruchirawat M, Satayavivad J (2016). Perfluorinated chemicals, PFOS and PFOA, enhance the estrogenic effects of 17β-estradiol in T47D human breast cancer cells. *J Appl Toxicol*. 36(6):790–801. doi:10.1002/jat.3210 PMID:26234195

Sørli JB, Låg M, Ekeren L, Perez-Gil J, Haug LS, Da Silva E, et al. (2020). Per- and polyfluoroalkyl substances (PFASs) modify lung surfactant function and pro-inflammatory responses in human bronchial epithelial cells. *Toxicol In Vitro*. 62:104656. doi:10.1016/j.tiv.2019.104656 PMID:31536757

Souders CL 2nd, Sanchez CL, Malphurs W, Aristizabal-Henao JJ, Bowden JA, Martyniuk CJ (2021). Metabolic profiling in human SH-SY5Y neuronal cells exposed to perfluorooctanoic acid (PFOA). *Neurotoxicology*. 85:160–72. doi:10.1016/j.neuro.2021.05.009 PMID:34029635

Specht IO, Hougaard KS, Spanò M, Bizzaro D, Manicardi GC, Lindh CH, et al. (2012). Sperm DNA integrity in relation to exposure to environmental perfluoroalkyl substances – a study of spouses of pregnant women in three geographical regions. *Reprod Toxicol*. 33(4):577–83. doi:10.1016/j.reprotox.2012.02.008 PMID:22449571

Spliethoff HM, Tao L, Shaver SM, Aldous KM, Pass KA, Kannan K, et al. (2008). Use of newborn screening program blood spots for exposure assessment: declining levels of perfluorinated compounds in New York State infants. *Environ Sci Technol*. 42(14):5361–7. doi:10.1021/es8006244 PMID:18754394

Sprengel J, Behnisch PA, Besselink H, Brouwer A, Vetter W (2021). In vitro human cell-based TTR-TRβ CALUX assay indicates thyroid hormone transport disruption of short-chain, medium-chain, and long-chain chlorinated paraffins. *Arch Toxicol*. 95(4):1391–6. doi:10.1007/s00204-021-02994-5 PMID:33555371

Starkov AA, Wallace KB (2002). Structural determinants of fluorochemical-induced mitochondrial dysfunction. *Toxicol Sci*. 66(2):244–52. doi:10.1093/toxsci/66.2.244 PMID:11896291

Starling AP, Liu C, Shen G, Yang IV, Kechris K, Borengasser SJ, et al. (2020). Prenatal exposure to per- and polyfluoroalkyl substances, umbilical cord blood DNA methylation, and cardio-metabolic indicators in newborns: the Healthy Start Study. *Environ Health Perspect*. 128(12):127014. doi:10.1289/EHP6888 PMID:33356526

Steenland K, Fletcher T, Stein CR, Bartell SM, Darrow L, Lopez-Espinosa M-J, et al. (2020). Review: evolution of evidence on PFOA and health following the assessments of the C8 Science Panel. *Environ Int*. 145:106125. doi:10.1016/j.envint.2020.106125 PMID:32950793

Stein CR, McGovern KJ, Pajak AM, Maglione PJ, Wolff MS (2016). Perfluoroalkyl and polyfluoroalkyl substances and indicators of immune function in children aged 12–19 y: National Health and Nutrition Examination Survey. *Pediatr Res*. 79(2):348–57. doi:10.1038/pr.2015.213 PMID:26492286

Steves AN, Turry A, Gill B, Clarkson-Townsend D, Bradner JM, Bachli I, et al. (2018). Per- and polyfluoroalkyl substances impact human spermatogenesis in a stem-cell-derived model. *Syst Biol Reprod Med*. 64(4):225–39. doi:10.1080/19396368.2018.1481465 PMID:29911897

Stiehl A (1974). Bile salt sulphates in cholestasis. *Eur J Clin Invest*. 4(1):59–63. doi:10.1111/j.1365-2362.1974.tb00373.x PMID:4819836

Su S, Billy LJ, Chang S, Gonzalez FJ, Patterson AD, Peters JM (2022a). The role of mouse and human peroxisome proliferator-activated receptor-α in modulating the hepatic effects of perfluorooctane sulfonate in mice. *Toxicology*. 465:153056. doi:10.1016/j.tox.2021.153056 PMID:34861291

Su Y, Santucci-Pereira J, Dang NM, Kanefsky J, Rahulkannan V, Hillegass M, et al. (2022b). Effects of pubertal exposure to butyl benzyl phthalate, perfluorooctanoic acid, and zeranol on mammary gland development and tumorigenesis in rats. *Int J Mol Sci*. 23(3):1398. doi:10.3390/ijms23031398 PMID:35163327

Suh KS, Choi EM, Kim YJ, Hong SM, Park SY, Rhee SY, et al. (2017). Perfluorooctanoic acid induces oxidative damage and mitochondrial dysfunction in pancreatic β-cells. *Mol Med Rep*. 15(6):3871–8. doi:10.3892/mmr.2017.6452 PMID:28440430

Suo C, Fan Z, Zhou L, Qiu J (2017). Perfluorooctane sulfonate affects intestinal immunity against bacterial infection. *Sci Rep*. 7(1):5166. doi:10.1038/s41598-017-04091-z PMID:28701769

Taibl KR, Schantz S, Aung MT, Padula A, Geiger S, Smith S, et al. (2022). Associations of per- and polyfluoroalkyl substances (PFAS) and their mixture with oxidative stress biomarkers during pregnancy. *Environ Int*. 169:107541. doi:10.1016/j.envint.2022.107541 PMID:36191484

Takacs ML, Abbott BD (2007). Activation of mouse and human peroxisome proliferator-activated receptors (alpha, beta/delta, gamma) by perfluorooctanoic acid

and perfluorooctane sulfonate. *Toxicol Sci*. 95(1):108–17. doi:10.1093/toxsci/kfl135 PMID:17047030

Takagi A, Sai K, Umemura T, Hasegawa R, Kurokawa Y (1991). Short-term exposure to the peroxisome proliferators, perfluorooctanoic acid and perfluorodecanoic acid, causes significant increase of 8-hydroxydeoxyguanosine in liver DNA of rats. *Cancer Lett*. 57(1):55–60. doi:10.1016/0304-3835(91)90063-N PMID:2025879

Tan F, Jin Y, Liu W, Quan X, Chen J, Liang Z (2012). Global liver proteome analysis using iTRAQ labeling quantitative proteomic technology to reveal biomarkers in mice exposed to perfluorooctane sulfonate (PFOS). *Environ Sci Technol*. 46(21):12170–7. doi:10.1021/es3027715 PMID:23046066

Tan Y, Taibl KR, Dunlop AL, Barr DB, Panuwet P, Yakimavets V, et al. (2023). Association between a mixture of per- and polyfluoroalkyl substances (PFAS) and inflammatory biomarkers in the Atlanta African American Maternal–Child Cohort. *Environ Sci Technol*. 57(36):13419–28. doi:10.1021/acs.est.3c04688 PMID:37649345

Tang L, Yu J, Zhuge S, Chen H, Zhang L, Jiang G (2022). Oxidative stress and Cx43-mediated apoptosis are involved in PFOS-induced nephrotoxicity. *Toxicology*. 478:153283. doi:10.1016/j.tox.2022.153283 PMID:35934180

Taylor BK, Dadia N, Yang CB, Krishnan S, Badr M (2002). Peroxisome proliferator-activated receptor agonists inhibit inflammatory edema and hyperalgesia. *Inflammation*. 26(3):121–7. doi:10.1023/A:1015500531113 PMID:12083418

Taylor KD, Woodlief TL, Ahmed A, Hu Q, Duncker PC, DeWitt JC (2023). Quantifying the impact of PFOA exposure on B-cell development and antibody production. *Toxicol Sci*. 194(1):101–8. doi:10.1093/toxsci/kfad043 PMID:37162486

Thomas RS, Paules RS, Simeonov A, Fitzpatrick SC, Crofton KM, Casey WM, et al. (2018). The US Federal Tox21 Program: a strategic and operational plan for continued leadership. *Altern Anim Exp*. 35(2):163–8. doi:10.14573/altex.1803011 PMID:29529324

Thompson J, Lorber M, Toms LL, Kato K, Calafat AM, Mueller JF (2010). Use of simple pharmacokinetic modeling to characterize exposure of Australians to perfluorooctanoic acid and perfluorooctane sulfonic acid. *Environ Int*. 36(4):390–7. doi:10.1016/j.envint.2010.02.008 PMID:20236705

Thottassery J, Winberg L, Youssef J, Cunningham M, Badr M (1992). Regulation of perfluorooctanoic acid-induced peroxisomal enzyme activities and hepatocellular growth by adrenal hormones. *Hepatology*. 15(2):316–22. doi:10.1002/hep.1840150223 PMID:1735536

Tian M, Peng S, Martin FL, Zhang J, Liu L, Wang Z, et al. (2012). Perfluorooctanoic acid induces gene promoter hypermethylation of glutathione-*S*-transferase Pi in human liver L02 cells. *Toxicology*. 296(1–3):48–55. doi:10.1016/j.tox.2012.03.003 PMID:22425687

Tien PT, Lin HJ, Tsai YY, Lim YP, Chen CS, Chang CY, et al. (2020). Perfluorooctanoic acid in indoor particulate matter triggers oxidative stress and inflammation in corneal and retinal cells. *Sci Rep*. 10(1):15702. doi:10.1038/s41598-020-72600-8 PMID:32973190

Timmermann CAG, Jensen KJ, Nielsen F, Budtz-Jørgensen E, van der Klis F, Benn CS, et al. (2020). Serum perfluoroalkyl substances, vaccine responses, and morbidity in a cohort of Guinea-Bissau children. *Environ Health Perspect*. 128(8):87002. doi:10.1289/EHP6517 PMID:32772733

Timmermann CAG, Pedersen HS, Weihe P, Bjerregaard P, Nielsen F, Heilmann C, et al. (2022). Concentrations of tetanus and diphtheria antibodies in vaccinated Greenlandic children aged 7–12 years exposed to marine pollutants, a cross sectional study. *Environ Res*. 203:111712. doi:10.1016/j.envres.2021.111712 PMID:34343554

Tirona RG, Lee W, Leake BF, Lan L-B, Cline CB, Lamba V, et al. (2003). The orphan nuclear receptor HNF4alpha determines PXR- and CAR-mediated xenobiotic induction of CYP3A4. *Nat Med*. 9(2):220–4. doi:10.1038/nm815 PMID:12514743

Toft G, Jönsson BAG, Bonde JP, Nørgaard-Pedersen B, Hougaard DM, Cohen A, et al. (2016). Perfluorooctane sulfonate concentrations in amniotic fluid, biomarkers of fetal Leydig cell function, and cryptorchidism and hypospadias in Danish boys (1980–1996). *Environ Health Perspect*. 124(1):151–6. doi:10.1289/ehp.1409288 PMID:26046833

Tojima H, Kakizaki S, Yamazaki Y, Takizawa D, Horiguchi N, Sato K, et al. (2012). Ligand dependent hepatic gene expression profiles of nuclear receptors CAR and PXR. *Toxicol Lett*. 212(3):288–97. doi:10.1016/j.toxlet.2012.06.001 PMID:22698814

Torres L, Redko A, Limper C, Imbiakha B, Chang S, August A (2021). Effect of perfluorooctanesulfonic acid (PFOS) on immune cell development and function in mice. *Immunol Lett*. 233:31–41. doi:10.1016/j.imlet.2021.03.006 PMID:33722553

Tsai M-S, Lin C-Y, Lin C-C, Chen M-H, Hsu SHJ, Chien K-L, et al. (2015). Association between perfluoroalkyl substances and reproductive hormones in adolescents and young adults. *Int J Hyg Environ Health*. 218(5):437–43. doi:10.1016/j.ijheh.2015.03.008 PMID:25921520

Tsang H, Cheung T-Y, Kodithuwakku SP, Chai J, Yeung WSB, Wong CKC, et al. (2013). Perfluorooctanoate suppresses spheroid attachment on endometrial epithelial cells through peroxisome proliferator-activated receptor alpha and down-regulation of Wnt signalling. *Reprod Toxicol*. 42(December):164–71. doi:10.1016/j.reprotox.2013.08.001 PMID:23978332

Tucker DK, Macon MB, Strynar MJ, Dagnino S, Andersen E, Fenton SE (2015). The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure. *Reprod Toxicol*. 54:26–36. doi:10.1016/j.reprotox.2014.12.002 PMID:25499722

Upham BL, Deocampo ND, Wurl B, Trosko JE (1998). Inhibition of gap junctional intercellular communication by perfluorinated fatty acids is dependent on the chain length of the fluorinated tail. *Int J Cancer*. 78(4):491–5. doi:10.1002/(SICI)1097-0215(19981109)78:4<491::AID-IJC16>3.0.CO;2-9 PMID:9797139

Upham BL, Park JS, Babica P, Sovadinova I, Rummel AM, Trosko JE, et al. (2009). Structure–activity-dependent regulation of cell communication by perfluorinated fatty acids using in vivo and in vitro model systems. *Environ Health Perspect*. 117(4):545–51. doi:10.1289/ehp.11728 PMID:19440492

US EPA (1979). Mutagenicity evaluation of T-2014 CoC in the Ames Salmonella/microsome plate test. Final report. Prepared by Litton Bionetics Inc., USA. Washington (DC), USA: US Environmental Protection Agency.

US EPA (1995a). Mutagenicity test with T-6342 in the Salmonella–*Escherichia coli*/mammalian-microsome reverse mutation assay. Laboratory No. 17073-0-409. Prepared by Lawlor TE for Corning Hazleton Inc., USA. EPA AR 226–0436. Washington (DC), USA: US Environmental Protection Agency (as cited in SIAR, 2006).

US EPA (1995b). Mutagenicity test on T-6342 in an in vivo mouse micronucleus assay. Study No. 17073-0-455. Prepared by Murli H for Corning Hazleton Inc., USA. EPA AR 226-0435. Washington (DC), USA: US Environmental Protection Agency.

US EPA (1996f). Mutagenicity test with T-6564 in the Salmonella–*Escherichia coli*/mammalian-microsome reverse mutation assay with a confirmatory assay. Final report. CHV study No. 17750-0-409R. EPA AR 226-0432. Prepared by Lawlor TE for Corning Hazleton Inc., USA. Washington (DC), USA: US Environmental Protection Agency (as cited in SIAR, 2006).

US EPA (1996a). Mutagenicity test on T-6342 measuring chromosome aberrations in human whole blood lymphocytes with a confirmatory assay with multiple harvests. Study No. 17073-0-449CO. Prepared by Murli H for Corning Hazleton Inc., USA. EPA AR 26-0433. Washington (DC), USA: US Environmental Protection Agency.

US EPA (1996b). Mutagenicity test on T-6564 measuring chromosomal aberrations in Chinese hamster ovary (CHO) cells with a confirmatory assay with multiple harvests. Study No. 17750-0-437CO. Prepared by Murli H for Corning Hazleton Inc., USA. EPA AR 226-0431. Washington (DC), USA: US Environmental Protection Agency.

US EPA (1996c). Mutagenicity test on T-6342 measuring chromosomal aberrations in Chinese hamster ovary (CHO) cells: with a confirmatory assay with multiple harvests. Study No. 17073-0-437CO. Prepared by Murli H for Corning Hazleton Inc., USA. EPA AR 226-0434. Washington (DC), USA: US Environmental Protection Agency.

US EPA (1996d). Mutagenicity test on T-6564 in an in vivo mouse micronucleus assay. Study number 17750-0-455. Prepared by Murli H for Corning Hazleton Inc., USA. EPA AR 226-0430. Washington (DC), USA: US Environmental Protection Agency.

US EPA (1996e). Mutagenicity test on T-6295 in an in vivo mouse micronucleus assay. Final report. CHV Study No. 17403-0-455. Prepared by Murli H for Corning Hazleton Inc., USA. Washington (DC), USA: US Environmental Protection Agency.

US EPA (1999a). Salmonella–*Escherichia coli*/mammalian-microsome reverse mutation assay with PFOS. Final report. Covance study No. 20784-0-409. Prepared by Mecchi MS for Covance Laboratories, USA. EPA AR 226-0133. Washington (DC), USA: US Environmental Protection Agency. Available from: https://cdn.toxicdocs.org/xj/xjJK9N7gNLR78LzpX4jXYVKOg/xjJK9N7gNLR78LzpX4jXYVKOg.pdf, accessed January 2025.

US EPA (1999b). Chromosomal aberrations in human whole blood lymphocytes with PFOS. Final report. Covance study No. 2784-0-499. Prepared by Murli H for Corning Hazleton Inc., USA. EPA-AR-226-0131. US Environmental Protection Agency.

US EPA (1999c). Unscheduled DNA synthesis in rat liver primary cell cultures with PFOS. Final report. Covance Study No. 207840447. Prepared by Cifone MA for Covance Laboratories, USA. EPA AR 226-0132. Washington (DC), USA: US Environmental Protection Agency.

US EPA (2002). CHO/HGPRT forward mutation assay – ISO (T6.889.7). Report No. 01-7019-G1. Prepared by Sadhu D for Toxicon Corporation, USA. EPA AR 226-1101 (as cited in SIAR, 2006). US Environmental Protection Agency.

US EPA (2003). Perfluorooctanoic acid: toxicokinetics in the rat. DuPont 7473. AR-226-149. Prepared by Kemper R, Jepson G, for Haskell Laboratory for Health and Environmental Sciences. Washington (DC), USA: United States Environmental Protection Agency.

US EPA (2021). CompTox Chemicals Dashboard. Washington (DC), USA: US Environmental Protection Agency. Available from: https://www.epa.gov/chemical-research/comptox-chemicals-dashboard, accessed January 2025.

US EPA (2023a). Perfluorooctanoic acid. 335-67-1. DTXSID8031865. CompTox Chemicals Dashboard V2.5.2 [online database]. Washington (DC), USA: US Environmental Protection Agency. Available from: https://comptox.epa.gov/dashboard/chemical/details/DTXSID8031865

US EPA (2023b). Perfluorooctanesulfonic acid. 1763-23-1. DTXSID3031864. CompTox Chemicals Dashboard V2.5.2 [online database]. Washington (DC), USA: US Environmental Protection Agency. Available from: https://comptox.epa.gov/dashboard/chemical/details/DTXSID3031864

van den Dungen MW, Murk AJ, Kampman E, Steegenga WT, Kok DE (2017a). Association between DNA methylation profiles in leukocytes and serum levels of persistent organic pollutants in Dutch men. *Environ Epigenet*. 3(1):dvx001. doi:10.1093/eep/dvx001 PMID:29492303

van den Dungen MW, Murk AJ, Kok DE, Steegenga WT (2017b). Persistent organic pollutants alter DNA methylation during human adipocyte differentiation. *Toxicol In Vitro*. 40:79–87. doi:10.1016/j.tiv.2016.12.011 PMID:28011154

van den Dungen MW, Rijk JCW, Kampman E, Steegenga WT, Murk AJ (2015). Steroid hormone related effects of marine persistent organic pollutants in human H295R adrenocortical carcinoma cells. *Toxicol In Vitro*. 29(4):769–78. doi:10.1016/j.tiv.2015.03.002 PMID:25765474

van Gerwen M, Alpert N, Alsen M, Ziadkhanpour K, Taioli E, Genden E (2020). The impact of smoking on the association between perfluoroalkyl acids (PFAS) and thyroid hormones: a National Health and Nutrition Examination Survey analysis. *Toxics*. 8(4):116. doi:10.3390/toxics8040116 PMID:33316920

Vanden Heuvel JP, Kuslikis BI, Peterson RE (1992). Covalent binding of perfluorinated fatty acids to proteins in the plasma, liver and testes of rats. *Chem Biol Interact*. 82(3):317–28. doi:10.1016/0009-2797(92)90003-4 PMID:1606626

Vanden Heuvel JP, Kuslikis BI, Van Rafelghem MJ, Peterson RE (1991). Tissue distribution, metabolism, and elimination of perfluorooctanoic acid in male and female rats. *J Biochem Toxicol*. 6(2):83–92. doi:10.1002/jbt.2570060202 PMID:1941903

Vanden Heuvel JP, Thompson JT, Frame SR, Gillies PJ (2006). Differential activation of nuclear receptors by perfluorinated fatty acid analogs and natural fatty acids: a comparison of human, mouse, and rat peroxisome proliferator-activated receptor-alpha, -beta, and -gamma, liver X receptor-beta, and retinoid X receptor-alpha. *Toxicol Sci*. 92(2):476–89. doi:10.1093/toxsci/kfl014 PMID:16731579

Verner M-A, Loccisano AE, Morken N-H, Yoon M, Wu H, McDougall R, et al. (2015). Associations of perfluoroalkyl substances (PFAS) with lower birth weight: an evaluation of potential confounding by glomerular filtration rate using a physiologically based pharmacokinetic model (PBPK). *Environ Health Perspect*. 123(12):1317–24. doi:10.1289/ehp.1408837 PMID:26008903

Verner M-A, Ngueta G, Jensen ET, Fromme H, Völkel W, Nygaard UC, et al. (2016). A simple pharmacokinetic model of prenatal and postnatal exposure to perfluoroalkyl substances (PFASs). *Environ Sci Technol*. 50(2):978–86. doi:10.1021/acs.est.5b04399 PMID:26691063

Vested A, Ramlau-Hansen CH, Olsen SF, Bonde JP, Kristensen SL, Halldorsson TI, et al. (2013). Associations of in utero exposure to perfluorinated alkyl acids with human semen quality and reproductive hormones in adult men. *Environ Health Perspect*. 121(4):453–8. doi:10.1289/ehp.1205118 PMID:23360585

Vestergren R, Cousins IT, Trudel D, Wormuth M, Scheringer M (2008). Estimating the contribution of precursor compounds in consumer exposure to PFOS and PFOA. *Chemosphere*. 73(10):1617–24. doi:10.1016/j.chemosphere.2008.08.011 PMID:18834614

Vriens A, Nawrot TS, Janssen BG, Baeyens W, Bruckers L, Covaci A, et al. (2019). Exposure to environmental pollutants and their association with biomarkers of aging: a multipollutant approach. *Environ Sci Technol*. 53(10):5966–76. doi:10.1021/acs.est.8b07141 PMID:31041867

Wan C, Han R, Liu L, Zhang F, Li F, Xiang M, et al. (2016). Role of miR-155 in fluorooctane sulfonate-induced oxidative hepatic damage via the Nrf2-dependent pathway. *Toxicol Appl Pharmacol*. 295:85–93. doi:10.1016/j.taap.2016.01.023 PMID:26844784

Wan HT, Zhao YG, Leung PY, Wong CK (2014). Perinatal exposure to perfluorooctane sulfonate affects glucose metabolism in adult offspring. *PLoS One*. 9(1):e87137. doi:10.1371/journal.pone.0087137 PMID:24498028

Wan HT, Zhao YG, Wong MH, Lee KF, Yeung WS, Giesy JP, et al. (2011). Testicular signalling is the potential target of perfluorooctanesulfonate-mediated subfertility in male mice. *Biol Reprod*. 84(5):1016–23. doi:10.1095/biolreprod.110.089219 PMID:21209418

Wan YJ, Li YY, Xia W, Chen J, Lv ZQ, Zeng HC, et al. (2010). Alterations in tumor biomarker GSTP gene methylation patterns induced by prenatal exposure to PFOS. *Toxicology*. 274(1–3):57–64. doi:10.1016/j.tox.2010.05.006 PMID:20621739

Wan Ibrahim WN, Tofighi R, Onishchenko N, Rebellato P, Bose R, Uhlén P, et al. (2013). Perfluorooctane sulfonate induces neuronal and oligodendrocytic differentiation in neural stem cells and alters the expression of PPARγ in vitro and in vivo. *Toxicol Appl Pharmacol*. 269(1):51–60. doi:10.1016/j.taap.2013.03.003 PMID:23500012

Wang C, Nie X, Zhang Y, Li T, Mao J, Liu X, et al. (2015c). Reactive oxygen species mediate nitric oxide production through ERK/JNK MAPK signalling in HAPI

microglia after PFOS exposure. *Toxicol Appl Pharmacol*. 288(2):143–51. doi:10.1016/j.taap.2015.06.012 PMID: 26086160

Wang C, Ruan T, Liu J, He B, Zhou Q, Jiang G (2015d). Perfluorooctyl iodide stimulates steroidogenesis in H295R cells via a cyclic adenosine monophosphate signalling pathway. *Chem Res Toxicol*. 28(5):848–54. doi:10.1021/tx5004563 PMID:25871633

Wang F, Liu W, Jin Y, Wang F, Ma J (2015b). Prenatal and neonatal exposure to perfluorooctane sulfonic acid results in aberrant changes in miRNA expression profile and levels in developing rat livers. *Environ Toxicol*. 30(6):712–23. doi:10.1002/tox.21949 PMID:24420840

Wang F, Liu W, Ma J, Yu M, Jin Y, Dai J (2012b). Prenatal and neonatal exposure to perfluorooctane sulfonic acid results in changes in miRNA expression profiles and synapse associated proteins in developing rat brains. *Environ Sci Technol*. 46(12):6822–9. doi:10.1021/es3008547 PMID:22594572

Wang H, Du H, Yang J, Jiang H, O K, Xu L, et al. (2019). PFOS, PFOA, estrogen homeostasis, and birth size in Chinese infants. *Chemosphere*. 221:349–55. doi:10.1016/j.chemosphere.2019.01.061 PMID:30641376

Wang H, Li W, Yang J, Wang Y, Du H, Han M, et al. (2023a). Gestational exposure to perfluoroalkyl substances is associated with placental DNA methylation and birth size. *Sci Total Environ*. 858(Pt 1):159747. doi:10.1016/j.scitotenv.2022.159747 PMID:36309289

Wang J, Pan Y, Cui Q, Yao B, Wang J, Dai J (2018a). Penetration of PFASs across the blood cerebrospinal fluid barrier and its determinants in humans. *Environ Sci Technol*. 52(22):13553–61. doi:10.1021/acs.est.8b04550 PMID:30362723

Wang J, Zhang Y, Zhang W, Jin Y, Dai J (2012a). Association of perfluorooctanoic acid with HDL cholesterol and circulating miR-26b and miR-199-3p in workers of a fluorochemical plant and nearby residents. *Environ Sci Technol*. 46(17):9274–81. doi:10.1021/es300906q PMID:22862179

Wang L, Wang Y, Liang Y, Li J, Liu Y, Zhang J, et al. (2014b). PFOS induced lipid metabolism disturbances in BALB/c mice through inhibition of low-density lipoproteins excretion. *Sci Rep*. 4(1):4582. doi:10.1038/srep04582 PMID:24694979

Wang P, Liu D, Yan S, Liang Y, Cui J, Guo L, et al. (2022b). The role of ferroptosis in the damage of human proximal tubule epithelial cells caused by perfluorooctane sulfonate. *Toxics*. 10(8):10. doi:10.3390/toxics10080436 PMID:36006114

Wang Q, Chen W, Zhang B, Gao Z, Zhang Q, Deng H, et al. (2022a). Perfluorooctanoic acid induces hepatocellular endoplasmic reticulum stress and mitochondrial-mediated apoptosis in vitro via endoplasmic reticulum–mitochondria communication. *Chem Biol Interact*. 354:109844. doi:10.1016/j.cbi.2022.109844 PMID:35123991

Wang T, Zhao X, Liu T, Zhang J, Qiu J, Li M, et al. (2023b). Transcriptional investigation of the toxic mechanisms of perfluorooctane sulfonate in rats based on an RNA-seq approach. *Chemosphere*. 329:138629. doi:10.1016/j.chemosphere.2023.138629 PMID:37030344

Wang X, Bai Y, Tang C, Cao X, Chang F, Chen L (2018b). Impact of perfluorooctane sulfonate on reproductive ability of female mice through suppression of estrogen receptor α-activated kisspeptin neurons. *Toxicol Sci*. 165(2):475–86. doi:10.1093/toxsci/kfy167 PMID:29939337

Wang X, Liu L, Zhang W, Zhang J, Du X, Huang Q, et al. (2017). Serum metabolome biomarkers associate low-level environmental perfluorinated compound exposure with oxidative /nitrosative stress in humans. *Environ Pollut*. 229:168–76. doi:10.1016/j.envpol.2017.04.086 PMID:28599201

Wang X, Zhao G, Liang J, Jiang J, Chen N, Yu J, et al. (2013). PFOS-induced apoptosis through mitochondrion-dependent pathway in human–hamster hybrid cells. *Mutat Res*. 754(1–2):51–7. doi:10.1016/j.mrgentox.2013.04.004 PMID:23618922

Wang Y, Aimuzi R, Nian M, Zhang Y, Luo K, Zhang J (2021b). Perfluoroalkyl substances and sex hormones in postmenopausal women: NHANES 2013–2016. *Environ Int*. 149:106408. doi:10.1016/j.envint.2021.106408 PMID:33548847

Wang Y, Wang L, Li J, Liang Y, Ji H, Zhang J, et al. (2014a). The mechanism of immunosuppression by perfluorooctanoic acid in BALB/c mice. *Toxicol Res (Camb)*. 3(3):205–13. doi:10.1039/c3tx50096a

Wang Y, Wang L, Liang Y, Qiu W, Zhang J, Zhou Q, et al. (2011). Modulation of dietary fat on the toxicological effects in thymus and spleen in BALB/c mice exposed to perfluorooctane sulfonate. *Toxicol Lett*. 204(2–3):174–82. doi:10.1016/j.toxlet.2011.04.029 PMID:21571052

Wang Y, Zhang X, Wang M, Cao Y, Wang X, Liu Y, et al. (2015a). Mutagenic effects of perfluorooctanesulfonic acid in *gpt* delta transgenic system are mediated by hydrogen peroxide. *Environ Sci Technol*. 49(10):6294–303. doi:10.1021/acs.est.5b00530 PMID:25875360

Wang Z, Li C, Shao Y, Xue W, Wang N, Xu X, et al. (2021a). Antioxidant defense system responses, lysosomal membrane stability and DNA damage in earthworms (*Eisenia fetida*) exposed to perfluorooctanoic acid: an integrated biomarker approach to evaluating toxicity. *RSC Advances*. 11(43):26481–92. doi:10.1039/D1RA04097A PMID:35479973

Wang Z, Shi R, Ding G, Yao Q, Pan C, Gao Y, et al. (2022d). Association between maternal serum concentration of perfluoroalkyl substances (PFASs) at delivery and acute infectious diseases in infancy. *Chemosphere*. 289:133235. doi:10.1016/j.chemosphere.2021.133235 PMID:34896425

Wang Z, Yao J, Guo H, Sheng N, Guo Y, Dai J (2022c). Comparative hepatotoxicity of a novel perfluoroalkyl ether sulfonic acid, nafion byproduct 2 (H-PFMO2OSA), and legacy perfluorooctane sulfonate (PFOS) in adult male mice. *Environ Sci Technol*. 56(14):10183–92. doi:10.1021/acs.est.2c00957 PMID:35786879

Watkins AM, Wood CR, Lin MT, Abbott BD (2015). The effects of perfluorinated chemicals on adipocyte differentiation in vitro. *Mol Cell Endocrinol*. 400:90–101. doi:10.1016/j.mce.2014.10.020 PMID:25448844

Watkins DJ, Wellenius GA, Butler RA, Bartell SM, Fletcher T, Kelsey KT (2014). Associations between serum perfluoroalkyl acids and LINE-1 DNA methylation. *Environ Int*. 63:71–6. doi:10.1016/j.envint.2013.10.018 PMID:24263140

Weaver YM, Ehresman DJ, Butenhoff JL, Hagenbuch B (2010). Roles of rat renal organic anion transporters in transporting perfluorinated carboxylates with different chain lengths. *Toxicol Sci*. 113(2):305–14. doi:10.1093/toxsci/kfp275 PMID:19915082

Webster GM, Rauch SA, Marie NS, Mattman A, Lanphear BP, Venners SA (2016). Cross-sectional associations of serum perfluoroalkyl acids and thyroid hormones in US adults: variation according to TPOAb and iodine status (NHANES 2007–2008). *Environ Health Perspect*. 124(7):935–42. doi:10.1289/ehp.1409589 PMID:26517287

Webster GM, Venners SA, Mattman A, Martin JW (2014). Associations between perfluoroalkyl acids (PFASs) and maternal thyroid hormones in early pregnancy: a population-based cohort study. *Environ Res*. 133:338–47. doi:10.1016/j.envres.2014.06.012 PMID:25019470

Wei KN, Wang XJ, Zeng ZC, Gu RT, Deng SZ, Jiang J, et al. (2021). Perfluorooctane sulfonate affects mouse oocyte maturation in vitro by promoting oxidative stress and apoptosis induced by mitochondrial dysfunction. *Ecotoxicol Environ Saf*. 225:112807. doi:10.1016/j.ecoenv.2021.112807 PMID:34562787

Weiss JM, Andersson PL, Lamoree MH, Leonards PEG, van Leeuwen SPJ, Hamers T (2009). Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin. *Toxicol Sci*. 109(2):206–16. doi:10.1093/toxsci/kfp055 PMID:19293372

Wen LL, Chen YT, Lee YG, Ko TL, Chou HC, Juan SH (2021). Perfluorooctane sulfonate induces autophagy-associated apoptosis through oxidative stress and the activation of extracellular signal-regulated kinases in renal tubular cells. *PLoS One*. 16(1):e0245442. doi:10.1371/journal.pone.0245442 PMID:33471797

Wen LL, Lin CY, Chou HC, Chang CC, Lo HY, Juan SH (2016). Perfluorooctanesulfonate mediates renal tubular cell apoptosis through PPARgamma inactivation. *PLoS One*. 11(5):e0155190. doi:10.1371/journal.pone.0155190 PMID:27171144

Wen LL, Lin LY, Su TC, Chen PC, Lin CY (2013). Association between serum perfluorinated chemicals and thyroid function in US adults: the National Health and Nutrition Examination Survey 2007–2010. *J Clin Endocrinol Metab*. 98(9):E1456–64. doi:10.1210/jc.2013-1282 PMID:23864701

Wen Y, Chen J, Li J, Arif W, Kalsotra A, Irudayaraj J (2020). Effect of PFOA on DNA methylation and alternative splicing in mouse liver. *Toxicol Lett*. 329:38–46. doi:10.1016/j.toxlet.2020.04.012 PMID:32320774

Wen Y, Rashid F, Fazal Z, Singh R, Spinella MJ, Irudayaraj J (2022). Nephrotoxicity of perfluorooctane sulfonate (PFOS)-effect on transcription and epigenetic factors. *Environ Epigenet*. 8(1):c010. doi:10.1093/eep/dvac010 PMID:35633893

Wielsøe M, Long M, Ghisari M, Bonefeld-Jørgensen EC (2015). Perfluoroalkylated substances (PFAS) affect oxidative stress biomarkers in vitro. *Chemosphere*. 129:239–45. doi:10.1016/j.chemosphere.2014.10.014 PMID:25455676

Wilson J, Berntsen HF, Zimmer KE, Verhaegen S, Frizzell C, Ropstad E, et al. (2016). Do persistent organic pollutants interact with the stress response? Individual compounds, and their mixtures, interaction with the glucocorticoid receptor. *Toxicol Lett*. 241:121–32. doi:10.1016/j.toxlet.2015.11.014 PMID:26599974

Winquist A, Lally C, Shin H-M, Steenland K (2013). Design, methods, and population for a study of PFOA health effects among highly exposed mid-Ohio valley community residents and workers. *Environ Health Perspect*. 121(8):893–9. doi:10.1289/ehp.1206450 PMID:23735518

Wisely GB, Miller AB, Davis RG, Thornquest AD Jr, Johnson R, Spitzer T, et al. (2002). Hepatocyte nuclear factor 4 is a transcription factor that constitutively binds fatty acids. *Structure*. 10(9):1225–34. doi:10.1016/S0969-2126(02)00829-8 PMID:12220494

Wolf CJ, Schmid JE, Lau C, Abbott BD (2012). Activation of mouse and human peroxisome proliferator-activated receptor-alpha (PPARα) by perfluoroalkyl acids (PFAAs): further investigation of C4–C12 compounds. *Reprod Toxicol*. 33(4):546–51. doi:10.1016/j.reprotox.2011.09.009 PMID:22107727

Wolf CJ, Takacs ML, Schmid JE, Lau C, Abbott BD (2008a). Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. *Toxicol Sci*. 106(1):162–71. doi:10.1093/toxsci/kfn166 PMID:18713766

Wolf DC, Moore T, Abbott BD, Rosen MB, Das KP, Zehr RD, et al. (2008b). Comparative hepatic effects of perfluorooctanoic acid and WY14,643 in PPAR-alpha knockout and wild-type mice. *Toxicol Pathol*. 36(4):632–9. doi:10.1177/0192623308318216 PMID:18467680

Wong F, MacLeod M, Mueller JF, Cousins IT (2014). Enhanced elimination of perfluorooctane sulfonic acid by menstruating women: evidence from population-based pharmacokinetic modeling. *Environ Sci Technol*. 48(15):8807–14. doi:10.1021/es500796y PMID:24943117

Woodcroft MW, Ellis DA, Rafferty SP, Burns DC, March RE, Stock NL, et al. (2010). Experimental characterization of the mechanism of perfluorocarboxylic acids' liver protein bioaccumulation: the key role of the neutral species. *Environ Toxicol Chem*. 29(8):1669–77. doi:10.1002/etc.199 PMID:20821618

Worley RR, Moore SM, Tierney BC, Ye X, Calafat AM, Campbell S, et al. (2017a). Per- and polyfluoroalkyl substances in human serum and urine samples from a residentially exposed community. *Environ Int*. 106:135–43. doi:10.1016/j.envint.2017.06.007 PMID:28645013

Worley RR, Yang X, Fisher J (2017b). Physiologically based pharmacokinetic modeling of human exposure to perfluorooctanoic acid suggests historical non drinking-water exposures are important for predicting current serum concentrations. *Toxicol Appl Pharmacol*. 330:9–21. doi:10.1016/j.taap.2017.07.001 PMID:28684146

Wu L-L, Gao H-W, Gao N-Y, Chen F-F, Chen L (2009). Interaction of perfluorooctanoic acid with human serum albumin. *BMC Struct Biol*. 9(1):31. doi:10.1186/1472-6807-9-31 PMID:19442292</jrn>

Xiao C, Grandjean P, Valvi D, Nielsen F, Jensen TK, Weihe P, et al. (2020). Associations of exposure to perfluoroalkyl substances with thyroid hormone concentrations and birth size. *J Clin Endocrinol Metab*. 105(3):735–45. doi:10.1210/clinem/dgz147 PMID:31665456

Xie MY, Sun XF, Wu CC, Huang GL, Wang P, Lin ZY, et al. (2023). Glioma is associated with exposure to legacy and alternative per- and polyfluoroalkyl substances. *J Hazard Mater*. 441:129819. doi:10.1016/j.jhazmat.2022.129819 PMID:36084455

Xie X, Weng X, Liu S, Chen J, Guo X, Gao X, et al. (2021). Perfluoroalkyl and polyfluoroalkyl substance exposure and association with sex hormone concentrations: results from the NHANES 2015–2016. *Environ Sci Eur*. 33(1):69. doi:10.1186/s12302-021-00508-9 PMID:36061407

Xu B, Chen X, Mao Z, Chen M, Han X, Du G, et al. (2013b). Perfluorooctane sulfonate disturbs Nanog expression through miR-490-3p in mouse embryonic stem cells. *PLoS One*. 8(10):e74968. doi:10.1371/journal.pone.0074968 PMID:24098361

Xu B, Ji X, Chen X, Yao M, Han X, Chen M, et al. (2015). Effect of perfluorooctane sulfonate on pluripotency and differentiation factors in mouse embryoid bodies. *Toxicology*. 328:160–7. doi:10.1016/j.tox.2014.12.010 PMID:25510869

Xu C, Jiang Z-Y, Liu Q, Liu H, Gu A (2017). Estrogen receptor beta mediates hepatotoxicity induced by perfluorooctane sulfonate in mouse. *Environ Sci Pollut Res Int*. 24(15):13414–23. doi:10.1007/s11356-017-8943-3 PMID:28386898

Xu D, Li C, Wen Y, Liu W (2013a). Antioxidant defense system responses and DNA damage of earthworms exposed to perfluorooctane sulfonate (PFOS). *Environ Pollut*. 174:121–7. doi:10.1016/j.envpol.2012.10.030 PMID:23257261

Xu J, Shimpi P, Armstrong L, Salter D, Slitt AL (2016). PFOS induces adipogenesis and glucose uptake in association with activation of Nrf2 signalling pathway. *Toxicol Appl Pharmacol*. 290:21–30. doi:10.1016/j.taap.2015.11.002 PMID:26548598

Xu M, Wan J, Niu Q, Liu R (2019). PFOA and PFOS interact with superoxide dismutase and induce cytotoxicity in mouse primary hepatocytes: a combined cellular and molecular methods. *Environ Res*. 175:63–70. doi:10.1016/j.envres.2019.05.008 PMID:31103794

Xu Y, Fletcher T, Pineda D, Lindh CH, Nilsson C, Glynn A, et al. (2020a). Serum half-lives for short-and long-chain perfluoroalkyl acids after ceasing exposure from drinking water contaminated by firefighting foam. *Environ Health Perspect*. 128(7):77004. doi:10.1289/EHP6785 PMID:32648786

Xu Y, Jurkovic-Mlakar S, Li Y, Wahlberg K, Scott K, Pineda D, et al. (2020b). Association between serum concentrations of perfluoroalkyl substances (PFAS) and expression of serum microRNAs in a cohort highly exposed to PFAS from drinking water. *Environ Int*. 136:105446. doi:10.1016/j.envint.2019.105446 PMID:31926437

Xu Y, Lindh CH, Fletcher T, Jakobsson K, Engström K (2022). Perfluoroalkyl substances influence DNA methylation in school-age children highly exposed through drinking water contaminated from firefighting foam: a cohort study in Ronneby, Sweden. *Environ Epigenet*. 8(1):c004. doi:10.1093/eep/dvac004 PMID:35308102

Yahia D, Haruka I, Kagashi Y, Tsuda S (2016). 8-Hydroxy-2′-deoxyguanosine as a biomarker of oxidative DNA damage induced by perfluorinated compounds in TK6 cells. *Environ Toxicol*. 31(2):192–200. doi:10.1002/tox.22034 PMID:25113910

Yan S, Wang J, Dai J (2015b). Activation of sterol regulatory element-binding proteins in mice exposed to perfluorooctanoic acid for 28 days. *Arch Toxicol*. 89(9):1569–78. doi:10.1007/s00204-014-1322-7 PMID:25092180

Yan S, Wang J, Zhang W, Dai J (2014). Circulating microRNA profiles altered in mice after 28 d exposure to perfluorooctanoic acid. *Toxicol Lett*. 224(1):24–31. doi:10.1016/j.toxlet.2013.10.017 PMID:24459700

Yan S, Zhang H, Guo X, Wang J, Dai J (2017). High perfluorooctanoic acid exposure induces autophagy blockage and disturbs intracellular vesicle fusion in the

liver. *Arch Toxicol*. 91(1):247–58. doi:10.1007/s00204-016-1675-1 PMID:26879310

Yan S, Zhang H, Wang J, Zheng F, Dai J (2015a). Perfluorooctanoic acid exposure induces endoplasmic reticulum stress in the liver and its effects are ameliorated by 4-phenylbutyrate. *Free Radic Biol Med*. 87:300–11. doi:10.1016/j.freeradbiomed.2015.06.043 PMID:26159507

Yang B, Zou W, Hu Z, Liu F, Zhou L, Yang S, et al. (2014). Involvement of oxidative stress and inflammation in liver injury caused by perfluorooctanoic acid exposure in mice. *BioMed Res Int*. 2014:409837. doi:10.1155/2014/409837 PMID:24724082

Yang CH, Glover KP, Han X (2010). Characterization of cellular uptake of perfluorooctanoate via organic anion-transporting polypeptide 1A2, organic anion transporter 4, and urate transporter 1 for their potential roles in mediating human renal reabsorption of perfluorocarboxylates. *Toxicol Sci*. 117(2):294–302. doi:10.1093/toxsci/kfq219 PMID:20639259

Yang J, Wang C, Nie X, Shi S, Xiao J, Ma X, et al. (2015). Perfluorooctane sulfonate mediates microglial activation and secretion of TNF-α through Ca^{2+}-dependent PKC-NF-κB signalling. *Int Immunopharmacol*. 28(1): 52–60. doi:10.1016/j.intimp.2015.05.019 PMID:26004316

Yang M, Lee Y, Gao L, Chiu K, Meling DD, Flaws JA, et al. (2022). Perfluorooctanoic acid disrupts ovarian steroidogenesis and folliculogenesis in adult mice. *Toxicol Sci*. 186(2):260–8. doi:10.1093/toxsci/kfac005 PMID:35104888

Yang M, Li L-Y, Qin X-D, Ye X-Y, Yu S, Bao Q, et al. (2021). Perfluorooctanesulfonate and perfluorooctanoate exacerbate airway inflammation in asthmatic mice and in vitro. *Sci Total Environ*. 766:142365. doi:10.1016/j.scitotenv.2020.142365 PMID:33601665

Yang Q, Abedi-Valugerdi M, Xie Y, Zhao XY, Möller G, Nelson BD, et al. (2002b). Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferator, perfluorooctanoic acid. *Int Immunopharmacol*. 2(2–3):389–97. doi:10.1016/S1567-5769(01)00164-3 PMID:11811941

Yang Q, Guo X, Chen Y, Zhang W, Ren J, Wang J, et al. (2020). Blood levels of perfluoroalkyl substances (PFASs), elements and their associations with metabolic syndrome (MetS) in Chinese male adults mediated by metabolic-related risk factors. *Sci Total Environ*. 742:140595. doi:10.1016/j.scitotenv.2020.140595 PMID:32629270

Yang Q, Wang W, Liu C, Wang Y, Sun K (2016). Effect of PFOS on glucocorticoid-induced changes in human decidual stromal cells in the first trimester of pregnancy. *Reprod Toxicol*. 63:142–50. doi:10.1016/j.reprotox.2016.06.003 PMID:27288094

Yang Q, Xie Y, Alexson SE, Nelson BD, DePierre JW (2002a). Involvement of the peroxisome proliferator-activated receptor alpha in the immunomodulation caused by peroxisome proliferators in mice. *Biochem Pharmacol*. 63(10):1893–900. doi:10.1016/S0006-2952(02)00923-1 PMID:12034374

Yang Q, Xie Y, Depierre JW (2000). Effects of peroxisome proliferators on the thymus and spleen of mice. *Clin Exp Immunol*. 122(2):219–26. doi:10.1046/j.1365-2249.2000.01367.x PMID:11091278

Yang Q, Xie Y, Eriksson AM, Nelson BD, DePierre JW (2001). Further evidence for the involvement of inhibition of cell proliferation and development in thymic and splenic atrophy induced by the peroxisome proliferator perfluorooctanoic acid in mice. *Biochem Pharmacol*. 62(8):1133–40. doi:10.1016/S0006-2952(01)00752-3 PMID:11597582

Yang YD, Tian R, Lu N (2023). Binding of serum albumin to perfluorooctanoic acid reduced cytotoxicity. *Sci Total Environ*. 876:162738. doi:10.1016/j.scitotenv.2023.162738 PMID:36906033

Yao PL, Ehresman DJ, Rae JM, Chang SC, Frame SR, Butenhoff JL, et al. (2014). Comparative in vivo and in vitro analysis of possible estrogenic effects of perfluorooctanoic acid. *Toxicology*. 326:62–73. doi:10.1016/j.tox.2014.10.008 PMID:25456267

Yao Q, Gao Y, Zhang Y, Qin K, Liew Z, Tian Y (2021). Associations of paternal and maternal per- and polyfluoroalkyl substances exposure with cord serum reproductive hormones, placental steroidogenic enzyme and birth weight. *Chemosphere*. 285:131521. doi:10.1016/j.chemosphere.2021.131521 PMID:34273704

Yao Q, Shi R, Wang C, Han W, Gao Y, Zhang Y, et al. (2019). Cord blood per- and polyfluoroalkyl substances, placental steroidogenic enzyme, and cord blood reproductive hormone. *Environ Int*. 129:573–82. doi:10.1016/j.envint.2019.03.047 PMID:31174145

Yao X, Zhong L (2005). Genotoxic risk and oxidative DNA damage in HepG2 cells exposed to perfluorooctanoic acid. *Mutat Res*. 587(1–2):38–44. doi:10.1016/j.mrgentox.2005.07.010 PMID:16219484

Yarahalli Jayaram V, Baggavalli S, Reddy D, Sistla S, Malempati R (2020). Effect of endosulfan and bisphenol A on the expression of SUMO and UBC9. *Drug Chem Toxicol*. 43(6):637–44. doi:10.1080/01480545.2018.1526179 PMID:30426790

Ye S, Liu Q, Huang K, Jiang X, Zhang X (2022). The comprehensive analysis based study of perfluorinated compounds – environmental explanation of bladder cancer progression. *Ecotoxicol Environ Saf*. 229:113059. doi:10.1016/j.ecoenv.2021.113059 PMID:34894427

Yeh MM, Bosch DE, Daoud SS (2019). Role of hepatocyte nuclear factor 4-alpha in gastrointestinal and liver diseases. *World J Gastroenterol*. 25(30):4074–91. doi:10.3748/wjg.v25.i30.4074 PMID:31435165

Yeung LW, Guruge KS, Taniyasu S, Yamashita N, Angus PW, Herath CB (2013a). Profiles of perfluoroalkyl substances in the liver and serum of patients with liver cancer and cirrhosis in Australia. *Ecotoxicol Environ*

Saf. 96:139–46. doi:10.1016/j.ecoenv.2013.06.006 PMID:23849467

Yeung LW, Robinson SJ, Koschorreck J, Mabury SA (2013b). Part II. A temporal study of PFOS and its precursors in human plasma from two German cities in 1982–2009. *Environ Sci Technol*. 47(8):3875–82. doi:10.1021/es4004153 PMID:23484930

Ylinen M, Hanhijärvi H, Jaakonaho J, Peura P (1989). Stimulation by oestradiol of the urinary excretion of perfluorooctanoic acid in the male rat. *Pharmacol Toxicol*. 65(4):274–7. doi:10.1111/j.1600-0773.1989.tb01172.x PMID:2587510

Ylinen M, Kojo A, Hanhijärvi H, Peura P (1990). Disposition of perfluorooctanoic acid in the rat after single and subchronic administration. *Bull Environ Contam Toxicol*. 44(1):46–53. doi:10.1007/BF01702360 PMID:2306537

Yu G, Wang J, Liu Y, Luo T, Meng X, Zhang R, et al. (2023). Metabolic perturbations in pregnant rats exposed to low-dose perfluorooctanesulfonic acid: an integrated multi-omics analysis. *Environ Int*. 173:107851. doi:10.1016/j.envint.2023.107851 PMID:36863164

Yu M, Hazelton WD, Luebeck GE, Grady WM (2020). Epigenetic aging: more than just a clock when it comes to cancer. *Cancer Res*. 80(3):367–74. doi:10.1158/0008-5472.CAN-19-0924 PMID:31694907

Yu N, Wei S, Li M, Yang J, Li K, Jin L, et al. (2016). Effects of perfluorooctanoic acid on metabolic profiles in brain and liver of mouse revealed by a high-throughput targeted metabolomics approach. *Sci Rep*. 6(1):23963. doi:10.1038/srep23963 PMID:27032815

Yu WG, Liu W, Jin YH, Liu XH, Wang FQ, Liu L, et al. (2009). Prenatal and postnatal impact of perfluorooctane sulfonate (PFOS) on rat development: a cross-foster study on chemical burden and thyroid hormone system. *Environ Sci Technol*. 43(21):8416–22. doi:10.1021/es901602d PMID:19924978

Yuan Y, Ding X, Cheng Y, Kang H, Luo T, Zhang X, et al. (2020). PFOA evokes extracellular Ca^{2+} influx and compromises progesterone-induced response in human sperm. *Chemosphere*. 241:125074. doi:10.1016/j.chemosphere.2019.125074 PMID:31627108

Zarei MH, Hosseini Shirazi SF, Aghvami M, Pourahmad J (2018). Perfluorooctanesulfonate (PFOS) induces apoptosis signalling and proteolysis in human lymphocytes through ROS mediated mitochondrial dysfunction and lysosomal membrane labialization. *Iran J Pharm Res*. 17(3):995–1007. https://pmc.ncbi.nlm.nih.gov/articles/PMC6094418/ PMID:30127822

Zeng HC, Zhang L, Li YY, Wang YJ, Xia W, Lin Y, et al. (2011). Inflammation-like glial response in rat brain induced by prenatal PFOS exposure. *Neurotoxicology*. 32(1):130–9. doi:10.1016/j.neuro.2010.10.001 PMID:20937303

Zeng HC, Zhu BQ, Wang YQ, He QZ (2021). ROS-triggered autophagy is involved in PFOS-induced apoptosis of human embryo liver L-02 cells. *BioMed Res Int*. 2021:6625952. doi:10.1155/2021/6625952 PMID:33880372

Zhang H, Cui R, Guo X, Hu J, Dai J (2016b). Low dose perfluorooctanoate exposure promotes cell proliferation in a human non-tumor liver cell line. *J Hazard Mater*. 313(5 August):18–28. doi:10.1016/j.jhazmat.2016.03.077 PMID:27045622

Zhang H, Xu C, Shi C, Zhang J, Qian T, Wang Z, et al. (2021a). Hypermethylation of heparanase 2 promotes colorectal cancer proliferation and is associated with poor prognosis. *J Transl Med*. 19(1):98. doi:10.1186/s12967-021-02770-0 PMID:33663522

Zhang H, Zhang C, Xu D, Wang Q, Xu D (2023b). Effects of subchronic exposure of perfluorooctane sulfonate on cognitive function of mice and its mechanism. *Environ Pollut*. 329:121650. doi:10.1016/j.envpol.2023.121650 PMID:37062406

Zhang J, Liang J, Zhu H, Li C, Wu Q (2013d). PFOS and PCB 153 have direct adverse effects on neonatal testis modeled using a coculture of primary gonocyte and Sertoli cells. *Environ Toxicol*. 28(6):322–31. doi:10.1002/tox.20723 PMID:21544924

Zhang J, Shao X, Zhao B, Zhai L, Liu N, Gong F, et al. (2020a). Neurotoxicity of perfluorooctanoic acid and post-exposure recovery due to blueberry anthocyanins in the planarians *Dugesia japonica*. *Environ Pollut*. 263(Pt B):114471. doi:10.1016/j.envpol.2020.114471 PMID:32268227

Zhang L, Krishnan P, Ehresman DJ, Smith PB, Dutta M, Bagley BD, et al. (2016a). Perfluorooctane sulfonate-choline ion pair formation: a potential mechanism modulating hepatic steatosis and oxidative stress in mice. *Toxicol Sci*. 153(1):186–97. doi:10.1093/toxsci/kfw120 PMID:27413108

Zhang L, Louie A, Rigutto G, Guo H, Zhao Y, Ahn S, et al. (2023e). A systematic evidence map of chronic inflammation and immunosuppression related to per- and polyfluoroalkyl substance (PFAS) exposure. *Environ Res*. 220:115188. doi:10.1016/j.envres.2022.115188 PMID:36592815

Zhang L, Ren XM, Guo LH (2013c). Structure-based investigation on the interaction of perfluorinated compounds with human liver fatty acid binding protein. *Environ Sci Technol*. 47(19):11293–301. doi:10.1021/es4026722 PMID:24006842

Zhang L, Ren X-M, Wan B, Guo L-H (2014b). Structure-dependent binding and activation of perfluorinated compounds on human peroxisome proliferator-activated receptor γ. *Toxicol Appl Pharmacol*. 279(3):275–83. doi:10.1016/j.taap.2014.06.020 PMID:24998974

Zhang N, Wang WS, Li WJ, Liu C, Wang Y, Sun K (2015b). Reduction of progesterone, estradiol and hCG secretion by perfluorooctane sulfonate via induction of apoptosis

in human placental syncytiotrophoblasts. *Placenta*. 36(5):575–80. doi:10.1016/j.placenta.2015.02.008 PMID:25748801

Zhang P, Qi C, Ma Z, Wang Y, Zhang L, Hou X (2022a). Perfluorooctanoic acid exposure in vivo perturbs mitochondrial metabolic during oocyte maturation. *Environ Toxicol*. 37(12):2965–76. doi:10.1002/tox.23652 PMID:36029293

Zhang Q, Wang J, Chen C, Kong Y, Yan H, Duan J, et al. (2019). Perfluorooctanoic acid induces migration and invasion and inhibits apoptosis through the PI3K/AKT signalling pathway in human rhabdomyosarcoma cells. *Oncol Rep*. 42(4):1558–68. doi:10.3892/or.2019.7265 PMID:31524277

Zhang R, Yao Y, Tu L, Luan T, Chen B (2021c). Non-targeted metabolomics of multiple human cells revealing differential toxic effects of perfluorooctanoic acid. *J Hazard Mater*. 409:125017. doi:10.1016/j.jhazmat.2020.125017 PMID:33421881

Zhang T, Qin X (2014). Assessment of fetal exposure and maternal elimination of perfluoroalkyl substances. *Environ Sci Process Impacts*. 16(8):1878–81. doi:10.1039/C4EM00129J PMID:24882725

Zhang T, Sun H, Lin Y, Qin X, Zhang Y, Geng X, et al. (2013a). Distribution of poly- and perfluoroalkyl substances in matched samples from pregnant women and carbon chain length related maternal transfer. *Environ Sci Technol*. 47(14):7974–81. doi:10.1021/es400937y PMID:23777259

Zhang T, Sun H, Qin X, Gan Z, Kannan K (2015a). PFOS and PFOA in paired urine and blood from general adults and pregnant women: assessment of urinary elimination. *Environ Sci Pollut Res Int*. 22(7):5572–9. doi:10.1007/s11356-014-3725-7 PMID:25367642

Zhang Y, Beesoon S, Zhu L, Martin JW (2013b). Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. *Environ Sci Technol*. 47(18):10619–27. doi:10.1021/es401905e PMID:23980546

Zhang Y, Cao X, Chen L, Qin Y, Xu Y, Tian Y, et al. (2020b). Exposure of female mice to perfluorooctanoic acid suppresses hypothalamic kisspeptin-reproductive endocrine system through enhanced hepatic fibroblast growth factor 21 synthesis, leading to ovulation failure and prolonged dioestrus. *J Neuroendocrinol*. 32(5):e12848. doi:10.1111/jne.12848 PMID:32307816

Zhang Y, Jiang W, Fang S, Zhu L, Deng J (2014a). Perfluoroalkyl acids and the isomers of perfluorooctanesulfonate and perfluorooctanoate in the sera of 50 new couples in Tianjin, China. *Environ Int*. 68:185–91. doi:10.1016/j.envint.2014.03.022 PMID:24747327

Zhang Y, Mustieles V, Sun Y, Oulhote Y, Wang YX, Messerlian C (2022b). Association between serum per- and polyfluoroalkyl substances concentrations and common cold among children and adolescents in the United States. *Environ Int*. 164:107239. doi:10.1016/j.envint.2022.107239 PMID:35447424

Zhang Y, Mustieles V, Wang YX, Sun Q, Coull B, Sun Y, et al. (2023d). Red blood cell folate modifies the association between serum per- and polyfluoroalkyl substances and antibody concentrations in US adolescents. *Environ Sci Technol*. 57(6):2445–56. doi:10.1021/acs.est.2c07152 PMID:36715557

Zhang Y, Zhang L, Bao J, Liu L, Wang X (2021b). Perfluorooctanoic acid exposure in early pregnancy induces oxidative stress in mice uterus and liver. *Environ Sci Pollut Res Int*. 28(46):66355–65. doi:10.1007/s11356-021-15453-6 PMID:34331232

Zhang YH, Wang J, Dong GH, Liu MM, Wang D, Zheng L, et al. (2013d). Mechanism of perfluorooctanesulfonate (PFOS)-induced apoptosis in the immunocyte. *J Immunotoxicol*. 10(1):49–58. doi:10.3109/1547691X.2012.691123 PMID:22953760

Zhang YM, Dong XY, Fan LJ, Zhang ZL, Wang Q, Jiang N, et al. (2017b). Poly- and perfluorinated compounds activate human pregnane X receptor. *Toxicology*. 380:23–9. doi:10.1016/j.tox.2017.01.012 PMID:28115241

Zhang Y-Z, Zeng X-W, Qian ZM, Vaughn MG, Geiger SD, Hu L-W, et al. (2017a). Perfluoroalkyl substances with isomer analysis in umbilical cord serum in China. *Environ Sci Pollut Res Int*. 24(15):13626–37. doi:10.1007/s11356-017-8954-0 PMID:28391466

Zhang Z, Tian J, Liu W, Zhou J, Zhang Y, Ding L, et al. (2023a). Perfluorooctanoic acid exposure leads to defect in follicular development through disrupting the mitochondrial electron transport chain in granulosa cells. *Sci Total Environ*. 905:166954. doi:10.1016/j.scitotenv.2023.166954 PMID:37722425

Zhang Z, Wen R, Zhang W, Ge N, Bo J (2023c). Efficacy and mechanism of lipoic acid in the treatment of reproductive injury caused by perfluorooctanoic acid. *Exp Ther Med*. 25(3):116. doi:10.3892/etm.2023.11815 PMID:36815965

Zhao B, Lian Q, Chu Y, Hardy DO, Li XK, Ge RS (2011b). The inhibition of human and rat 11β-hydroxysteroid dehydrogenase 2 by perfluoroalkylated substances. *J Steroid Biochem Mol Biol*. 125(1–2):143–7. doi:10.1016/j.jsbmb.2010.12.017 PMID:21237268

Zhao C, Wang S, Zhai Y, Wang M, Tang Y, Li H, et al. (2023). Direct inhibition of human and rat 11β-hydroxysteroid dehydrogenase 2 by per- and polyfluoroalkyl substances: structure–activity relationship and in silico docking analysis. *Toxicology*. 488:153484. doi:10.1016/j.tox.2023.153484 PMID:36878351

Zhao G, Wang J, Wang X, Chen S, Zhao Y, Gu F, et al. (2011a). Mutagenicity of PFOA in mammalian cells: role of mitochondria-dependent reactive oxygen species. *Environ Sci Technol*. 45(4):1638–44. doi:10.1021/es1026129 PMID:21194205

Zhao H, Xie J, Wu S, Sánchez OF, Zhang X, Freeman JL, et al. (2022). Pre-differentiation exposure of PFOA induced persistent changes in DNA methylation and mitochondrial morphology in human dopaminergic-like neurons. *Environ Pollut*. 308:119684. doi:10.1016/j.envpol.2022.119684 PMID:35764183

Zhao W, Zitzow JD, Ehresman DJ, Chang SC, Butenhoff JL, Forster J, et al. (2015). Na+/taurocholate cotransporting polypeptide and apical sodium-dependent bile acid transporter are involved in the disposition of perfluoroalkyl sulfonates in humans and rats. *Toxicol Sci*. 146(2):363–73. doi:10.1093/toxsci/kfv102 PMID:26001962

Zhao Y, Tan YS, Haslam SZ, Yang C (2010). Perfluorooctanoic acid effects on steroid hormone and growth factor levels mediate stimulation of peripubertal mammary gland development in C57BL/6 mice. *Toxicol Sci*. 115(1):214–24. doi:10.1093/toxsci/kfq030 PMID:20118188

Zheng L, Dong GH, Jin YH, He QC (2009). Immunotoxic changes associated with a 7-day oral exposure to perfluorooctanesulfonate (PFOS) in adult male C57BL/6 mice. *Arch Toxicol*. 83(7):679–89. doi:10.1007/s00204-008-0361-3 PMID:19015834

Zheng L, Dong GH, Zhang YH, Liang ZF, Jin YH, He QC (2011). Type 1 and Type 2 cytokines imbalance in adult male C57BL/6 mice following a 7-day oral exposure to perfluorooctanesulfonate (PFOS). *J Immunotoxicol*. 8(1):30–8. doi:10.3109/1547691X.2010.537287 PMID:21299352

Zheng XQ, Shi YJ, Lu Y-L, Xu AB (2016). Growth inhibition and DNA damage in the earthworm (*Eisenia fetida*) exposed to perfluorooctane sulphonate and perfluorooctanoic acid. *Chem Ecol*. 32(2):103–16. doi:10.1080/02757540.2015.1116524

Zhong SQ, Chen ZX, Kong ML, Xie YQ, Zhou Y, Qin XD, et al. (2016). Testosterone-mediated endocrine function and TH1/TH2 cytokine balance after prenatal exposure to perfluorooctane sulfonate: by sex status. *Int J Mol Sci*. 17(9):1509. doi:10.3390/ijms17091509 PMID:27626407

Zhou Y, Hu L-W, Qian ZM, Chang J-J, King C, Paul G, et al. (2016). Association of perfluoroalkyl substances exposure with reproductive hormone levels in adolescents: by sex status. *Environ Int*. 94:189–95. doi:10.1016/j.envint.2016.05.018 PMID:27258660

Zhou Y, Hu L-W, Qian ZM, Geiger SD, Parrish KL, Dharmage SC, et al. (2017). Interaction effects of polyfluoroalkyl substances and sex steroid hormones on asthma among children. *Sci Rep*. 7(1):899. doi:10.1038/s41598-017-01140-5 PMID:28420867

Zhou Y, Li H, Lin C, Mao Y, Rao J, Lou Y, et al. (2020). Perfluorooctanoic acid (PFOA) inhibits the gap junction intercellular communication and induces apoptosis in human ovarian granulosa cells. *Reprod Toxicol*. 98(December):125–33. doi:10.1016/j.reprotox.2020.09.005 PMID:32971237

Zhou YT, Li R, Li SH, Ma X, Liu L, Niu D, et al. (2022). Perfluorooctanoic acid (PFOA) exposure affects early embryonic development and offspring oocyte quality via inducing mitochondrial dysfunction. *Environ Int*. 167:107413. doi:10.1016/j.envint.2022.107413 PMID:35863238

Zhou Z, Shi Y, Vestergren R, Wang T, Liang Y, Cai Y (2014). Highly elevated serum concentrations of perfluoroalkyl substances in fishery employees from Tangxun lake, China. *Environ Sci Technol*. 48(7):3864–74. doi:10.1021/es4057467 PMID:24588690

Zhu J, Qian W, Wang Y, Gao R, Wang J, Xiao H (2015). Involvement of mitogen-activated protein kinase and NF-κB signalling pathways in perfluorooctane sulfonic acid-induced inflammatory reaction in BV2 microglial cells. *J Appl Toxicol*. 35(12):1539–49. doi:10.1002/jat.3119 PMID:25677194

Zhu TR, Cao J, Hong JW, Li J (2020). [Effects of PFOS on inflammatory factors in human placental trophoblast cells]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi*. 38(7):481–4. doi:10.3760/cma.j.cn121094-20190917-00380 PMID:32746565 [Chinese]

Zhu Y, Qin XD, Zeng XW, Paul G, Morawska L, Su MW, et al. (2016). Associations of serum perfluoroalkyl acid levels with T-helper cell-specific cytokines in children: by gender and asthma status. *Sci Total Environ*. 559:166–73. doi:10.1016/j.scitotenv.2016.03.187 PMID:27060656

Zhu Y, Shin H-M, Jiang L, Bartell SM (2022). Retrospective exposure reconstruction using approximate Bayesian computation: a case study on perfluorooctanoic acid and preeclampsia. *Environ Res*. 209:112892. doi:10.1016/j.envres.2022.112892 PMID:35149111

Zota AR, Geller RJ, Romano LE, Coleman-Phox K, Adler NE, Parry E, et al. (2018). Association between persistent endocrine-disrupting chemicals (PBDEs, OH-PBDEs, PCBs, and PFASs) and biomarkers of inflammation and cellular aging during pregnancy and postpartum. *Environ Int*. 115:9–20. doi:10.1016/j.envint.2018.02.044 PMID:29533840

Zou W, Liu W, Yang B, Wu L, Yang J, Zou T, et al. (2015). Quercetin protects against perfluorooctanoic acid-induced liver injury by attenuating oxidative stress and inflammatory response in mice. *Int Immunopharmacol*. 28(1):129–35. doi:10.1016/j.intimp.2015.05.043 PMID:26054880