($p < 0.05$ and $p < 0.001$, respectively). The group of younger participants (25 ± 5 years) presented higher PFHxS values and lower FOSA values than did the older participants [73].

Rylander et al. [84] also registered higher concentrations of PFOS, PFOA, PFHxS, and PFHpS in male Norwegian participants than in women. Here, also increasing concentrations of PFOS, PFHxS, and PFHpS were observed with increasing age.

A study of 245 blood samples of donors from China showed that lower concentrations of PFOS were detected in infants, young children, children, and adolescents (2.52 to 5.55 μg/L) than in adults (8.07 μg/L), and correlations of PFOS ($r = 0.468$) and PFHxS ($r = 0.357$) with age were reported. In contrast, PFOA concentrations in blood of the children and adolescents were higher (1.23 to 2.42 μg/L) than in adults (1.01 μg/L), showing a negative correlation with age ($r = -0.344$). The composition of the PFC concentration profiles also varied between age groups, suggesting different sources of exposure. Gender specific differences in PFC concentration could not be determined in any of the groups [52].

Fromme et al. [77] carried out a study of PFC concentrations in blood of participants in Germany. Concentrations of PFOA and PFOS were measured in 356 blood plasma samples. The mean values of 10.9 μg/L PFOS and 4.8 μg/L PFOA were determined for women. The values for men were higher (13.7 μg/L PFOS and 5.7 μg/L PFOA). Higher blood PFC concentrations correlated with increasing age in students; however, this correlation was only statistically significant for female students [77]. A second German study also confirmed age as having an effect on PFC concentrations is plasma. The age of men correlated positively with the plasma concentrations of PFOS, PFOA, and PFHxS. In the case of women, this was only true for PFOA [19]. In a US American study, the mean PFOS and PFHxS concentrations were significantly lower in participants who were younger than 40 years than in the group over 40 years [85]. The values from this study are shown in Table 14.

According to the EFSA [15], none of the studies included show a clear difference in relationship to PFOS and/or PFOA serum concentrations in relation to age or gender of the participants. Fromme et al. [38] had come to the conclusion, however, that the majority of the studies show gender-specific differences in serum concentrations of PFOS and PFOA. In regard to age dependency, however, they agree with the EFSA [15] that there is no significant correlation between age and PFC blood concentrations although it must be assumed that these compounds accumulate in the body over time.

Since human biomonitoring studies showed higher PFOS blood concentrations for men than for women, Liu et al. [62,86,87] investigated the effect of pregnancy, menstruation, and periodic exposure to PFOS concentration in the blood of mice. The animals received 50 μg/L PFOS in their drinking water. Pregnancy or menstruation led to lower PFOS concentrations in the blood. Every additional individual exposure to PFOS increased the concentration of the substance in blood.

Geographic and ethnic differences

Geographical differences have been detected in the PFOS and PFOA concentrations in serum of blood donors in diverse countries. Kannan et al. [71] reported differences in the occurrence of PFOS and PFOA among blood donors in nine different countries. Harada et al. [82] detected differences in the PFOS and PFOA serum concentrations for both genders in Japan. The concentrations of PFOS and PFOA in blood measured in Germany were lower than the values from a study in the USA and Canada [77].

Fromme et al. [38] came to the conclusion that serum concentrations of the US population are higher than those of inhabitants of Europe, Asia, or Australia. The same is true of PFHxS [38] (Table 15).

Concentrations of 29 μg/L PFOS, 3.9 μg/L PFOA, 0.5 μg/L PFHxS, 0.8 μg/L PFNA, and 1.1 μg/L PFHpS (mean values) were detected in 95% of all blood samples from Norwegians [84]. In another Norwegian study of 315 women, concentrations of 20 μg/L PFOS, 4.4 μg/L PFOA, 1.0 μg/L PFHxS, and 0.81 μg/L PFNA were found in 90% of the plasma samples [88].

Kärrman et al. [83] did not find a difference in PFC serum concentrations for participants from rural or urban regions of Australia. Mean values for PFOS (20.8 μg/L), PFOA (7.6 μg/L), and PFHxS (6.2 μg/L) measured in this study were similar to the values determined for

Table 14 PFOS, PFOA, and PFHxS concentrations in blood (μg/L) according to age group

Substance	< 40 years	40 to 60 years	> 60 years
PFOS	24.8/33.3	31.7/33.6	35.3/35.1
PFOA	2.2/5.3	2.4/5.5	2.4/6.0
PFHxS	0.7/1.8	1.9/2.5	2.2/3.0

Values from 1974/1989 of Olsen et al. [85].

Table 15 Serum and plasma PFC concentrations (μg/L) according to geographical origin of participants

Substance	US population	EU population	Asian population
PFOS	656	1 to 116	n.r.
PFOA	88	0.5 to 40	n.r.
PFHxS	< 0.4 to 712	< 0.4 to 40	0.1 to 20.9

Adapted from Fromme et al. [38]; n.r., not reported.

serum concentrations in Europe and Asia, or higher, but lower than in the USA.

In an African study, concentrations of 1.6 μg/L PFOS, 1.3 μg/L PFOA, and 0.5 μg/L PFHxS were measured in the blood of mothers who were tested. Fifty eight percent of the PFOS molecules present were in the linear form. The highest PFC concentrations were detected in the blood of people from urban and semi-urban regions, which are areas with the highest quality of living conditions [89].

Hemat et al. [90] determined a lower internal PFC contamination of people in Afghanistan. PFOS concentrations of 0.21 to 11.8 μg/L were detected in blood, and PFOA and PFHxS concentrations were below the LOD of 0.5 μg/L. In drinking water, as well, PFOA or PFOS concentrations were not detected at levels above the LOD (0.03 and 0.015 μg/L). The studies cited here are shown in Figure 2.

The study of Kannan et al. [71] in which samples were obtained from nine different countries showed differences in levels of PFOS in relation to the country of the donors. The US study [91] showed that non-Hispanic whites had statistically significantly higher concentrations of PFOS than both non-Hispanic blacks and Mexican Americans; Mexican Americans had statistically significantly lower concentrations than non-Hispanic blacks. Genetic variability, diet, lifestyle, or a combination of all these factors may contribute to the different patterns of human exposure to PFOS observed among the population groups [15].

Dietary influences

A Swedish study in which samples of blood from 108 women were analyzed showed a correlation between increased consumption of predatory fish (pike, perch, zander) and PFOS concentration in the blood. This correlation could not, however, be shown for total fish consumption or for other groups of foodstuffs ([92] cited in EFSA [15]). A Polish study established a correlation between increased fish consumption and the highest serum concentrations measured in 45 test candidates for 10 fluorochemicals (including PFOS and PFOA) [93].

In a study of 60 participants in Norway, Rylander et al.[84] determined significantly lower concentrations of PFOS and PFOA in the blood of candidates who stated that they had consumed 150 g of vegetables and fruits per week over the past year. In contrast, an increase consumption of oily fish (150 g/week) led to significantly higher concentrations of these substances in the blood.

In another study, Rylander et al. [88] examined blood from 315 Norwegian women between the ages of 48 and 62 years. Participants who consumed larger

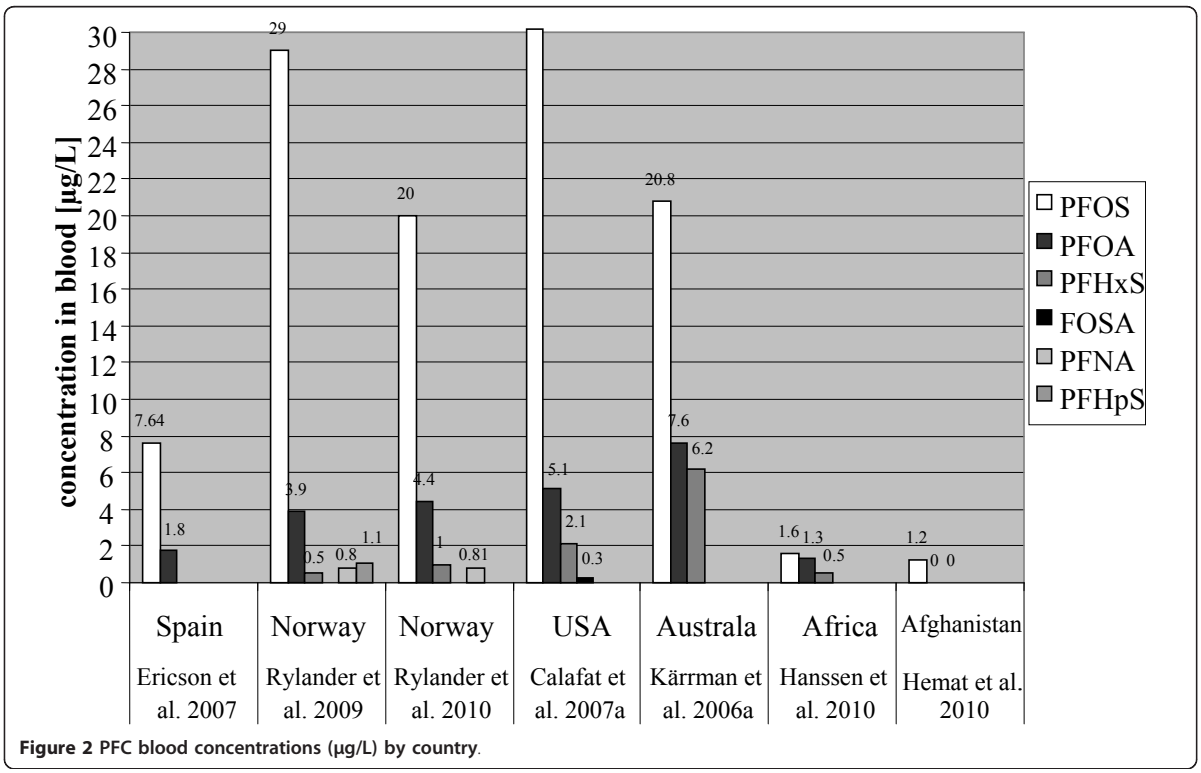

Figure 2 PFC blood concentrations (μg/L) by country.

amounts of fish had higher PFOS, PFNA, and PFHxS concentrations in their blood than did younger women with larger households and a more western diet of rice, pasta, water, white and red meat, chocolate, snacks, and pastry. No specific cluster of foods could be correlated with higher PFOA blood concentrations [88].

Time trends

A study of 178 US serum samples shows an increase in PFOS and PFOA concentrations between 1974 and 1989. The mean values of serum concentrations of PFOS, PFOA, and PFHxS from 1974 and 1989 are shown in Table 16. Serum samples collected in 2001 did not show any further increase in PFC concentrations [69,85].

A Japanese study established an increase in PFOS and PFOA concentrations in serum samples over the last 25 years. PFOS concentrations increased by a factor of 3, and PFOA concentrations by as much as a factor of 14 [82].

A continual increase in PFOA and PFOS over time was also shown in a Chinese study in which serum samples from 1987, 1990, 1999, and 2002 were analyzed [94]. The changes in serum concentrations over time as shown in this study are presented in Figure 3.

On the other hand, another study showed the decline of serum concentrations of PFOS by 32%, of PFOA by 25%, and of PFHxS by 10% (data from the NHANES from 1999 to 2000). These changes can probably be attributed to the change in production of PFOS and perfluorooctane sulfonylfluoride compounds. The PFNA concentrations increased by 100% [95]. These values are also shown in Table 16. The concentrations listed by Olsen [69,85] are mean values, while those from Calafat et al. [95] are geometric mean values, making a comparison of the results difficult or impossible.

Studies from the Sauerland region of Germany show constant PFOS and PFOA concentrations between 1997 and 2004; however, the plasma concentrations of PFHxS have risen continuously since 1977 [21].

Differences dependent upon the isomery of the compounds

Studies have shown that the linear form of PFOS [L-PFOS] is more plentiful than the branched isomers in the human serum and plasma samples. L-PFOS was seen to account for 58% to 70% of the total PFOS in samples from Australia, 68% from Sweden, and 59% from the UK. The disparities are presumably the result of different sources of exposure in the various countries. For example, a standard PFOS product produced by electrochemical fluoridation [ECF] consists of 76% to 79% L-PFOS [72].

A study by De Silva and Mabury [96] showed that 98% of the PFOA in the serum of the participants was linear PFOA [L-PFOA], so only 2% was present in the branched form. The same is true of PFNA and PFUnA. A standard PFOA product produced by ECF consists of 80% L-PFOA. The high proportion of L-PFOA in serum can probably be attributed to the exposure and metabolism of FTOH and alkanes [38].

Table 16 PFC concentration in blood (μg/L) by year

Substance	Olsen et al. [69,85]			Calafat et al. [95]	
	1974	1989	2001	1999 to 2000	2003 to 2004
PFOS	29.5	34.7	35.8	30.4	20.7
PFOA	2.3	5.6	4.7	5.2	3.9
PFHxS	1.6	2.4	1.5	2.1	1.9

Toxicology of perfluorinated compounds

Toxicokinetics of perfluorinated compounds

Uptake

Data from animal experiments show that PFC uptake can occur by oral, inhalation, or dermal exposure [97-102].

Oral uptake of PFOS and PFOA results in rapid and almost complete assimilation. Ninety five percent of the radioactively labeled PFOS dose (4.3 mg/kg BW) and 93% of the labeled PFOA-dose (11 mg/kg BW) were resorbed by male rats within 24 h. The authors found 5% and 7% of the total radioactivity in feces and in the digestive tract and concluded that the remainder is the resorbed portion. These resorption data are from Gibson and Johnson [97] and were determined using ^{14}C-labeled PFOS and PFOA [17].

After 10 inhalations of 84 mg/m^3 APFO, a mean concentration of 108 mg/L was measured in the blood of male rats. The APFO blood concentration declined to 0.84 mg/L 84 days after the treatment [100].

Uptake via dermal exposition appears to be somewhat weaker [101]. A study by Kennedy [99] showed a dose-dependent increase in blood concentration of organofluoro compounds in rats after dermal application of APFO. The subchronic dermal treatment with 2, 000 mg APFO/kg resulted in blood concentrations of 118 mg/L.

In rats, an uptake of 8:2 FTOH via the skin was relatively low. After 6 h of exposure, 37% of the substance evaporated or was removed by washing. The evaporated portion was trapped by a device attached to the skin and was consequently analyzed. The treated area of skin was washed with a soap-ethanol mixture, and the 8:2 FTOH concentration in the solvent was measured. In these experiments, a single 8:2 FTOH dose of 125 mg/kg^c in 0.5% methyl cellulose was applied. The 8:2 FTOH was labeled with ^{14}C (3-^{14}C 8:2 FTOH) and applied to the shaved area of skin (10 μL/cm^2) [102].

Distribution

PFOS and PFOA are weakly lipophilic, very water soluble, and bind preferentially to proteins. The principle

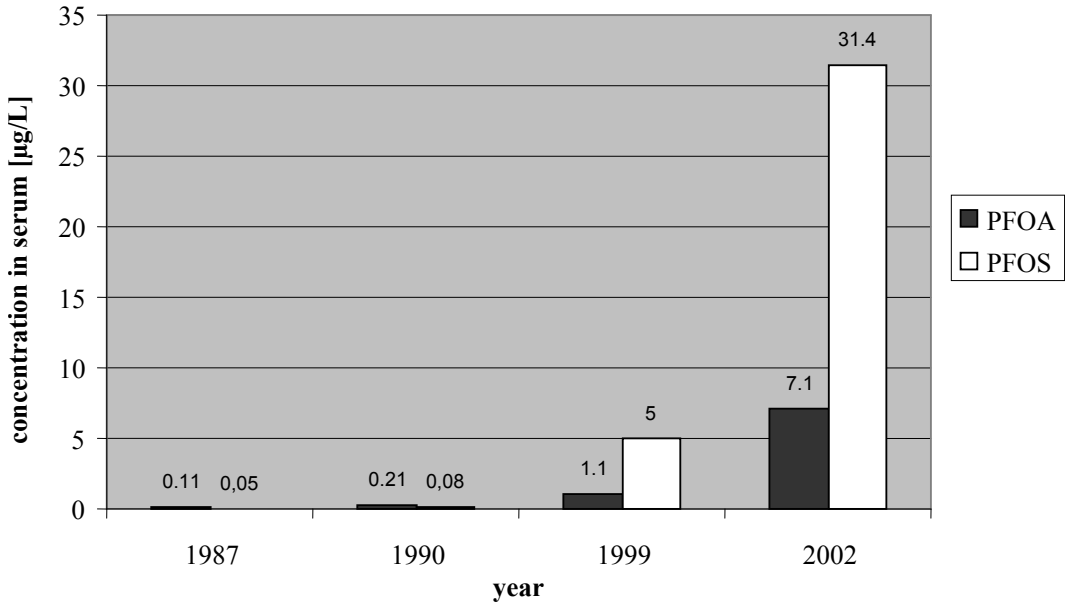

Figure 3 PFOA and PFOS serum concentrations over time in China. (By Jin et al. [94]).

binding partner is albumin [61,103]; however, it also binds to β-lipoproteins or fatty acid binding proteins in the liver [L-FABP] [104].

Approximately 90% to 99% of the perfluoridated carboxylic acids in the blood are bound to serum albumin [103,105]. The chain length and the functional group of the PFCs have an influence on the preferential binding site and binding affinity [80]. PFCs have the same binding site and a similar affinity to serum albumin as fatty acids [80].

Qin et al. [106] used spectrometry to determine the influence of the length of the carbon chain of perfluorinated carboxylic acids on the binding to bovine serum albumin. They determined that the binding strength increased with the increasing chain length of the perfluorinated compound. The changes in enthalpy and entropy indicate that Van-der-Waals' forces and hydrogen bonds are the dominant intermolecular forces [106]. Bischel et al. [79] also confirmed the high affinity interactions between perfluorinated compounds and serum albumin, in particular at low molar ratios. PFOS and PFOA are primarily extracellular and accumulate primarily in the liver, blood serum, and kidneys. Small amounts of the substances are found in other tissues as well. According to studies by Austin et al. [107] and Seacat et al. [108], the liver to serum ratio for PFOS is about 2.5. PFOS and PFOA were also found primarily in the liver and kidneys of chickens [109] and Han et al. [110] found an active uptake mechanism for PFO (the anion of PFOA) in rat hepatocytes.

In addition, differences in distribution patterns may be dose dependent. In experiments with rats, Kudo et al. [111] found that 2 h after a single intravenous injection of low-dosage PFOA (0.041 mg/kg BW), a larger proportion of the substance is found in the liver (52%) than with a higher dosage (27% for a dosage of 16.56 mg/kg BW). Apparently, PFOA is distributed to the blood or other tissues as soon as the level in the liver reaches 4 mg/kg. The study does not provide an immediate explanation of these results; however, a dose-dependent difference in intracellular distribution between the membrane fraction and the cytosol was observed for the two different dosages of 0.041 mg/kg BW and 4 mg/kg BW. Injection of the higher dosage resulted in PFOA primarily in the cytosolic fraction. If the liver concentration remained under 4 mg/kg, PFOA was found almost completely in the membrane fraction with a remainder of 3% in the cytosol. Kudo et al. [111] concluded that this indicates a preferred bond of PFOA to membrane components that are not unlimitedly available. As a consequence, higher dosages of PFOA are distributed in the blood or other tissues. Elimination via the bile rose with higher doses were administered, suggesting transport of unbound PFOA from the cytosolic fraction of the cell to the bile. A biliary elimination rate of 0.07 mL/hr/kg BW was determined[d]. The rate of elimination rose in a dose-dependent manner; however, the differences of the rates between the administered doses were not significant [111].

Tan et al. [112] discovered differences in distribution patterns dependent upon the perfluorinated compound,

species (rat or monkey), and gender. PFOS, probably because of its higher liver to blood distribution coefficient, seemed to remain in the tissue longer than PFOA. The maximal transport capacity of renal resorption in monkeys was 1, 500 times greater than that of rats, and the clearance of renal filtrate in the central compartment was about 10 times greater. Male rats showed a slower renal elimination of PFOA than female animals; however, low PFOA concentrations (< 0.1 μg/mL) were eliminated at a similarly slow rate by females [112].

In addition, Liu et al. [113] studied age-dependent differences in the toxicokinetics of PFOS in mice. The concentrations and distribution ratios of PFOS in the blood, brain, and liver of mice after a single subcutaneous application of 50 mg PFOS/kg BW differed significantly between the individual postnatal developmental stages. With increasing age, the differences became more evident. Gender-specific differences were greater in older mice. A study demonstrated the following distribution pattern of FTOH:

Four to seven percent of the ^{14}C-labeled 8:2 FTOH was recovered in the tissue of rats 7 days after oral applications (125 mg/kg), principally in the fat, liver, thyroid, and adrenal tissues [102]. PFCs are also distributed in the milk and via the placenta, as described in the 'Pre- and postnatal exposures' section.

PFOS could also be detected in the livers of rat fetuses [114]. Additionally, on the basis of studies of rats, it was possible to estimate that the PFOA plasma concentration of the fetus amounts to half the steady state concentration in the plasma of the mother animal. In the transition of PFOA to the milk of the mother animal, the steady state concentration in the milk was 1/10 lower than the level in plasma ([58] cited in EFSA [15,115]). Peng et al. [116] determined that the ratio of concentrations in the eggs of sturgeons to the concentration in the liver of the mother sturgeon was 0.79 for PFOA and 5.5 for perfluorotridecanoic acid.

Contamination with PFOA may have also resulted from corresponding precursor substances. It has, for example, been demonstrated that PFOA can be formed from FTOH [31,32]. Following a single dose of 30 mg/kg BW 8:2 FTOH on the eighth gestational day [GD] (GD 8) in mice, the PFOA concentrations in the fetus rose from 45 ± 9 μg/kg (GD 10) to 140 ± 32 μg/kg (GD 18). Furthermore, PFNA was also detected at a concentration of 31 ± 4 μg/kg (GD 18). For the mice that were not contaminated with 8:2 FTOH *in utero*, but rather through nursing, concentrations of 57 ± 11 μg PFOA/L were detected on the third and 58 ± 3 μg PFOA/L on the 15th day after birth. This indicates that the progeny became contaminated with PFOA by nursing from the mother animal that had been exposed to FTOH [117].

Metabolism

As far as it is known, PFOS and PFOA are not metabolized in mammals. Thus, PFOA is not subject to defluorination nor to phase-II metabolism of biotransformation [101]. According to Fromme et al. [2], only FTOH comes into question regarding metabolism.

For example, Fasano et al. [102] could detect glucuronide and glutathione conjugates in the bile as well as perfluorooctanoate and perfluorhexanoate in excrements and in the plasma of male and female rats that had received a single oral dose of 5 and 125 mg/kg ^{14}C-labeled 8:2 FTOH. This implies that FTOH is metabolized and that a removal of CF_2 groups takes place.

Other studies have also shown possible formation of PFCA from FTOH [31,32,117]. It is generally assumed that oxidation of the alcohol group takes place to form fluorotelomer aldehyde, followed by oxidation to saturated fluorotelomer compounds (fluorotelomer saturated carboxylate [FTCA]). Butt et al. [118] examined in greater detail the biotransformation pathway for 8:2 FTOH in rainbow trout, in particular, from the metabolic intermediates 8:2 FTOH unsaturated carboxylate [FTUCA] and 7:3 FTOH saturated carboxylate [FTCA]. The authors administered these intermediates as well as 8:2 FTCA to the trout for 7 days and then identified the compound in the blood and liver for a further 10 days. Exposure to 7:3 FTCA resulted in lower concentrations of 7:3 FTUCA and perfluorohepatanoate (PFHpA) and did not result in an accumulation of PFOA. Furthermore, 8:2 FTCA and 8:2 FTUCA were generated. PFOA was formed when 8:2 FTCA and 8:2 FTUCA were administered. These results suggest a β-oxidation beginning with 8:2 FTUCA to 7:3 keto acid and 7:2 ketone for the PFOA formation [118].

The emerging metabolic products are often more toxic than the original substance itself. This was also shown for FTOH in a study by Martin et al. [119]. In tests in which isolated rat hepatocytes were incubated with FTOH of various chain lengths, the shortest (4:2 FTOH) and longest (8:2 FTOH) lengths showed a greater toxicity, in terms of the LC_{50} than did, e.g., 6:2 FTOH.

Treatment with 8:2 FTOH led to a decline in glutathione [GSH] levels and an increase in protein carbonylation and lipid peroxidation. The addition of aminobenzotriazol, an inhibitor of cytochrome P450, diminished the cytotoxicity of all tested FTOH and decreased protein carbonylation and lipid peroxidation of 8:2 FTOH. Preincubating the hepatocytes with hydralazine or aminoguanidine (a carbonyl trap with nucleophilic amino groups that form adducts with aldehydes) also reduced the cytotoxicity of 8:2 FTOH. Likewise, a GSH-reactive α/β-unsaturated acid which is a result from the metabolism proved more toxic than the corresponding FTOH compound. It can be concluded from

this that the toxicity of FTOH is the result of electrophonic aldehydes or acids, GSH decrease, and protein carbonylation [119].

Excretion

Since PFOS and PFOA cannot be metabolized by mammals, excretion is the only means by which the toxic activity of these compounds can be eliminated once they have been taken up by the body [17].

Measurements of PFC concentrations in urine and feces yielded an elimination half-life of more than 90 days for PFOS in rats. The half-life of PFOA is markedly shorter and exhibits gender-dependent differences, 2 to 4 h for female rats and 4 to 6 days for male rats [115].

Because of albumin binding of a large portion of PFCs in the blood, the glomerular filtration rate is low. However, an active excretory mechanism via transport proteins has been described in rats. This so-called organic anion transporter [OAT] (OATs 2 and 3) enables the uptake of PFOA from the blood by the proximal tubule cells in the kidneys [120]. The expression of OAT 2 and 3 in the kidneys correlates with the excretion of PFOA by rats and is presumably regulated by sex hormones. This may explain why female rats have excreted 91% of the applied dose of ^{14}C-labeled PFOA after 24 h via urine, while only 6% of the administered ^{14}C-labeled PFOA can be detected in the urine of male rats. An active excretory mechanism has not yet been described for PFOS ([121] cited in EFSA [15]).

Weaver et al. [122] confirmed the involvement of the basolateral OATs 1 and 3 in renal secretion of C7-C9 PFCA in rats. On the other hand, the apical organic anion transport polypeptide [OATP] 1a1 contributes to the reabsorption of C8-C10 PFCA in the proximal tubule cells of the rat, with the highest affinity to C9 and C10. The OATP 1a1 expression is heightened in the kidneys of male rats and might therefore also help explain the gender-specific differences in renal PFCA excretion.

Experiments by Johnson et al. [123] show the presence of an enterohepatic circulation of PFCs. Increased fecal excretion of ^{14}C-labeled PFOA and PFOS in rats was observed after multi-day administration cholestyramine *per os*, accompanied by a concurrent reduction in concentrations of the substances in the liver and plasma. Cholestyramine is an anion-exchange resin; it is not resorbed and carries PFOA and PFOS to the intestines to be excreted. The rates of excretion for PFOA and/or PFOS in rats that had received APFO (13.3 mg/kg) or the potassium salt of PFOS (3.4 mg/kg) intravenously were increased by 9.8 times and 9.5 times, respectively, after a 14- or 21-day administration of a 4% cholestyramine mixture in their feed [123].

Cui et al. [124] examined PFOS and PFOA excretions in male rats during a 28-day consecutive administration of PFOS and PFOA. Urine was confirmed as the primary path of excretion of PFOS and PFOA in rats in this study. In particular, PFOA excretion rates were greater in urine than in feces. Within the first 24 h after the start of oral application of PFOA or PFOS, 24.7% to 29.6% PFOA and 2.6% to 2.8% PFOS of the oral dosage (5 and 20 mg/kg BW/day) were excreted in the urine and feces. The rate of excretion over this period of time increased with the increasing dosage. The higher rate of elimination indicates a lower accumulation capacity. The rapid, almost total uptake and relatively weak elimination of PFOA and PFOS facilitate the bioaccumulation in the body [124].

In experiments on chickens, Yoo et al. [109] determined a rate of elimination for PFOA six times higher than for PFOS. The authors administered 0.1 or 0.5 g/L PFOA or 0.2 or 0.1 g/L PFOS to the 6-week-old male chickens for 4 weeks. A 4-week excretion phase for PFOA and PFOS followed. The data from the study can be seen in Table 17[109].

In primates, the half-life of PFCs is longer than in other experimental animals such as mice and rats. The elimination half-life is 14 to 42 days in male or female cynomolgus monkeys after oral and intravenous applications. The PFOA concentrations after a 4-week oral application are shown in Table 18. Urine was the principle path of excretion for PFOA in monkeys [125].

In contrast, the half-life of PFOA in Japanese macaques is notably shorter (2.7 to 5.6 days) ([101] as cited by Harada et al. [126]). A half-life of 110 to 130 days was determined for nonhuman primates after a single, intravenous application [127].

The elimination half-time for PFOS in male cynomolgus monkeys was found to be about 200 days [128]. In addition to species-specific differences, the structure of the PFCs can also influence excretion.

Benskin et al. [129] administered a single dose of 500 μg/kg BW PFOS, PFOA, and PFNA or 30 μg/kg BW PFHxS to seven male Sprague-Dawley rats. Urine, feces, blood, and tissue samples were taken over the following 38 days, and PFC concentrations were determined by high performance liquid chromatography coupled with tandem mass spectroscopy. It was found that all PFC branch-chained isomers had a lower half-time in the blood than the corresponding linear isomers. The only exception was the PFOS isomer that had an α-perfluoro methyl chain (1m-PFOS). This was probably less readily excreted than the linear isomer of PFOS due to spatial shielding of the hydrophilic sulfonate moiety. The authors therefore reasoned that the property of PFOS, PFOA, PFNA and PFHxS chain branching, in general, lowers the half-life in the blood and increases excretion rates. However, different kinetic data may arise depending upon gender, dosage, and species [129].

Table 17 Excretion of PFOS and PFOA by young chickens

Parameter	PFOS	PFOA
Excretion half-life	125 days	4.6 days
Residual blood concentration at the end of the 4-week excretion phase	48% to 52%	2% to 3%
Excretion rate constant	0.023% ± 0.004% per day	0.150% ± 0.010% per day

Excretion was after a 4-week exposure and a 4-week excretion phase (from Yu et al. [171]).

Part two of this study examined the same circumstances under the more realistic conditions of a subchronic exposure. PFCs were mixed with the feed and administered to male and female rats over a period of 12 weeks, followed by a 12-week excretion phase. The feed contained 0.5 μg/g of the ECF products PFOA (approximately 80% linear), PFOS (approximately 70% linear), and PFNA (linear form and isopropyl-PFNA). Blood samples that were collected during the exposure phase showed a preferential accumulation of the linear form of PFOA and PFNA over the branched chain isomers. Thus, most of the branched chain PFCA isomers were more quickly eliminated than were the linear forms. No statistically significant differences in rate of elimination of branched chain or linear isomers of PFOS were found. Additional exceptions for two small ECF PFOA isomers and 1m-PFOS exist. In general, female rats excrete PFCs more rapidly than male rats [130].

Olsen et al. [131] studied the pharmacokinetic behavior of PFBS in rats, monkeys, and humans. Rats received an intravenous PFBS dose of 30 mg/kg BW and monkeys, a dose of 10 mg/kg BW. Serum and urine samples were collected from the animals following application of the substance. Human participants in the study were workers who were occupationally exposed to PFBS. The elimination half-life of PFBS can be seen in Table 19. PFBS is apparently excreted more rapidly than PFHxS and PFOS by rats, monkeys, and humans, whereby species specific differences were observed. This indicates, also for humans, that the capacity for accumulation of PFBS in serum is lower than for long-chain homologues. PFBS excretion for humans was shown to be via the urine [131].

Additional human PFC half-life values were calculated on the basis of serum concentrations from 26 workers in the fluorochemical industry. The mean time was 5.4 years for PFOS, 3.8 years for PFOA, and 8.5 years for PFHxS [132].

Table 18 PFOA steady-state concentrations in matrices following daily oral administration of APFO dosages on cynomolgus monkeys

Sample matrix	Dose		
	3 mg/kg	10 mg/kg	20 mg/kg
Serum	81 mg/L	99 mg/L	156 mg/L
Urine	53 mg/L	166 mg/L	181 mg/L
Feces	7 mg/kg	28 mg/kg	50 mg/kg
Liver	16 mg/kg	14 mg/kg	50 mg/kg

from Butenhoff et al. [283]

The renal clearance values for PFOS are 0.012 mL/kg/day for men and 0.019 mL/kg/day for women, which are low in comparison with the values for the animals studied. The values for renal clearance of PFOA are somewhat higher [126]. The corresponding data are summarized in Table 20.

Renal clearance of PFOS and PFOA is therefore weak, and the compounds have a markedly long half-life in the human body when compared with those in other species. This hinders the translation of results from animal experiments to humans. A gender-dependent excretion of PFOS and PFOA via a hormone-regulated mechanism seems unlikely in humans [126]. This mechanism would also not be expected in mice or rabbits. In the animal model, excretion is mainly through urine and, to a smaller extent, through feces [133,134]. Protein binding and the formation of transporters are decisive factors in the distribution and excretion of PFCs [15,115]. Table 19 presents a summary of the elimination half-life values for various species of PFCs.

Toxicodynamics of perfluorinated compounds

Acute toxicity

In animal models, PFOS and PFOA demonstrate a moderate acute toxicity. The lethal dose with 50% lethality [LD_{50}] for PFOS is 251 mg/kg BW for a single oral dose in rats. LD_{50} values for PFOA range from 430 to 680 mg/kg BW with an average of 540 mg/kg BW per day [15,17]. The lethal concentration with 50% lethality [LC_{50}] for 1 h inhalation of airborne dust contaminated with PFOS was 5.2 mg/L for rats. Kennedy et al. [100] determined an LC_{50} of 0.98 mg/L for inhalation of PFOA. Inhalation of this concentration over one 4-hour period resulted in enlargement of the liver and corneal opacity in rats.

Glaza et al. [135] determined a dermal LC_{50} of 2, 000 mg PFOA/kg BW in rabbits [15]. Rats and rabbits were tested in another study on the dermal toxicity of APFO by Kennedy [99]. Dermal application of 0.5 g APFO for 24 h caused light skin irritation in rabbits.

Skin irritation was less pronounced in rats than in rabbits. Irritation of the skin and eyes by PFOS was not observed in albino New Zealand rabbits. ([136] cited in

Table 19 Elimination half-lives by various species according to perfluorinated compound and gender

Species	PFOS	PFOA		PFHxS		PFBA		PFBS	References
	M/F	F	M	F	M	F	M	M/F	
Chicken Chick	125 days 16 days	-	4.6 days 3.9 days	-	-	-	-	-	Yoo et al. [109]; Yeung et al. [284]
Rat	100 days	2 to 4 h	4 to 6 days	-	-	1.6 to 1.8 h	7 to 9 h	4.5 h (M) 4 h (F)	Chang et al. [285]; Johnson et al. [98]; Olsen et al. [131]
Mouse	-	17 days	19 days	-	-	3 h	7 h	-	Chang et al. [285]; Lau et al. [161]
Rabbit	-	7 h	5.5 h	-	-	-	-	-	Hundley et al. [133]
Dog	-	8 to 13 days	20 to 30 days	-	-	-	-	-	Hanhijarvi et al. [286]
Monkey	150 days	30 days	21 days	87 days	141 days	1.7 days	-	3.5 to 4 days 4 days (M) 3.5 days (M)	Butenhoff et al. [125]; Chang et al. [285]; Buttenhoff et al. [283]; Olsen et al. [85]; Seacat et al. [128]; Olsen et al. [131]
Human	5.4 years	-	3.8 years	-	8.5 years	-	-	30 days 25.8 days	Olsen et al. [85,131,132]

F, female; M, male; PFBA, perfluorobutane acid; (from Lau et al. [115] amended and expanded).

EFSA [15]). PFOS was shown to be more toxic than PFOA in studies of fresh water organisms such as water flea, water snails, shrimp, and planaria. Ji et al. [137] even alluded to a toxicity of PFOS 10 times higher than PFOA in such organisms. The lowest LC_{50} for fish is a 96-h LC_{50} of 4.7 mg/L to the fathead minnow *Pimephales promelas* for the lithium salt [134]. Table 21 summarizes the various LD_{50} and LC_{50} values.

Subacute and subchronic toxicities

Studies have shown that the primary effects of subacute and/or subchronic toxicities induced by repetitive applications of PFOS and PFOA varied according to species: hypertrophy and vacuolization of the liver, reduction of serum cholesterol, reduction of triglycerides in serum, reduction in body weight gain or body weight, and increased mortality.

The most sensitive target organs for repetitive oral application of PFOS over a period of 4 weeks to 2 years in rats and cynomolgus monkeys were the liver and thyroid. The liver was also the most sensitive target organ for repetitive applications of PFOA in mice, rats, and primates. The effects observed include increased weight of liver, increases in enzymatic activity of transaminases in serum (alanine aminotransferase [ALT], aspartate aminotransferase [AST]), hepaticellular hypertrophy, vacuolization, and liver necrosis (17, [127] cited in EFSA [15]). A 28-day study on the oral toxicity of PFOA showed increased mortality, dose-dependent reduction in weight gain and increase in liver weight in rats and mice that had received 30 mg/kg in their feed or 50 mg/L in their drinking water ([138,139]; [140] cited in EFSA [15]).

No evidence of disease or increase in mortality rate was observed in a 90-day study (13 weeks) on male rats. An increase in weight loss was observed in the group which received the highest dosage of APFO (6.5 mg/kg

Table 20 PFOS and PFOA renal clearance and serum half-life in humans compared with monkeys and rats

Substance	Species	Gender	Renal clearance (mL/day/kg)	Serum half-life in days
PFOS	Human	Female (48 kg)	0.019	n.r.
		Male (61 kg)	0.012	3, 165
	Cynomolgus monkey	Male	n.r.	200
PFOA	Human	Female (48 kg)	0.027	n.r.
		Male (61 kg)	0.033	1, 573
	Japanese macaque	Female	32	2.7
		Male	15	5.6
	Wistar rat	Female	1, 054	0.08
		Male	46.1	5.63

Adapted from Harada et al. [126]; n.r., not reported.

Table 21 LD_{50} and LC_{50} values for PFOS and PFOA (from [15]; BfR [17] enhanced)

Criterion	PFOS	PFOA	Animal species	Reference
LD_{50}	251 mg/kg BW	430 to 680 mg/kg BW	Rat	Dean and Jessup [287]
LC_{50}	5.2 mg/L, inhalative	0.98 mg/L, inhalation	Rat	Kennedy [100]
LD_{50}	n.r.	4, 300 mg/kg APFO, dermal	Rabbit	Kennedy [99]
LD_{50}	n.r.	7, 000 mg/kg 7, 500 mg/kg, dermal	Male rats Female rats	Kennedy [99]
LC_{50}	n.r.	2, 000 mg/kg, dermal	Rabbit	Glaza et al. [135]
48 h-LC_{50} 96 h-LC_{50}	27 to 233 mg/L 10 to 178 mg/L	181 to 732 mg/L 337 to 672 mg/L	Four fresh water species (water flea, water snail, shrimp, planarian)	Li [288]
LC_{50}	18 mg/L	200 mg/L	Japanese water flea	Ji et al. [137]

n.r., Not reported.

BW/day), at a dosage of 0.64 mg/kg BW/day, and increased levels of palmitoyl-CoA-oxidase activity, a marker for peroxisome proliferation.

In addition, liver weight increased. Histopathological changes included hypertrophy and necrosis of the liver cells. Levels of estradiol, testosterone, and luteinizing hormone [LH] remained unchanged. The PFOA concentrations in serum, measured after treatment with various APFO doses, are shown in Table 22. The 'no observed adverse effect level' [NOAEL] determined in this study was 0.06 mg/kg since a dose of 0.64 mg/kg BW/day and above resulted in reversible changes to the liver [141].

Liver toxicity was also described in rats after inhalation and dermal uptake of PFCs. An increase in mortality rates was observed after inhalation exposure to PFOA. Based on non-neoplastic effects in the liver at the next higher dosage, the NOAEL was noted as 0.14 to 0.16 mg/kg BW/day [127].

Further studies show that the toxicity profiles of L-PFOA, 80% linear and 20% branched chain PFOA, as well as 100% branched chain PFOA are similar. However, the branched chain form is less effective than the pure linear form. The 'lowest observed adverse effect level' [LOAEL] in rats was higher for linear and branched chain isomers (1 mg/kg BW/day) than the LOAEL for the purely linear application form of PFOA (0.3 mg/kg BW/day). The LOAEL in these studies was based on the reduction of cholesterol and triglyceride levels in the blood of rats. This LOAEL was equivalent to a PFOA serum concentration of 20 to 51 mg/L in rats ([142] cited in EFSA [15]). These observations are in agreement with the conclusion drawn above that branched chain isomers are generally excreted more rapidly than the linear forms [129,130].

Table 22 PFOA serum concentrations of male rats after a 90-day exposure to various dosages of APFO

Dosage (mg APFO/kg feed/day)	PFOA serum concentration (mg/L)
0.06	7.1
0.64	41
1.94	70
6.5	138

Adapted from Perkins et al. [141].

Seacat et al. [108] assumed a NOAEL for PFOS of 0.34 to 0.4 mg/kg BW/day when ingested by rats with their food. This was the lowest dose for which an effect could be observed over a time period of 14 weeks in male rats. Nonetheless, this dose was denoted as NOAEL, whereby the observed hepatocellular hypertrophy and vacuolization were marginal [108].

Curran et al. [143] undertook a detailed and extensive study of subacute toxicity of PFOS in rats. The authors exposed Sprague-Dawley rats to doses of 2, 20, 50, or 100 mg PFOS/kg in the feed over a period of 28 days. At low dosages, PFOS accumulated primarily in the liver and at lower concentrations, in other organs such as the spleen and heart, as well as in the serum. The PFOS concentrations in the serum and other organs were seen to rise at higher dosages (50 and 100 mg/kg food). The results of this study confirm that the liver is the target organ for PFOS. Hepatomegaly, reduced triglyceride and cholesterol levels in serum, increased the expression of the gene for acyl-coenzyme A-oxidase 1 (ACOX1) and of cytochrome P450 4A22 (CYP4A22) are all indications of exposure to a peroxisome proliferator. Changes in fatty acid profiles in the liver encompass an increase in the total amount of simple unsaturated fatty acids, a loss in the total amount of polyunsaturated fatty acids as well as an increase in linoleic acid concentration and a reduction of long-chain fatty acids. These changes also portend to a weak peroxisome proliferator. The authors suggest that the fatty acid dysfunctions in the liver may possibly be the cause of changes in the

cell membranes in red blood cells, seen as an increase in lysis and cell fragility. Concentrations of the thyroid hormones tri-iodo thyronine [T_3] and thyroxine [T_4] were lowered in PFOS-exposed rats. The kidneys and the cardiovascular system do not seem to be influenced by PFOS. The LOAEL in this study was 20 mg PFOS/kg feed for male rats and 2 mg PFOS/kg feed for female rats based on increased liver weight and reduced body weight. At these dosages, the animals had serum concentrations of 13.5 or 1.5 mg PFOS/kg, respectively [143].

In a study on the subacute toxicity of PFCs in rats, Cui et al. [144] determined that the liver, the lungs, and the kidneys were the main target organs for these substances. They exposed Sprague-Dawley rats to PFOS or PFOA at concentrations of 5 and 20 mg/kg BW/day, respectively, for 28 days. Changes were observed in the group with the highest PFOS dose (20 mg/kg/day) including reduced activity, lethargy, reduced food uptake, and an apparent loss of body weight. Hypertrophy and cytoplasmic vacuolization of the liver and epithelial cells induced pleural thickening. The highest PFOA concentrations after a 28-day exposure were measured in the kidneys (228 ± 37 mg/kg at a dosage of 5 mg/kg/day). The highest PFOS concentrations were 648 ± 17 mg/kg in the liver following an exposure of 20 mg/kg/day for 28 days. The increased accumulation of PFOS may explain the higher toxicity of this substance [144].

In a 90-day study on the oral toxicity of PFOA in rhesus monkeys, all four of the animals in the group that received 100 mg/kg BW/day died within 5 weeks, and three monkeys of the group that received 30 mg/kg BW/day died in the 13th week. Loss of heart and brain masses was detected in female animals that received 10 mg/kg BW/day. PFOA-induced organ damage could be observed in animals that received 3 to 10 mg/kg BW/day. The only change seen in the monkeys that received the lowest dosage (3 mg/kg BW/day) was a slight diarrhea [145,15].

In a study, a six-month oral APFO exposure of cynomolgus monkeys indicated a dose-dependent increase in liver weight in association with a proliferation of the mitochondria. No histological evidence of liver damage was observed in the low-dosage range (3 to 10 mg/kg BW/day). In addition, no changes were observed in clinical parameters of hormones, urine, or blood composition that could be attributed to treatment with APFO. It must be noted, however, that the groups were made up of only four to six animals, whereby one monkey from the group receiving the lowest dosage was replaced because of bacterial septicemia, and the highest dosage was lowered retroactively due to weight loss and a reduction in food uptake by the animals [146].

In a study by Seacat et al. [128], doses of 0.03, 0.15, and 0.75 mg PFOS/kg BW/day were applied directly to the stomach of cynomolgus monkeys for 26 weeks. Histopathological changes were detected in the liver at the highest dosage. At the lowest dosages, changes in serum concentrations of thyroid hormones (thyroid stimulating hormone [TSH], T_3) were observed. High-density lipoprotein [HDL] and cholesterol levels were also changed. The observed effects in dependence upon dosage in male and female monkeys are shown in Table 23. The clinical changes and the effects on the liver had completely disappeared 211 days after treatment. This reversibility of the effects was accompanied by a significant reduction in PFOS concentration in the serum and in the liver [128].

In both the cynomolgus monkey and in the rat studies, a steep dose-effect relationship for PFOS was conspicuous. The dose-effect curve for PFOA in rats was less steep than that for PFOS ([17] cited Perkins et al. [141]).

Subacute toxic effects of PFC exposure were also observed in fish. Yang [147] put Japanese Girardinus guppies in sea water containing 10, 50, or 100 mg/L PFOA for 7 days. Neither survival rate nor relative liver and gonad size or growth was affected by this concentration. Peroxisomal acyl-CoA-oxidase activity was, however, increased at the highest dosage. This was accompanied by a significant increase in the peroxisome proliferator activated receptor [PPAR]α expression. PFOA induced a significant inhibition of catalase activity at a high dosage, without causing changes in the superoxide dismutase or glutathione peroxidase level in the liver. This suggests that PFOA causes an induction of the peroxisomal fatty acid oxidation and an increase in oxidative stress by changing the cellular oxidative homeostasis in the liver. Furthermore, PFOA increases the mRNA concentration of proinflammatory cytokines such as IL-6, TNF-α, and IL-1β suggesting that inflammation and tissue damage may be involved [45].

Table 23 Changes in male and female cynomolgus monkeys after daily application of various dosages of PFOS

Change	PFOS dosage (mg/kg BW)			
	0	0.03	0.15	0.75
Increased mortality				×[a]
Vacuolization and hypertrophy of the liver				× ○
TSH level increased			×	× ○
T_3 level lowered		○	×	× ○
HDL concentration lowered		×	○	× ○
Bilirubin concentration dropping				×
Cholesterol concentration dropping				× ○

Changes in male (cross) and female (empty circle) monkeys; [a]two of six animals. Adapted from Seacat et al. [128]; BfR [17].

Fang et al. [148] found that a 14-day exposure of rare minnows to PFOA caused a change in the expression of apolipoproteins and upstream genes (PPARα, PPARγ, HNF4α). These changes in gene expression can influence lipid metabolism or other physiological functions in fish. Results from studies on subacute and subchronic toxicities of PFCs are summarized in Table 24.

Chronic toxicity and carcinogenicity

In a study on chronic toxicity and carcinogenicity of PFOS, groups of 40 to 70 male and female rats were fed with the potassium salt of PFOS in doses of 0.5, 2, 5, and 20 mg/kg mixed with their feed for 104 weeks. An additional comparison group received the maximum PFOS dose for 52 weeks, followed by 52 weeks of control diet without PFOS exposure. Hepatotoxic and carcinogenic effects were observed in the rats after PFOS exposure. Based on the hepatotoxic effects, a NOAEL of 2 mg/kg feed or 0.14 mg/kg BW/day was calculated for male and female rats ([17,149] cited in EFSA [15]). The observed effects in rats according to dose and frequency are shown in detail in Table 25.

A study by Sibinski [150] on chronic exposure to PFOA showed an increased incidence of Leydig cell adenomas. The incidence of breast fibroadenomas was not significantly or dose-dependently increased over the control values. The 50 male and 50 female rats[e] were fed 30 or 300 mg/kg APFO with their feed for a period of 2 years. A dose-dependent decrease in weight gain was observed in male rats and, to a lesser extent, in female rats. The decrease was statistically significant for both male and female animals that received the maximum dosage. Comparison of survival rates, urinalyses, and opthalmological examinations did not show any significant differences from the control animals. Additional effects observed after exposure to APFO are presented in Table 26. The biological significance of ovarian damage was questioned by the authors due to the lack of evidence of tumorigenesis. According to an evaluation by Mann and Frame [151], the effects on the ovaries were in the form of gonadal hyperplasias and/or adenomas. The NOAEL for male rats, based on increased liver weight and liver anomalies, was 1.3 mg PFOA/kg BW. For females, the NOAEL was listed as 1.6 mg PFOA/kg BW/day since higher dosages led to reduced body weight and changes in blood values [15].

A pathology work group evaluated the appearance of proliferative injury to mammary glands in female rats that had been fed APFO for 2 years. Using documents

Table 24 Data from studies on subacute and subchronic toxicities of PFOS and PFOA

Substance	Exposure period	Species	Target organ/Effect	Effective dosage[a]	NOAEL[a]	Reference
PFOS	28 days	Rats	Body weight ↓, liver mass ↑, and altered gene expression and fatty acid metabolism in the liver, T_3, and T_4 ↓	2 to 20 mg/kg feed	n.r.	Curran et al. [143]
	14 weeks	Rats (male)	Hypertrophy and vacuolization of the liver	n.r.	0.37	Seacat et al. [108]
	26 weeks	Cynomolgus monkey	Centrilobular vacuolization, hypertrophy of the liver, T_3 ↓, TSH ↑, HDL ↓, and bilirubin, cholesterol concentrations ↓	n.r.	0.03	Seacat et al. [128]
	1 and 4 months	Fresh water larvae of small dragonflies	Deterioration of behavioral and activity parameters (larvae were less active, less able to avoid attackers, or less efficient in foraging)	> 10 µg/L	10 µg/L	Van Gossum et al. [289]
PFOA	7 days	Japanese guppies	Activity of peroxisomal acyl-CoA-oxidase ↑ and significant inhibition of catalase activity, mRNA concentration proinflammatory cytokines such as IL-6, TNF-α and IL-1β ↑	50 and 100 mg/L	n.r.	Yang [147]
	14 days	Minnows	Changes in the expression of apolipoproteins and upstream genes (PPARα, PPARγ, HNF4α)	n.r.	n.r.	Fang et al. [148]
	90 days	Rats (male)	Liver mass ↑ and hepatocellular necrosis	1.7	0.6	Goldenthal [290]
APFO	14 days	Rats	Body weight gain ↓, liver mass ↑, and serum enzyme activity ↑	84 mg/m^3	1 mg/m^3	Kennedy et al. [100]
	90 days	Rats (male)	Absolute and relative liver mass ↑, hepatocellular hypertrophy, and effects were reversible	0.64	0.06	Perkins et al. [141]
	90 days	Rhesus monkey	Gastrointestinal effects and mortality	3 to 100	n.r.	Goldenthal [145]
	26 weeks	Cynomolgus monkey	Liver mass and mortality ↑	3 to 30	n.r.	Butenhoff et al. [146]
PFOS/ PFDA/ PFOA	3 weeks	Chicks (male)	No significant effects	n.r.	> 1	Yeung et al. [284]

[a]Effective dosage and NOAEL without further specifications are presented in milligrams per kilogram per body weight per day. Upward arrow, increased; downward arrow, decreased; n.r., not reported; T_3: tri-iodo thyronine; T_4, thyroxin.

Table 25 Observed effects in a study of chronic exposure of rats to PFOS

Category	Effects	Occurrence, dosage, and frequency (Cases/Total number)
Hepatotoxicity	Increase in centrolobular hypertrophy, eosinophilic granuloma, and vacuolization of liver cells	(a) Male rats, 2 mg/kg of feed (b) Male and female rats, 5 or 20 mg/kg of feed
Electron microscopic results	Mild to moderate hyperplasia of the smooth endoplasmic reticulum, minimal to mild hypertrophy of liver cells, and no peroxisome proliferation	In a proportion of the animals that received 20 mg/kg of feed
Neoplastic effects	Significant increase in the incidence of (a) adenomas of the liver, (b) follicular adenomas of the thyroid, (c) hepatocellular adenomas, carcinomas, (d) follicular adenomas and carcinomas of the thyroid, (e) fibrocystic breast adenomas/adenomas, and (f) fibrocystic breast adenomas/adenomas/carcinomas	(a) Male rats, 20 mg/kg of feed (7/60), control (0/60) (b) Comparison group (9/39), control (3/60), group with highest dosage (4/59) (c) Female rats, 20 mg/kg of feed (d) Female rats, 5 mg/kg of feed (3/50), control (0/60) (e) Female rats with the exception of the group with the highest dosage (f) Female rats, 0.5 mg/kg of feed (36/50), 2 mg/kg (31/48), but not for the group with 5 and 20 mg/kg of feed (29/50 and 24/60), control (29/60)

Adapted from Thomford [149] cited in EFSA [15].

from the study of Sibinski [150], they came to the conclusion that the incidence of mammary gland tumors was not changed by chronic exposure to APFO. Feeding female rats (see Table 26) as much as 300 mg/kg APFO did not result in an increase in proliferative damage to breast tissue [152].

In an additional study on the carcinogenicity of APFO, rats were fed 300 mg APFO/kg of food, equivalent to *ca*. 14 mg/kg BW/day for 2 years. The study encompassed 153 rats, and an additional 80 animals formed the control group. Hormone status, cell proliferation, and peroxisome proliferation were measured. Increases in liver weight and β-oxidation activity of the liver were statistically significant throughout the whole test period, whereas increases in weight of the testicles only occurred at 24 months. No differences were detected between the exposed rats and the control animals in regard to serum concentrations of testosterone, follicle-stimulating hormone [FSH], LH, or prolactin. An increased incidence of Leydig cell adenomas was seen in the exposed group (8/76) when compared with the control group (0/80) as well as liver adenomas (10/76 vs. 2/80) and pancreas cell tumors (7/77 vs. 0/80). The numbers in brackets show the observed cases and total number of animals in the groups of exposed and control animals [153]. Further studies showed that an APFO dosage of 14.2 mg/kg BW/day increases the incidence of damage to proliferating pancreas cells; however, it does not increase the incidence of adenomas or carcinomas ([17,154] cited in EFSA [15]).

Sibinski [150] and Biegel et al. [153] both showed that PFOA or PFOS induces liver-cell adenomas, Leydig cell adenomas, and hyperplasia of acinar pancreas cells. Furthermore, it could be shown that PFOA functions as promoter in liver carcinogenesis of male Wistar rats. The rats were treated with 0.02% APFO in their feed, and 200 mg/kg BW/day of diethylnitrosamine served as initiator ([155,156] cited in EFSA [15]).

Genotoxicity and epigenetic effects

In various *in vitro* and *in vivo* test systems, PFOS and PFOA did not appear to be genotoxic. Therefore, it can be assumed that the carcinogenic effects are the result of an epigenetic mechanism and that the trigger is a threshold concentration, i.e., apparently a dosage exists beneath which a carcinogenic effect would not be expected [17].

Based on a number of *in vitro* and *in vivo* tests concerning gene and/or chromosome mutagenicity or the induction of unscheduled gene repair, the EFSA also assumes that PFOS is not genotoxic. PFOS does not

Table 26 Effects on rats after a 104-week application of APFO (from Sibinski [150]; EFSA [15])

Category	Effects	Animal, dosage, in part frequency (Cases/Total number)
Non-neoplastic effects	(a) Slight reduction in number of erythrocytes and packed cell volume, liver mass, liver nodules, Leydig cell mass ↑; (b) number of white blood cells ↑ and AST, ALT, alkaline phosphatase, and creatine phosphokinase ↑; (c) breast tissue mass ↑ and tubular hyperplasia of the ovarian stroma; (d) kidney mass ↑ and changes in the liver (hepatomegalocytosis, necrosis, mononuclear cell infiltration, cystoid degeneration)	(a) Male rats, 300 mg/kg (b) Male rats, 30 mg/kg (c) Female rats, 30 mg/kg (d) Female rats, 300 mg/kg
Carcinogenicity	(a) Incidences of testicular Leydig cell adenomas ↑ and (b) fibrocystic breast adenomas ↑	(a) Male rats, 0 mg/kg (0/50), 30 mg/kg (2/50), 300 mg/kg (7/50) (b) Female rats, 0 mg/kg (10/46), 30 mg/kg (19/45), 300 mg/kg (21/44)

Upward arrow, increased.

induce gene mutation with or without metabolic activation in a bacterial test system, does not cause chromosome aberrations in human lymphocytes, and does not induce unscheduled DNA synthesis in rat hepatocytes. PFOS does not cause formation of micronuclei in a mouse's bone marrow cells *in vivo*. Various *in vitro* and *in vivo* genotoxicity tests for precursors of PFOS and *N*-ethylperfluorooctyl sulfonamide ethanol [*N*-EtFOSE], *N*-EtFOSA, *N*-methylperfluorooctyl sulfonamide ethanol were also negative. APFO also failed to induce back mutations in tests with *Salmonella typhimurium* or *Escherichia coli*, both with or without metabolic activation. APFO did not cause chromosome aberrations in human lymphocytes or in ovary cells of Chinese hamsters, with or without metabolic activation, nor did it lead to cell transformation in mouse embryo fibroblasts. An *in vivo* micronuclear test on mice treated with PFOA was also negative [15].

Murli et al. [157] twice tested the potential of APFO to cause chromosome aberrations in cells of the Chinese hamster. In the first test, the results were positive, both with and without metabolic activations, i.e., chromosome damage was observed. In the second test, APFO induced chromosome aberrations and polyploidy only without activation. However, these effects were only observed at cytotoxic concentrations of APFO [15].

In the study by Yao and Zhong [158], PFOA was seen to induce not only DNA strand breaks, but also increased concentrations of reactive oxygen species and 8-hdroxydesoyguanosine [8-dG]. This result suggests that the observed genotoxic effects are induced by an oxidative damage to the DNA or by intracellular ROS. Takagi et al [159] also detected significantly increased 8-dG concentrations.

Reproductive and developmental toxicity

PFOS and PFOA neither interfered with reproduction nor did they lead to any appreciable teratogenic effects. Both substances did, however, show developmental toxicity when the mother animal was exposed during pregnancy, i.e., they led to a reduced increase in body weight after birth and reduced the number of live births and the viability of the progeny in the first five days after birth [15,17,115,134,160,161].

For example, in a study by Lau et al. [162], all live-born young rats, born to a mother that was exposed to 10 mg PFOS/kg BW/day during gestation, were pallid, inactive, became moribund within 30 to 60 min, and died shortly thereafter. The offspring of mother animals that received 5 mg PFOS/kg BW/day, survived for 8 to 12 h. This could also be observed in progeny of mother animals that received 20 or 15 mg/kg BW/day. However, 95% of these progeny died within the first 24 h after birth. Approximately 50% of the progeny died when the mother animal received 3 mg PFOS/kg BW/day (rat) or 10 mg/kg BW/day (mouse). Wet nursing the progeny by a non-exposed control animal did not improve their viability. Prenatally exposed rats and mice that did survive showed delays in growth and opening of the eyes. Exposed young mice had significantly higher liver weight and lower T_4 concentrations in serum but unchanged T_3 and TSH concentrations when compared with non-PFOS-exposed animals [162].

In a two-generation study on rats, Lübker et al. [163] found fertility parameters unchanged after oral application of the maximal PFOS concentration was tested (3.2 mg/kg BW/day).

In another two-generation study on rats, the progeny of PFOS-exposed mother animals (LOAEL = 0.4 mg/kg BW/day) were found to gain body weight more slowly in the F1 generation and to have reduced birth weight in the F2 generation. The serum concentrations of the animals (F0) on the 21st day of gestation were 26.2 mg/kg and of the fetuses, 34.4 mg/kg (liver- and serum-pooled). The NOAEL was calculated to be 0.1 mg/kg BW/day ([164] cited in EFSA [15]).

Unaltered fertility parameters were found in yet another two-generation study of PFOA-exposed rats by Buttenhoff [165]. The highest dosage in this study was 30 mg/kg BW/day. No signs of maternal toxicity were observed in the animals at exposures up to this dosage. However, compared with those in control animals, the adult body weight of the progeny was lower and liver and kidney weights were higher even at the lowest dosage tested, 1 mg/kg BW/day. The mortality rate of the progeny was increased at 30 mg/kg BW/day, which is the highest dosage tested [17].

Because of allusions to a correlation between PFOA serum concentrations with a reduced sperm count in young Danish adults and/or a longer period before pregnancy occurred, York et al. [166] reevaluated these two-generation studies. Testicular and sperm structures and functions, however, were unchanged in APFO-treated rats with an average PFOA serum concentration as high as 50, 000 μg/L. Since the PFOA concentration in the Danish cohort was 5 μg/L, the authors assume that there is no causal relationship between PFOA concentrations in serum and a reduction in sperm count in these men [166].

Lau et al. [161] carried out studies on the developmental toxicology of PFOA using mice since the excretion of PFOA in female rats is so rapid that these animals were not considered appropriate experimental subjects for these tests. Effects (increased liver weight) were observed in the mother animals exposed to a dosage of 1 mg/kg BW/day or higher. Increased resorption of fetuses and reduction of survival rate and body weight gain of the live-born progeny were observed when mother animals received dosages of 3 mg/kg BW/

day. These effects exhibited a steep dose-response curve. The resorption of all of the fetuses in a litter during gestation (full-litter resorption) which resulted from a dosage of 5 mg PFOA/kg BW/day or higher was particularly striking [17,161].

Grasty et al. [167] set out to determine a critical time period of gestation for effects of prenatal exposure using Sprague-Dawley rats. The authors administered 25 mg/kg BW of the potassium salt of PFOS on GD 2 to 5, 6 to 9, 10 to 13, 14 to 17, and 17 to 20 or 25 or 50 mg/kg BW on day19 to 20. Neonatal mortality was observed for all of the time periods; however, the incidence of stillbirths increased with the PFOS exposure at later periods of gestation, reaching 100% for prenatal exposure on GD 17 to 20. Exposure to PFOS in the late phases of gestation is apparently adequate to induce effects that are toxic to reproduction. This result suggests that PFOS damages the organs that develop in the last phases of gestation. Grasty et al. [168] therefore examined the lungs of newborn rats and discovered thickening of the alveolar walls of prenatal PFOS-exposed young animals. However, as a result of the normal phospholipid profile of the lungs and the fact that treatment with dexamethasone or retinylpalmitate did not ameliorate the situation, it must be concluded that the neonatal mortality is not due to the immaturity of the lungs [15]. Lau et al. [115] mentioned studies that suggest an effect of PFCs on the pulmonary surfactants, e.g., dipalmitoylphosphatidylcholine. In a study in which PFOA was exclusively applied in the late phase of gestation, it was also shown that this treatment was adequate to trigger developmental toxic effects in mice ([169] cited in BfR [17]).

In a cross-fostering study, Lübker et al. [170] observed that neonatal mortality was also high in progeny that had been exposed to PFOS *in utero* but which had not been exposed to any further PFOS in milk. Compared with control animals, a diminished gain in body weight was also noted in animals that were only exposed to PFOS via the milk they drank, but were not the progeny of PFOS-treated mother animals [17,115].

Yu et al. [171], in another cross-fostering study, observed that both pre- and postnatal PFOS exposures (3.2 mg/kg feed) lower the T_4 concentration in the prenatally exposed progeny. On days 21 and 35 after birth, the T_4 concentrations were reduced by 20.3% or 19.4%, and in postnatally exposed rats, by 28.6% or 35.9% compared with control animals.

Liu et al. [113] injected young mice with 50 mg/kg BW PFOS on different days after birth. They then measured, among other things, the concentration of maleic acid dialdehyde, superoxide dismutase [SOD] activity, and the total antioxidative capacity [T-AOC] as parameters of oxidative damage that might be occurring. PFOS induced a loss of body weight in mice and an increase in the relative weight of the liver. It also suppressed SOD activity and diminished the T-AOC in the brain and liver. Younger mice were more sensitive to the effects of PFOS than older animals [113].

Abbott et al. [172] studied the influence of PPARα on the PFOA-induced developmental toxicity using wild-type and PPARα knockout mice. The authors administered oral dosages of 0.1, 0.3, 0.6, 1, 3, 5, 10, and 20 mg/kg BW on the 1st to the 17th GD. (The effects are described in Table 17). Resorption of all fetuses of a mother animal through the administration of 5 mg PFOA/kg BW/day occurred as frequently in the PPARα-deficient mice as in the wild-type animals. The effects of PFOA cannot therefore be attributed fully to the activation of PPARα. PPARα does, however, seem to play a role in the delayed opening of eyes and the postnatal reduction in weight gain [15,17,172]. Abbott et al. [173] came to the conclusion that the developmental toxicity effects are not dependent upon the activation of PPARα by PFOS. The wild-type mice were just as sensitive to the effects of neonatal lethality as were the PPARα-knockout mice. Furthermore, it can be seen from this publication that PPARα, β, and γ are expressed in early developmental phases in embryos of rodents and humans. The expression patterns depend upon the developmental stage and the type of tissue, leading to the assumption that PPARα, β, and γ play important functions in many cell types and organs during development [173].

The influences on reproduction by PFOS and PFOA are not limited to mammals but have, for example, also shown to affect chickens [174-176], quail, mallard duck [177], frogs, and fish ([178,179] cited in Lau et al. [115]). The following observations stem from studies on the developmental and reproductive toxicity of other PFCs:

The toxic effects of *N*-Et-FOSE are similar to those of PFOS. This may be explained by the transformation of *N*-Et-FOSE into PFOS; however, *N*-Et-FOSE was also seen to increase the number of stillbirths and mortality of the newborn in the F2 generation of rats ([163,164] cited in Lau et al. [115]). The effects of 8:2 FTOH on rats were slightly similar to those of PFOA into which FTOH can be transformed. The NOAEL for 8:2 FTOH was determined to be 200 mg/kg BW/day ([58] cited in Lau et al. [115]). PFBS did not elicit a verifiable developmental effect in rats [115]. In contrast to observations on PFOS and PFOA, exposure of pregnant mice to PFBA was not found to have adverse effects on survival of newborn or their postnatal growth [180]. Although PFHxS, compared with PFBS, PFOS and PFOA, has the longest half-life in humans, no effects on reproduction or survival and growth of the progeny was observed in rats. The NOAEL for developmental toxicity of PFHxS

was determined to be 10 mg/kg BW/day ([181] cited in Lau et al. [115]). Perfluorodecanoic acid, like other PFCs, did not induce deformations and also did not elicit any other developmental toxic effects [182].

PFNA led to cell apoptosis in testicles of male rats. The animals received oral doses of 1, 3, and 5 mg/kg/day for 14 days. The results imply that the 'death receptor pathway' is the chief mediator for apoptosis in the kidneys which is a result of PFNA exposure. It is not yet known whether PFNA induces the changes in Fas and FasL expressions directly or whether the imbalance between testosterone and estradiol, which causes germ cell apoptosis, is involved in the Fas/FasL pathway [183]. Table 27 presents a survey of the studies on reproduction and developmental toxicity of PFOS, PFOA, and other PFCs.

Neurotoxicity

A study by Austin et al [107] showed that PFOS can have an influence on the neuroendocrine system in rats. The authors discovered reduced food intake and body weight, influence on the ovarian cycle, increased corticosterone concentration, and decreasing leptin concentration in serum as effects of PFOS exposure. In addition, noradrenaline concentrations in the paraventricular nucleus of the hypothalamus were elevated.

In an *in vitro* study, Harada et al. [184] observed that PFOS increases the negative charge density in the cell membrane of Purkinje cells, e.g., nerve cells in the cerebellum, of rats. It also reduced the membrane potential, leading to hyperpolarization and thus influencing activation and inactivation of the ion channels. This appears to indicate that PFOS has an effect on the action potential in nerve cells [185].

Slotkin et al. [186] tested the neurotoxicity of PFOS, PFOA, FOSA, and PFBS in an *in vitro* experiment on undifferentiated and differentiated PC12 cells. After addition of the substances, the authors examined the cells for inhibition of DNA production, deficits in cell numbers and growth, oxidative stress, reduced viability, as well as changes in the production of the neurotransmitters, dopamine and acetylcholine. They came to the conclusion that the different PFCs do not exhibit the same influence on neurons and that it is unlikely that a simple, mutual mechanism is behind all of the neurotoxic effects. FOSA exhibited the strongest effects on the cells, followed by PFOS and PFBS, and finally, PFOA. FOSA depressed DNA production, caused oxidative stress, and reduced the viability of the cells. An explanation for the stronger toxic potential of FOSA is most likely the increased hydrophobicity of this compound and the inherently enhanced access to the cell membrane [186].

In their study, Liao et al. [187] also came to the conclusion that the effects of PFCs on the neurons of the hippocampus of rats are dependent upon the length of the carbon chains and on the functional groups on the alkyl chains. The influence of PFCs on synaptic transmission, calcium current, and neurite growth were examined. Longer chain compounds or such that have a sulfonate group appeared to have stronger effects than short-chain PFCs with a carboxylate group. For example, the experiments with PFOS and PFTDA displayed the highest frequency and strongest amplitude of spontaneous miniature postsynaptic currents [187].

Ten-day old mice received a single dose of 0.75 or 11.3 mg PFOS/kg BW, 0.58 or 8.7 mg PFOA/kg BW, or 0.72 or 10.8 mg PFDA/kg BW in their stomachs. Their spontaneous behavior, defined as movement, breeding behavior, and total activity, as well as their habits were then observed at 2 and 4 months. Behavioral abnormalities were observed in the mice that were exposed to PFOS and PFOA. These appeared as a reduced or deficient adaptability and hyperactivity of the adult mice. These effects became stronger with age. An effect on the cholinergic system was examined using the nicotine-induced spontaneous behavior test on 4-month old animals. The response to nicotine was hypoactivity in exposed animals in contrast with a hyperactive response to nicotine in control animals. Based on the response to nicotine, the effects appear to be mediated by the cholinergic system. These neurotoxic changes are similar to those induced by other POPs such as PCB [15,188]. In a subsequent study on mice, Johansson et al. [189] also showed that PFOS and PFOA increased the concentrations of proteins that are necessary for normal brain development, the tau protein and synaptophysin. Tau proteins play a role in the pathogenesis of Alzheimer's disease, and synaptophysin is a membrane protein of synaptic vesicles [190]. Altered concentrations of these proteins could possibly explain the behavioral changes described above [189].

According to the results of Sato et al. [191], a single dose of PFOS (≥250 mg/kg in rats, ≥125 mg/kg in mice) caused tonic spasms; however, ultrasound stimulus was required as trigger. Even with ultrasound stimulus, PFOA was not found to cause spasms. Changes in neurotransmitter concentrations in the brain or damage to nerve cells did not occur. Therefore, it was not possible to finally elucidate the mechanism responsible for the spasms. PFOS concentrations in the brain (20 to 25 mg/kg) were always lower than those in the liver, kidneys, or serum and increased with passing time after application [191].

The developmental neurotoxic effects were studied in a further *in vivo* study. Rats were fed 7.2 or 14.4 mg PFOS/kg of feed from the beginning of gestation until 30 days after birth. The cross-fostering method was used to differentiate between pre- and postnatal

Table 27 Studies on the reproduction and developmental toxicity of PFCs (from EFSA [15] and enhanced)

Substance	Experimental animal	Tested dosage and time point	Effects	NOAEL LOAEL[a]	Reference
PFOS	Rats	1, 5, and 10 mg/kg BW/day; GD 6 to 15	Body mass of the maternal animal ↓and lens abnormalities	5 10	Gortner [291]
	Rats	5 and 10 mg/kg BW/day; GD 6 to 15	Weight loss of the maternal animal and developmental toxic effects: birth weight ↓, inner organ abnormalities, delayed ossification, and skeletal anomalies	1 5	Wetzel [292]
	Rats	1, 2, 3, 5, and 10 mg/kg BW/day; GD 2 to 21	Weight gain of the maternal animal ↓; serum T_4 and T_3 of the maternal animal ↓; at the highest dosage: body weight of the fetus ↓, cleft palate, anasarca, death rate 4 to 6 h after birth ↑; and from 2 mg/kg BW/day: postnatal growth rate, delayed opening of eyes, T_4 ↓	n.r.	Lau et al. [162]; Thiobodeaux et al. [114]
	Mice	1, 5, 10, 15, and 20 mg/kg BW/day; GD 1 to 18	Delayed opening of eyes and from 5 mg/kg BW/day: liver mass ↑	n.r.	Lau et al. [162]; Thiobodeaux et al. [114]
	Rats	0.4, 0.8, 1, 1.2, 1.6, and 2 mg/kg BW/day; 6 weeks before mating, during gestation, and up to the fourth day of nursing	From 0.8 mg/kg BW/day: gestation time and viability of the young animals ↓	n.r.	Lübker et al. [163,170]
	Rats	0.1, 0.4, 1.6, and 3.2 mg/kg BW/day; 42 days before mating, during gestation, and nursing	At highest dosage: gestation time, number of implantation points, and litter size ↓; and at the second-highest dosage (F1): reduced viability, body weight of the newborn, delayed reflexes and physical development, food uptake after weaning ↓ and dosage (F2): birth weight ↓	0.1 0.4	Christian et al. [164]
	Rabbits	0.1, 1, 2.5, and 3.75 mg/kg BW/day; GD 6 to 20	Weight gain of the maternal animal ↓;and at the second highest dosage: birth weight ↓ and delayed ossification	0.1 1 1 2.5	Case et al. [293]
	Mice	1, 10, and 20 mg/kg BW/day; GD 0 to the end of the study	At the highest dosage (maternal animal): weight gain, feed ↓, water uptake ↑, liver mass ↑; body weight of the fetus ↓, enlargement of the neck, skeletal deformity; and newborn weak and inactive, lung atelectasis, aneurism of intracranial arteries, respiratory dysfunction to death	n.r.	Yahia et al. [294]
	Leghorn chickens	1, 2.5, and 5 mg/kg egg; before incubation	No effect on hatching rate, spleen mass ↑, right wings shorter, frequent occurrence of brain asymmetry, immunoglobulin (IgM, IgY) ↓, plasma lysozyme activity ↑; at the highest dosage: liver mass ↑; and at the highest dosage: body weight ↑	(1 mg/kg egg to 154 ng/g in serum)	Peden-Adams et al. [175]
	Mallard ducks and quail	10, 50, and 150 mg/kg of feed	Viability of the 14-day-old progeny↓; at the lowest dosage: slight increase in incidences of small testes (length); however, spermatogenesis and fertility were not affected	Quail, 10 mg/kg feed	Newsted et al. [177]
PFOA	Rats	1, 3, 10, and 30 mg/kg BW/day	Body weight ↓, liver and kidney mass ↑; and at the highest dosage: birth weight ↓, mortality after weaning ↑, delayed puberty	n.r.	Butenhoff et al. [125,165]
	Mice	1, 3, 5, 10, 20, and 40 mg/kg BW/day; during gestation	Liver enlargement; full-term gestation, viable fetuses, fetus weight, postnatal viability ↓; and growth deficit, delayed opening of eyes, accelerated sexual maturity of male progeny	n.r.	Lau et al. [161]
	Mice	3 to 20 mg/kg BW/day	Liver mass of the maternal animal ↑; body weight gain ↓; and application during GD 7 to 17 and 10 to 17: delayed opening of eyes and growth of coat	n.r.	Wolf et al. [169]
	Mice	5 mg/kg BW/day; GD 1 to 17, 8 to 17, and 12 to 17	Body weight of young animals ↓ and abnormal development of the nursing process to retarded growth of progeny	n.r.	White et al. [295]