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

	Chickens	5, 20, 40 mg/kg egg; before incubation	Impaired hatching rate, high prevalence of splayed legs, and chicks with partial or complete loss of yellow pigment in the down	n.r.	Yanai et al. [174]
PFBS	Rats	30 to 1, 000 mg/kg	No impairment of fertility or reproduction, no developmental toxic effects aside from a slight delay in onset of puberty, and weight gain in male F1 rats of the group with the highest dosage	n.r.	Lau et al. [115]
PFHxS	Rats	n.r.	No effect on fertility, reproduction, or viability and growth of the progeny	10	York [181] cited in Lau et al. [115]
N-Et-FOSE	Rats	n.r.	Toxic effects similar to those of PFOS, still birth, and mortality in the first three days ↑; in addition, increase in the number of stillbirths and mortality in the F2 generation	n.r.	Christian et al. [164]; Lübker et al. [163] cited in Lau et al. [115]
PFBA	Mice	35, 175, and 350 mg/kg BW/day; GD 1 to 17	No adverse effects in regard to survival rate of the progeny or their postnatal growth, delayed opening of eyes, at the two highest dosages: delayed onset of puberty, and at the highest dosage: loss of complete litter	n.r.	Das et al. [180]
PFNA	Rats	1, 3, and 5 mg/kg/day; for 14 days	Cell apoptosis in the testes and imbalance between testosterone and estradiol	n.r.	Feng et al. [183]
PFDA	Mice	0.25, 0.5, 1, 2, 4, 8, and 16, 32 mg/kg BW/day; GD 10 to 13 and 0.03, 0.3, 1, 3, 6.4, and 12.8 mg/kg BW/day; GD 6 to 15	No deformities or other developmental toxic effects; the applied dosages also did not show toxic effects in the maternal animal	n.r.	Harris and Birnbaum [182]
8:2 FTOH	Rats	n.r.	Only mild effects similar to those caused by PFOA	200	Mylchreest et al. [58] cited in Lau et al. [115]

GD, gestation day; n.r., not reported; upward arrow, increased; downward arrow, decreased. [a]NOAEL/LOAEL, not reported in milligrams per kilogram body weight per day.

exposures. The progeny were placed in a water labyrinth, and immunohistochemical analysis was undertaken. The authors came to the conclusion that pre- and postnatal exposures to PFOS impair spatial cognition and memory. The mechanism could be related to a reduction in *N*-methyl-D-aspartate receptor 2B [NR2B] concentration in the cortex and hippocampal region of the brain [192].

In a subsequent study, the authors investigated the effects of PFOS exposure on gene expression of calcium-dependent signal molecules in the hippocampus during gestation and in the lactation period on Wistar rats. By use of the cross-foster method, rats were pre- and postnatally exposed to 3.2 mg PFOS/kg of feed. The expression of NR2B, calmodulin, Ca^{2+}/calmodulin-dependent kinase II α, and cAMP-response element binding protein were examined by real-time reverse-transcriptase polymerase chain reaction. Changes in gene expression of these molecules were detected at various time points during exposure to PFOS. It is therefore possible that perinatal PFOS exposure during a critical phase of brain development exerts a neurotoxic effect on the central nervous system via the molecules of the calcium signal pathway [193].

Pinkas et al. [194] also confirmed the existence of neurotoxic properties of PFOS and PFOA in developing chickens. The authors observed the impairment of cognitive performance in hatched chicks that had been exposed to PFOS or PFOA (5 or 10 mg/kg) *in ovo*. Imprinting behavior was tested on the day of hatching, and impairment was observed after treatment with both of the substances. In order to learn more about the mechanism behind these effects, experiments were undertaken on protein kinase C [PKC] isoforms (α, β, γ) in the intermedial part of the *hyperstriatum ventrale*, the region most closely associated with imprinting. Exposure to PFOA resulted in significant increases in the cytosolic PKC concentration of all three isoforms. In spite of the general increase in PKC expression, the membrane-associated PKC remained unaffected, suggesting a defect in PKC translocation. In contrast, PFOS exposure resulted in reduction of cytosolic PKC, particularly in the β- and γ-isoforms, but again without any changes in the membrane-associated enzyme. Based on these results, PFCs do appear to be developmentally toxic. They lowered the cognitive performance after hatching. The synaptic mechanisms behind these effects seem to be different for PFOS and PFOA [194].

Effects on the endocrine system

The first reports of the effect of PFCs on thyroid hormones were from Langley and Pilcher [195] and Gutshall et al. [196].

Rats that had received a dose of PFDA were found to have significantly reduced T_4 and T_5 concentrations, lower body temperature, and a slower heartbeat than control animals. Treatment with T_4 was not able to reverse the hypothermia. Other studies on rats also showed that PFOS exposure resulted in a reduction of T_4 and T_3 in serum. There is, however, no increase in TSH, a hormone that enhances formation of T_4 and T_3. There is evidence that PFOS, similarly to PFDA, displaces the thyroid hormone from its binding protein as it circulates in the blood [115].

Weiss et al. [197] examined this subject and discovered that PFCs compete with T_4 in binding to the thyroid hormone transport protein transthyretin. This may explain the decline in thyroid hormone levels after treatment with PFCs. The binding potential of PFCs to transthyretin decreases in the order of PFHxS > PFOS/PFOA > perfluoroheptanoic acid > perfluor-1-octanoic sulfinate > perfluorononanoic acid and was approximately 12.5 to 50 times lower than that of the natural ligand of T_4.

When looking at the expression of the thyroid hormone-related mRNA, Yu et al. [171] only observed changes in the expression of mRNA for transthyretin. The transcription level for transthyretin was 150% higher in PFOS-exposed rats than in control animals.

Chang et al. [198] discovered that the oral application of PFOS in rats results in increased tissue availability of thyroid hormone and an increased turnover of T_4 in connection with a reduction in the total amount of T_4 in the serum. Under these conditions, PFOS neither induced hypothyreosis nor did it alter the activity of the hypothalamus-pituitary-thyroid axis.

Moreover, there is evidence that PFCs alter the biosynthesis of gender-specific steroid hormones. For example, application of PFOA to male rats for 14 days led to a reduction in serum and testicular testosterone and an increase in estradiol concentration in serum. Consequently, an increase took place in hormone synthesis in the liver via induction of aromatase. These hormonal changes most likely are connected to the occurrence of Leydig cell adenomas observed in chronic exposure to PFOA [115,185].

Benninghoff et al. [199] described an estrogenic mechanism for PFOA that could promote carcinomas in the liver of rainbow trout. In addition, PFNA, PFDA, and PFUnA behaved like estrogens in the *in vivo* vitellogenin-induction-bioassay. In the meantime, there is evidence that PFCs may act as weak xenoestrogens in the environment [115].

Wei et al. [200] described effects of PFOA on estrogen responsive genes in the liver of minnows. The fish were exposed to 3, 10, or 30 mg/L PFOA for 28 days. PFOA interfered with the function of estrogen in the male fish by inducing vitellogenin and the estrogen receptor β in the liver. It also caused a degeneration of the ovaries in female animals. Zhao et al. [201] showed that PFOA inhibits 3β-hydroxysteroid-dehydrogenase and 17β-hydroxysteroid-dehydrogenase in rat Leydig cells.

Furthermore, PFOA appears to stimulate the development of mammary glands in C57B1/6 mice by promoting steroid hormone production in the ovaries and by increasing the concentration of a number of growth factors in the mammary glands. The results of this study suggest an indirect estrogen effect of PFOA, the possible utility of progesterone biomarker for PFOA exposure of girls and women, and an independence of the PPARα expression, for example, during tumorigenesis of the liver [202]. Maras et al. [203] established an estrogenic effect of 6:2 and 8:2 FTOH *in vitro*; however, it must be assumed that a different mechanism is responsible for this potential xenoestrogen than for the reference substance 17β-estradiol.

In a study of zebrafish (*Danio rerio*), Liu et al. [86] determined that 8:2 FTOH exposure interferes with sex hormone synthesis and impairs reproduction resulting in diminished hatching rates. Four-month-old zebrafish were subjected to 8:2 FTOH concentrations of 10, 30, 90, or 270 μg/L for 4 weeks. Testosterone [T] and estradiol [E2] concentrations in the plasma of the female fish increased significantly, whereas T and E2 concentrations in males decreased or increased. Furthermore, egg numbers and sperm production were reduced; the eggshells were thinner; and the protein content and egg diameter were lower. Histological examination showed the promotion of egg-cell maturation and delayed spermiation. Gene transcription of FSH β and LH β in the pituitary gland was upregulated in female and downregulated in male fish. Increased gene transcription for vitellogenin and *zona pellucida* protein 2 in males is evidence of estrogen activity. In females, the gene transcription for these markers was reduced and was associated with reduced fertility [86].

It was shown in a study by Shi et al. [204] that PFDoA interferes with the reproductive function, testicular structure, and the genes for steroidgenesis in male rats. The rats were treated orally with 1, 5, or 10 mg PFDoA/kg BW/day.

Subsequent testing for chronic, oral exposure to PFDoA (over a period of 110 days) also showed inhibition of steroidgenesis in the testicles and of the expression of certain genes. Significantly lower testosterone concentrations in serum were detected in rats that received 0.2 and 0.5 mg PFDoA/kg BW orally per day. Many factors may play a role in inhibition of testosterone by PFDoA since these dosages of PFDoA reduced levels of the steroidogenic acute regulatory protein, cholesterol side-chain cleavage enzyme, mRNA

concentrations for insulin-like growth factor I [IGF-I], IGF-I-receptor, and interleukin 1α [IL-1α] and altered genes of the hypothalamic-neurohypophysial system [205].

The EFSA assumes that thyroid tumors result secondarily due to hormone imbalances. It was not possible to draw a clear conclusion about the mammary gland tumors. Estradiol-activated growth factors may play a role in the development of Leydig cell tumors [15].

Immunotoxicity

Yang et al. [206-208] reported on the immunotoxic potential of PFOA in mice. Addition of a high dose of 0.02% PFOA to the feed for 7 to 10 days led to a loss of body weight and reduced mass of the thymus and the spleen. Thymus and spleen cells were reduced by more than 90% and by approximately 50%, respectively, probably as a result of inhibition of cell proliferation. The immature $CD4^+$ and $CD8^+$ populations of the thymus cells were most noticeably reduced. The T and B cells were affected in the spleen. An increase in liver weight and peroxisome proliferation occurred in a similar time course as the thymus and the spleen atrophy. Exposure to PFOA (50 to 200 μM) for 24 hours *in vitro*, however, had no effect on the thymus and spleen cells [206].

Yang et al. [208] were also able to establish immunosuppressive properties of PFOA in *in vitro* and *ex vivo* experiments. Oral administration of PFOA in mice (10 days, 0.02% in feed) inhibited an increase in plaque formation by anti-IgM-IgG as well as an increase in serum concentration of IgM and IgG that normally occurs upon immunization with horse red blood cells. An attenuation of spleen cell proliferation by PFOA was demonstrated *ex vivo*. The T- and B-cell activators, lipopolysaccharide and concanavalin, serve as triggers for proliferation of spleen cells; however, no PFOA induced changes in proliferation were observed in spleen cells *in vitro* [208].

Fang et al. [209] discovered toxic effects of PFNA on the lymphatic organs, T cells, and secretion of cytokines by lymphocytes in mice. These are likely due to the activation of PPARα and also PPARγ. The hypothalamus-pitutitary-adreneal axis also appears to play a role since increased serum concentrations of adrenocorticotropic hormone and cortisol were detected in exposed mice. Likewise, cell-cycle arrest and apoptosis were observed in the spleen and thymus after PFNA exposure [209].

Peden-Adams et al. [210] administered six different PFOS dosages to mice for 28 days. However, the authors reported an increase in activity of natural killer cells only in male mice, and they saw a drop in IgM concentration. Lymphocyte proliferation remained unchanged in the male and female mice. In this study, it was also shown that PFOS induces immunotoxic effects at concentrations that have also been detected in humans under special conditions of exposure (serum 91.5 μg/kg; dose 1.66 μg/kg BW/day). The NOAEL of suppression of the sheep red blood cell specific IgM production (plaque forming cell-response) was 0.166 μg/kg BW/day for male animals. The PFOS serum concentration at this dosage was 17.8 ± 4.24 μg/kg. It can be assumed that B cells are the target location for PFOS-induced immunotoxicity [210].

Keil et al. [211] came to similar conclusions in a study of the immunotoxic effects on the developing immune system in the F1 generation of exposed mice. The immunotoxicity of PFOS resulted in functional deficits in the congenital and humoral immune systems of adult animals born to mothers that had been orally administered 0.1, 1, and 5 mg PFOS/kg/day between the 1st and 17th day of gestation, a significantly reduced function of the natural killer cells. A reduced production of IgM was observed in the F1 generation from the eight week of life onwards. The male progeny were significantly more sensitive to the effects triggered by PFOS than the female animals [211].

Qazi et al. [212] showed that even a comparatively short exposure over 10 days with high dosages of PFOS or PFOA (0.02% in the feed) in mice also suppresses adaptive immunity and increases the inflammatory reactions to lipopolysaccharides.

In a subsequent study, the authors found that under the conditions mentioned above, the immune modulating effects of PFOS are in part the result of PPARα activation. For example, hepatomegaly (enlargement of the liver) occurs independently of PPARα; the changes in the thymus are partially dependent upon PPARα; and the effects to the spleen are for all practical purposes eliminated in the absence of the receptors [213]. Further information on the study by Qazi et al. can be found in Table 28.

Guruge et al. [214] exposed female mice to 5 or 25 μg PFOS/kg BW/day for 21 days and then infected them with influenza virus A/PR/8/34 (H1N1). The mice were then examined for their defense against influenza A virus infection. The PFOS concentrations in the blood plasma, spleen, thymus, and lungs increased clearly after exposure to the substance (lungs ≈ plasma > spleen ≈ thymus). A significant loss of weight and mortality were observed as reactions to the virus. Twenty days after infection, the survival rate of the mice was 46% (control group), 30% (5 μg/kg BW/day), and 17% (25 μg/kg BW/day). The average survival time was 14.1 days (control group), 13.2 days (5 μg/kg BW/day), and 11.4 days (25 μg/kg BW/day). Studies that dealt with immunotoxicity are presented in Table 28.

DeWitt et al. [215] wrote a summary article on the immunotoxicity of PFOS and PFOA as well as the role of PPARα in the process. There is a consensus that

Table 28 Studies on the immunotoxicity of PFOS and PFOA

Substance	Experimental animal	Dosage and length of exposure	Effects/dosage	Serum concentration according to dosage	Reference
PFOS	Mouse (C57BL/6)	5, 20, and 40 mg/kg BW/day; 7 days	Food intake, body weight ↓; liver mass, serum corticosterone concentration ↑; and from 20 mg/kg: lymphatic cells↓, depression of natural killer cell activity, lymphocyte proliferation, and antibody forming plasma cells	110.5 to 338 mg/L	Zheng et al. [296]
	Male mouse (C57BL/6)	8.33, 83.3, 416, 833, and 2, 083 μg/kg BW/day; 60 days	From 83.3 μg/kg BW/day: liver mass ↑, altered lymphocyte proliferation, and activity of natural killer cells: depression of antibody forming plasma cells NOAEL: 8.33 μg/kg BW/day, LOAEL: 83.3 μg/kg BW/day	0.674 ± 0.166 mg/L and 7.132 ± 1.039 mg/L[a]	Dong et al. [297]
	Mouse (B6C3F1)	0.166, 1.66, 3.31, 16.6, 33.1, and 166 μg/kg BW/day; 28 days	At the three highest dosages: activity of the natural killer cells ↑ in male mice, altered T cells, and IgM ↓ LOAEL: 1.66 and 16.6 μg/kg BW/day for male or female animals, respectively	0.092 ± 0.022 mg/kg[b] and 0.67 ± 0.11 mg/kg, respectively	Peden-Adams et al. [210]
	Female mouse (B6C3F1)	0.005 and 0.025 mg/kg BW/day	Body weight, immune resistance, and survival rate with influenza A virus infection ↓	2.1 ± 0.3 mg/L (control), 189 ± 14 mg/L, and 670 ± 47 mg/L	Guruge et al. [214]
	Male mouse (C57BL/6)	0.001% to 1% in feed; 10 days	At > 0.02%: clinical effects; at 0.02%: weight ↓, hepatomegaly, atrophy of the thymus, spleen, and fat tissue, thymus and spleen cells ↓ by 84% and 43%	50.8 ± 2.5 mg/L to 340 ± 16 mg/L	Qazi et al. [213]
	Mouse (B6C3F1)	7 mg/kg; 28 days	Weight gain ↓, liver mass ↑, and no detrimental effects on the adaptive immune system	11 mg/L	Qazi et al., [298]
	Rat	0.14 to 7.58 mg/kg BW/day; 28 days	Body weight ↓, liver weight ↑, lymphocyte apoptosis in the thymus ↑, T helper cells ↑, and B cells ↓	0.4 to 30 mg/kg male and 1 to 43 mg/kg female rats	Lefebvre et al. [299]
PFOS or PFOA	Male mouse (C57BL/6)	0.02% in feed; 10 days	Total number of white blood cells ↓, lymphocyte number ↓, neutrophilic granulocytes ↓ (only with PFOA), macrophage number in the bone marrow ↓ (but not in the spleen or abdominal cavity), and tumor necrosis factor α and interleukin 6 production ↑ mildly (*ex vivo*)	340 ± 16 mg/L (PFOS) and 152 ± 8.6 mg/L (PFOA)	Qazi et al. [212]
PFOA	Male mouse (C57B1/6)	0.02% in feed; 7 to 10 days	Body weight ↓, liver mass ↑, spleen and thymus mass ↓, and peroxisome proliferation ↑	n.r.	Yang et al. [206]
	Male mouse (C57BL/6)	0.02% in feed; 10 days	Immunosuppressive: plaque formation ↓, IgM- and IgG serum concentrations ↓, and proliferation of spleen ↓	n.r.	Yang et al. [208]
	Mouse	3.73, 7.5, 15, and 30 mg/kg BW/day; 10 days	T-cell dependent antibody reaction ↓ and no consequences of a stress-induced corticosterone production	n.r.	DeWitt et al. [216]
	Mouse	30 mg/kg BW/day; 10 to 15 days	IgM synthesis ↓	74 mg/L[c] at 3.75 mg/kg BW/day	DeWitt et al. [300]

n.r., Not reported; upward arrow, increase; downward arrow, decrease; [a]50 times higher than that of a human work-related exposure; [b]14 times lower than that of an exposed worker; [c]150 times higher than that of a heavy work exposure or environmentally contaminated human.

PFOA and PFOS influence the immune system. The immune modulation induced by PFOS and PFOA as observed in animal experiments involve changes in inflammatory response, production of cytokines and reduction in weight of the lymphatic organs, and changes in antibody synthesis. Additionally, there are indications from experimental studies that PFOA influences IgE-dependent allergic asthma. Furthermore, the role of corticosterone in PFOA-induced immunosuppression is questioned since the increased corticosterone concentration is accompanied by reduced IgM antibody titers, suggesting an immune response triggered by stress reaction. It was, however, shown by DeWitt et al. [216] that the suppression of antibody synthesis is not the result of liver toxicity nor of stress-induced corticosterone production.

In addition, it must be noted that different animal species show varying degrees of sensitivity to immunological effects. It has been shown that certain mouse strains are the most sensitive animals for immune modulatory effects of PFOA and PFOS. A few strains already showed changes at PFOA or PFOS serum concentrations that were about 100 times higher (for PFOA) or 15 times lower (for PFOS) than the concentrations that had been measured in exposed workers. This indicates that detailed studies on immunotoxicity in humans are necessary [215].

Hepatotoxicity and mode of action

Effects on the liver have often been observed in toxicological studies. For example, liver enlargement was seen in connection with hypertrophy and vacuolization of the liver cells and an increase in liver weight in studies on

subchronic and chronic toxicity. Most generally, rodents and nonhuman primates have been exposed to PFCs. In addition, hepatocellular adenomas occurred in rats.

In particular, liver tumors have been traced to the activation of PPARα [115]. PPARα occurs primarily in the liver and can be activated by long-chain polyunsaturated fatty acids or fibrate. As a consequence, there is an increase in the production of enzymes for fatty acid recovery, a formation of ketone bodies, and a reduction in protein synthesis for liponeogenesis [120]. Rats have a higher susceptibility to the PPARα-based mechanism than humans. However, hepatocarcinogenicity can also be only partially attributed to this mechanism. This is corroborated by the fact that exposure to PFOA also caused an increase in liver weight in the PPARα knockout mice comparable to that in wild-type mice ([15,207,208] cited in BfR [17]). *In vitro* studies showed the following:

In Hep G2 cells, PFOA and PFOS (50 to 200 μmol/L) induced the production of reactive oxygen species [ROS], the dissipation and/or scattering of the membrane potential of the mitochondria and apoptosis. The activity of the SOD, catalase, and glutathione reductase was increased; however, the activity of glutathione-*S*-transferase and glutathione peroxidase was lowered. The glutathione content was reduced. A differential gene expression was observed after PFC exposure. The mechanism behind this could be an overload of antioxidative systems, stimulation of ROS formation, an influence on mitochondria, and interference of gene expression for apoptosis regulators that initiate the apoptosis program [217].

In the study by Eriksen et al. [218] on the genotoxic potential of PFCs in human HepG2 cells, an increase in intracellular ROS was only detected for PFOS, PFOA, and PFNA. However, PFOS and PFOA were not found to cause damage to DNA, and the increase in ROS was not concentration dependent. PFBS and PFHxA evoked neither ROS nor DNA damage. Only PFNA led to a weak increase in DNA damage at cytotoxic concentrations. However, this cannot be accounted for by generation of ROS [218].

Qian et al. [219] exposed human microvascular endothelial cells to PFOS. They found that PFOS induced ROS production in the cells which resulted in a reorganization of actin filaments and an increased endothelial permeability.

It must be assumed that PFOS and PFOA can function as agonists of PPARα. In *in vitro* experiments, PFOS activated PPARα [220,221] and led to peroxisome proliferation, as had been previously shown only in studies on rodents [108,222,223]. The hepatotoxic effects of PFOA in studies on rodents may also have resulted from the activation of peroxisome proliferation [222-224]. This mechanism is more likely to apply to PFOA than to PFOS. In a study on rats, a concentration of 0.64 mg PFOA/kg BW/day and above was found to induce peroxisome proliferation, clearly illustrating the effect of PFOA as a PPARα agonist ([141] cited in EFSA [15]). The activation of PPARα leads to the expression of genes that are involved in lipid metabolism, energy homeostasis, cell differentiation, and peroxisome proliferation [225]. This mechanism can result in tumor induction by non-genotoxic carcinogens.

The fact that the PPARα from mice, rats, and humans can be activated by PFOS and PFOA was also shown in a study by Vanden Heuvel et al. [221]. In these experiments, the respective PPAR expression plasmid was transfected with a luciferase reporter plasmid in mouse 3T3-L1 cells. The relative luciferase activity was measured after addition of increasing concentrations of possible PPAR agonists (e.g., 1 to 200 μM PFOA). PFOS and PFOA had little or no influence on the induction of PPARβ or PPARγ. The human PPARα reacted most strongly, and the rat PPARα, most weakly to PFOS and PFOA. Compared with the naturally occurring PPAR ligands, i.e., long-chain fatty acids such as linoleic and α-linoleic acid, PFOS and PFOA show only a weak effect on PPAR [221].

Shipley et al. [225] were also able to show the activation of human and mouse PPARα by PFOS and FOSA. The test systems used were a COS-1-cell (green monkey kidney cell)-based luciferase reporter gene transactivation test and a rat liver cell model. The mean effective concentration (EC_{50}) was 13 to 15 μM for PFOS with a little difference between PPARα from mice or humans. Maloney and Waxman [226], using a similar test system, determined the maximum activity of mouse PPARα by 10 μM PFOA and humans by 20 μM PFOA. These results were confirmed by a more recent study using similar methods. PFOS appeared less effective than PFOA for mice or human PPARα. Neither PFOA nor PFOS could be shown to have a significant activating effect on PPARγ [227]. In studies using transgenic mice, Nakamura et al. [228] indicated that the human PPARα at relatively low concentrations (0.1 or 0.3 mg/kg) reacts less strongly to PFOA than the mouse PPARα.

It is also possible that PFCs affect PPARα by changes in lipid metabolism and transport. The metabolism of lipids and lipoproteins takes place in part in the liver, where PPARα is also expressed. Additionally, long-chain fatty acids are the natural ligands for PPARα. Thus, Lübker et al. [104] were able to show *in vitro* that PFOS, *N*-EtFOSA, *N*-EtFOSE, and PFOA could interfere with the binding affinity of the L-FABP to endogenous ligands (fatty acids), in the same manner as a strong peroxisome proliferator.

The connection between the activation of PPARα by PFOS and the occurrence of hepatotoxic effects is, however, unclear since a number of inconsistencies appeared in regard to the dose-dependent changes. For example, liver toxicity and hepatocarcinogenicity were seen at PFOS dosages that were lower than those (200 to 500 mg/kg) that induced peroxisome proliferation in short-term studies of rats. Stimulation of peroxisome proliferation was not detected in rats with high cumulative PFOS tissue concentrations. This can likely be explained by an adaptive downregulation of hepatic peroxisome proliferation that resulted from PFOS treatment *in vivo* [115]. This mechanism also does not seem to be responsible for the observed liver toxicity following PFOS exposure in monkey. For example, in a study using cynomolgus monkeys, hypertrophy and lipid vacuolization was observed in the group that received 0.75 mg PFOS/kg/day but without peroxisome proliferation or increase in palmitoyl-CoA-oxidase activity [128].

In addition, induction of a number of liver enzymes (carboxylesterase, cytochrome P450, acyl-CoA-oxidase and -dehydrogenase, as well as carnitine-acetyl-tranferase) was observed. Reduction of 3-hydroxy-3-methylglutaryl-Co A reductase could explain the decrease in cholesterol and triglyceride concentrations [229]. Gene expression studies on rat liver cells showed that PFOS causes changes especially in the genes that play roles in peroxisomal fatty acid metabolism, hormone regulation, and transcription of various cytochrome P450 forms [230].

In regard to PFOA, the correlation of hepatotoxic effects and activation of PPARα is also not consistent. For example, in a study on the cynomolgus monkey, liver mass was seen to increase in association with mitochondrial proliferation at the lowest applied dosage (3 mg/kg/day for 26 weeks). The underlying mechanism could not be explained because the peroxisomal markers remained unchanged ([146] cited in EFSA [15]). In addition, the results of another study suggest a PPARα-independent mechanism for induction of hepatomegaly by PFOA in mice. The increase in liver weight correlated with the exposure to PFOA or a classical peroxisome proliferator in wild-type mice. This effect did not occur in the PPARα knockout mice; however, this was only true for the peroxisome proliferator, not for PFOA. The hepatomegaly observed in the PPARα knockout mice could, however, also be the result of an accumulation of lipid droplets or PFOA in the liver. PFOA also interferes with lipid and lipoprotein metabolism by activating the PPARα. The normal lipid metabolism equilibrium in mammals is disrupted by the induction of enzymes ([230] cited in EFSA [15]). Studies on gene expression in the rat liver show that exposure to PFOA causes induction of all genes that are connected with metabolism and transport of lipids, in particular fatty acids [230-233]. For example, PPARα activation upregulates a gene that is responsible for the formation of lipid droplets in many cell types. An increase in the number of lipid droplets in the liver that resulted from the changes in lipoprotein metabolism could be detected in the PPARα knockout mice and might explain the rise in liver weight after exposure to PFOA [234].

In the study by Minata et al. [235], a 4-week application of APFO (12.5, 25, and 50 μmol/kg/day) to PPARα null mice caused damage to hepatocytes and the bile duct. In wild-type mice, dosages of 25 and 50 μmol/kg/day resulted in more severe dose-dependent hepatocellular damage and less striking impairment of the biliary tract. PPARα null mice that had been exposed to PFOA exhibited marked fat accumulation, severe damage to the biliary tract, hepatocellular damage, and apoptotic cells, most prevalently in the biliary tract. At 50 μmol/kg/day, the oxidative stress was also increased by a factor of 4 in these animals; and at 25 μmol/kg/day, TNF-α mRNA was upregulated by a factor of 3. The bile acid/phospholipid ratio was higher in these animals than that in wild-type mice. These results suggest that PPARα may actually protect against effects of PFOA and plays a critical role in xenobiotic-induced hepatobiliary damage [235].

A further study by Elcombe et al. [236] indicates that PFOA possesses the properties of a mixed enzyme inducer. It induces various cytochrome P450 types in liver microsomes. This induction profile implies a reaction of PFOA with various receptors of the super family of nuclear hormone receptors, in particular with PPARα, constitutive androstane receptor [CAR], and pregnane-X receptor [PXR] [236]. Ren et al. [237] were able to show the activation of PPARα, CAR, and PXR by PFCs in rats, but not in chickens or fish. Furthermore, the PFOS-induced gap junctional intercellular communication [GJIC] and *in vivo* (GJIC) inhibition observed *in vitro* in the rat liver may be of importance in liver carcinogenesis [115,238]. This process is used by cells to exchange ions, secondary messengers, and other small molecules. In multicellular organisms, GJIC plays a role in tissue homeostasis, normal growth, development, and differentiation. An *in vitro* study by Upham et al. [239] showed a dysregulation of GJIC by PFOA that resulted from the activation of the extracellular receptor kinase and phosphatidylcholine specific phospholipase. This evidence suggests that PFOA may also have an effect on GJIC *in vivo*. Inhibition of GJIC appears to only be a property of PF with a chain length of 7 to 10 carbon atoms. For example, PFPeA did not exhibit inhibitory effects on GJIC and did not cause hepatomegaly [239]. The meaning of this widespread and reversible mechanism in carcinogenesis of PFOS or PFOA is, however,

still unclear [15,115,185]. Experiments on the importance of the length of the carbon chain of different PFCs for liver toxicity and peroxisome proliferation in mice and rats suggest that longer chain molecules are more toxic due to their accumulation in the liver [115,240-242].

Combination effects

Wei et al. [243] studied the combined action of PFCs (PFOA, PFNA, PFDA, PFDoA, PFOS, 8:2 FTOH at a concentration of 5 mg/L each). The authors created a gene expression profile using a DNA microarray to detect changes in cultivated hepatocytes from minnows. The fish were treated with four mixtures of these substances or with each substance individually. The study showed that, in fact, certain genes were regulated by the mixture that were unaffected by the individual substances. The effected genes are involved in fatty acid metabolism and transport, in xenobiotic metabolism, in the immune response, and in the emergence of oxidative stress [243].

In another study exposure to a mixture of PFOS and PFOA (50 to 200 μmol/L each) induced and expedited cell apoptosis more effectively than did exposure to the individual substances. This suggests summation effects that, however, appear to be neither synergistic nor antagonistic. Therefore, the effect on the cells was stronger with the mixture than with PFOS or PFOA alone [217].

Using a micronuclear test, Jernbro et al. [244] investigated whether the presence of PFOS increased the genotoxic potential of cyclophosphamide [CPP] in hamster V79 lung cells. Up to a concentration of 12.5 μg/mL PFOS did not show any genotoxic effects. However, after metabolic activation, a combination of PFOS and two different dosages of CPP (1.25 and 2.5 μg/mL) resulted in a greater number of micronucleus containing cells than in cells treated with CPP alone. PFOS induced changes to the cell membrane, and the ensuing changes in the uptake of toxic substances may play a role in these observations [244].

Watanabe et al. [245] examined the effect of co-exposure to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin 2, 3, 7, 8-[TCDD] and PFOS or PFOA on expression of cytochrome P450 isoforms (monooxygenases) in a chicken embryo hepatocyte culture. PFOS or TCDD alone did not induce mRNA production of CYP4V2; however, administration of 40 to 50 μM PFOS plus 0.3 nM TCDD did. A combination of TCDD and PFOA behaved exactly the same way. PFOS caused an increase in CYP1A4 mRNA with or without TCDD co-exposure. The authors suspect a complex gene response to the combined exposure of multiple xenobiotics [245].

Epidemiological studies

Epidemiological studies have been primarily carried out on groups of people who are occupationally exposed to PFCs. These took place, for example, in the course of medical monitoring studies of workers in the fluorochemical industry. The workers were principally from the PFC manufacturing company, 3M, in Decatur, Alabama, USA and Antwerp, Belgium. In particular, biochemical parameters for liver damage or interference with lipid metabolism were examined. Furthermore, hormonal changes and cancer death rates and/or tumor incidence were at the center of interest. Recent studies have also examined possible reproductive toxicities. The significance of these epidemiological studies is, however, limited because of the small number of participants, mostly males due to the working structure in the companies, and the problematic classification of exposure [17,115].

Studies on biochemical parameters and mortality rates

An epidemiological study was carried out on 2, 083 workers in a factory in Decatur in which perfluorooctane sulfonylfluoride [POSF] is manufactured and processed. The workers had been employed for at least one year in the company and had been exposed to POSF-based materials by inhalation, skin contact, and/or ingestion. The various workplaces were categorized according to the amount of exposure as determined by analysis of serum samples of the individual workers. A total of 982 people (47%) were subjected to a relatively high POSF exposure at their workplaces. The proportion of males in this subcohort was 84%. Of a total of 145 deaths, 65 (45%) were of these workers with high PFOS serum concentrations (*ca*. 0.6 to 2 mg/L). This group had a risk of urinary bladder cancer 13 times higher than did the general population of Alabama. This is based on three deaths compared to 0.23 expected cases of bladder cancer (standard mortality rate [SMR] = 12.8). The workers with cancer carried out maintenance work in the factory and worked in the area of the incinerator and sewage treatment plant. It is, however, possible that the workers were also exposed to other bladder cancer-producing compounds outside of the workplace. Other mortality rates in this study were below the statistically expected number of deaths. For example, there were five cases of liver cirrhosis among the total number of participants (SMR 0.85). In animal experiments, however, the liver was identified as the target organ of PFCs [15,246].

In the follow-up study, 11 cases of bladder cancer were documented among the total of 1, 588 participants. Eight cases would be expected statistically. Three of the bladder cancer victims had worked for more than a year at jobs with high PFOS exposure (mean serum concentration 1.3 to 1.97 mg/L). Nonetheless, the correlation between PFOS exposure and increased risk of bladder cancer was not significant [247].

Grice et al. [248] were unable to detect an association between occupational PFOS exposure and the

occurrence of skin, breast, prostate, or intestinal cancer in workers at a PFC-producing company. In addition, there was no correlation between PFOS exposure and the state of health or the course of pregnancies and birth weight. This study was undertaken with the use of questionnaires and medical reports and, as in the study by Alexander et al. [246], encompassed 2, 083 workers of a POSF-processing factory in Decatur [248].

The first retrospective cohort study on mortality of employees of the PFOA-producing factory of 3 M was carried out by Gilliland and Mandel [249]. Participants in the study (2, 788 men and 749 women) were required to have worked for at least 6 months in the factory between 1947 and 1983. In this time period, 398 workers died (348 men and 50 women). Eleven and 148 of the deceased women and men, respectively, had been exposed to APFO. The SMR for prostate cancer (2.03) was increased, based on four deaths out of 148 cases of APFO-exposed workers. The expected frequency would have been 1.97. The relative prostate cancer risk for a one year longer period of employments was 1.13 and rose to 3.3 for workers who had been employed there for over 10 years. The SMR value for other causes of death was not significantly increased [249].

An update of this study was undertaken by Alexander [250] in order to record the deaths that occurred by 1997. The 3, 992 employees were divided into three exposure categories: definite exposure, possible exposure, and exposure not expected. The 607 deaths were allocated to these three categories (46, 267, 294). Regarding all workers, the highest SMR (1.31) was for bladder cancer. A few of the SMR values were elevated in the group that was definitely exposed. For example, two deaths resulted from colon cancer (SMR 1.67), one from pancreatic cancer (SMR 1.34), and one from prostate cancer (SMR 1.3). In the group with possible exposure, elevated SMR values were obtained for cancers of male sexual organs (2.75), pancreas (1.24), and skin (1.42). The results are difficult to interpret because of the changing, more specific exposure categories. The previously determined significant association between prostate cancer and time of employment could not be confirmed in this study [15].

A recent epidemiological study was undertaken by Lundin et al. [251]. The authors examined the correlation between the APFO exposure of 3, 993 workers in the 3 M factory in Minnesota and the rates of mortality. This study differed from that of Gilliland and Mandel [249] because of newer data and increased exclusion of workers with only short times of exposure. The workers were divided into three exposure categories: definite exposure, possible exposure, and workplace without exposure. APFO exposure was not associated with liver, pancreas, or testicular cancer but presumably with prostate cancer, cerebral vascular disease, and diabetes mellitus [251].

Studies by DuPont [252] provide little information about correlations of PFOA exposure at the workplace and death rates or incidence of cancer since data on exposure, contact with other substances, and lifestyle were missing. Significant differences regarding exposure were shown for bladder and kidney cancer [15,115]. A subsequent study indicated increased SMR values for kidney, liver, and bladder cancers; however, the only significant increase in rate of death was from diabetes mellitus ([253] cited in Lau et al. [115]). No significant liver toxicity was found in a further study by Gilliland and Mandel [254] of 115 males, occupationally PFOA-exposed study participants with PFOA serum concentrations of (0 to 26 mg/L; mean 3.3 mg/L), although hepatotoxic effects often occurred in studies of rats. Total fluorine values were measured since 90% of those in serum are made up of PFOA. The enzymes of the liver (serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, gamma glutamyltransferase), the lipoproteins (LDL, HDL), and the cholesterol values were within the normal zone of fluctuation. Possible disturbance variables such as body mass index [BMI] and tobacco and alcohol consumption were taken into account. Based on the enzyme profiles, the authors suspected that PFOA possibly modulates the reaction of the liver to obesity and xenobiotics [254].

Olsen et al. [68] examined similar parameters in connection with the PFOS serum concentrations in workers involved in the production of fluororganic compounds at 3 M. In 1995, the PFOS mean concentration for 178 male workers was 2.19 mg/L, and in 1997, the concentration for 149 workers was 1.75 mg/L. No explicit changes in liver enzymes, cholesterol, or lipoproteins in serum could be detected in the serum of workers with PFOS concentrations below 6 mg/L [68].

One year later, a study was published by Olsen et al. [255] on workers in the production of APFO. The mean PFOA serum concentration was 5 mg/L (1993), 6.8 mg/L (1995), and 6.4 mg/L (1997). Cholecystokinin concentration in plasma was also determined. It was assumed that pancreatic adenomas of the acinar cells observed in animal studies were caused by a steady increase in cholecystokinin that resulted from hepatic cholestasis (bile congestion). However, the cholecystokinin values (average 28.5 ng/L) were within the reference range and were negatively coordinated with increasing PFOA concentrations. The measured PFOA concentrations, therefore, did not appear to cause a hepatotoxic effect. The previous suspicion that PFOA modulates the reaction of the liver to obesity and alcohol consumption could not be confirmed. The weak points in the study were the small number of workers (17), the relevant experimental

data for whom encompassed only three years, the low rate of participation in the study (50% to 70%), and the low number of participants with serum concentrations over 10 mg/L [255].

Olsen et al. [69] then linked PFOS and PFOA serum concentrations with data from medical examinations of workers in one plant. The mean serum concentrations of PFOS and PFOA of the 263 workers in Decatur were found to be 1.32 mg/L and 1.78 mg/L, respectively. The serum concentrations of the 255 workers at the plant in Antwerp were approximately 50% lower. After taking into consideration possible disturbance variables (age, BMI, cigarette, and alcohol consumption per day) no conspicuous changes in the blood, lipid, liver, thyroid, or urine parameters were noted [69].

Possible weak points in the study of Olsen et al. [69] were taken into consideration in a following study by Olsen and Zobel [256]. Since the intention was to measure cholesterol levels, persons who took cholesterol-lowering drugs were excluded. The calculations of LDL were not coupled to triglyceride values, and data on PFOA were also gathered both in Antwerp and Decatur. The study comprised 506 workers at 3 M factories in Antwerp, Minnesota and Alabama who took part in a 'fluorochemical medical surveillance program.' PFOA serum concentrations were between 0.007 and 92.0 mg/L. The total cholesterol or LDL concentrations were not significantly altered with increasing PFOA concentrations in the serum after removal of the disturbance variables such as age, BMI, and alcohol consumption ($P > 0.05$). HDL was negatively correlated with PFOA in the workers at all three locations, but not in employees at each individual location. This may be explained by demographic differences between the locations. The positive correlation of triglyceride values with PFOA serum concentrations of the workers appeared similar. There was no statistically significant correlation between the concentrations of PFOA and liver enzyme activities. Only for the employees at one of the locations was a weak positive association apparent. Results were inconsistent for thyroid hormones. TSH and T_4 concentrations showed no correlation to PFOA levels. Free T_4 was negatively associated to PFOA, whereby increases in T_3 concentrations were correlated with increased PFOA values. Nonetheless, thyroid hormone levels were within the range of reference values [132].

Olsen et al. [257] looked for potential correlations between PFOA exposure and changes in hormone levels in male PFOA production workers. The focus of the study was on a drop in T_3 and T_4 levels and estrogen-like effects. The study showed an increase in mean estradiol concentration of approximately 10% at the highest PFOA serum concentrations (> 0.03 mg/L) in the 191 workers. This relationship, however, could have been influenced by the BMI. No PFOA concentration relationships were found for other hormones. The weak points in this study were the cross-sectional design that led to the low number of participants with high blood PFOA concentrations [257].

In regard to blood lipids, liver enzymes, and occupational exposure to APFO, Sakr et al. [258] detected an increase in total cholesterol (10.6 mg/L per 1 mg/L PFOA) and aspartate aminotransferase (0.35 U per 1 mg/L PFOA) in serum. After adjusting for possible influencing factors, an association of PFOA concentrations and triglyceride or lipoprotein levels was no longer recognizable. Total bilirubin was reduced by 0.08 mg/L per 1 mg PFOA/L serum in exposed persons.

Because of the correlation between APFO exposure and increased lipid concentrations in serum, Sakr et al. [259] examined the association of APFO exposure and incidence of ischemic heart disease. The study cohort comprised 4, 747 workers from the DuPont Washington Works. However, no evidence was found of an increased mortality risk for ischemic heart disease in the exposed workers [259].

Costa et al. [260] evaluated medical surveillance reports for the years 1978 to 2007 from workers who had been employed in the PFOA-producing industry. The study population comprised 53 male workers who had received a medical examination each year and for whom blood tests for various parameters and for PFOA concentrations were available. In the most recent study from 2007, the PFOA concentrations of workers presently employed were 0.2 to 47 mg/L (mean value 5.71 mg/L), and for previously exposed workers, 0.53 to 18.7 mg/L (mean value 4.43 mg/L). No clinical evidence was found to indicate a correlation between PFOA concentrations and illness in the 30 years of observation. Biochemical parameters for liver, kidneys, and hormonal functionality were also within the reference range. However, significant correlations between the PFOA serum concentration and the total cholesterol and uric acid levels were detected, as had also been previously reported by Sakr et al. [258]. This is suggestive of an effect of PFOA on purine metabolism in the liver [260].

Very few epidemiological studies exist with data from the general population. In a study by Bloom et al. [261], they compared concentrations of various PFCs (PFDA, PFNA, PFHpA, PFHxS, PFOA, PFOS, FOSA, PFUnA) as well as free T_4 [FT_4] in the blood of 31 fishermen from New York. The background of this study is the crucial role played by the thyroid hormones in the neuronal development of human beings. A statistically significant correlation was not found between any of the PFCs examined or the sum of all the substances and TSH or FT_4 concentration. There is a possibility, however, that a weakly positive correlation exists between FT_4, PFDA,

and PFUnA, detected in low concentrations (0.21 and 0.2 μg/L) in the blood of the fishermen.

Based on possible effects of PFCs on the thyroid, Pirali et al. [262] examined PFOS and PFOA in surgical thyroid samples from patients with diseases of the thyroid. The substances were detected in all samples. The mean concentration for PFOS was 5.3 μg/kg, and for PFOA, 2.0 μg/kg. There was no correlation between the intrathyroidal PFC concentrations and the occurrence of thyroid diseases. For PFOS, a correlation between the tissue and serum concentrations was established. The serum concentrations were always higher than the respective surgical tissue samples.

Melzer et al. [263] also investigated a correlation between the occurrence of thyroid diseases and PFOS and PFOA in 3, 974 adults from the NHANES. The authors came to the conclusion that higher PFOA and PFOS serum concentrations were associated with a thyroid disease in adults of the general US population. More women with blood concentrations of ≥5.7 μg PFOA/L were found to have currently treated thyroid disease than women with ≤ 4.0 g/L of blood levels. More men with blood concentrations of PFOS ≥36.8 μg/L also had diseases of the thyroid than men with concentrations ≤ 25.5 μg/L. The correlation with PFOS in women was not significant [263].

Test persons from the general public that had been contaminated with higher concentrations of PFOA[f] in drinking water had distinctly higher serum values (mean 354 μg/L) than the average population in the US (mean 4 to 5 μg/L). A blood count was made; biochemical parameters were recorded; and the subjects were interviewed. No statistically significant correlations could be found between the PFOA serum concentration and the values from liver and kidney tests, or with cholesterol or TSH concentrations in serum or with the blood count in regard to red or white blood cells or thrombocytes when compared to standard reference values from the general population. PFOA concentrations were not elevated in persons with liver or thyroid illnesses ([264] cited in BfR [17]).

Nelson et al. [265] established a positive association between PFOS, PFOA, and PFNA and total cholesterol, LDL, and very LDL [VLDL] levels. The participants from the highest PFOS quartile (44.8 ± 28.0 μg/L) had 13.4 mg/dL higher total cholesterol concentrations than the participants from the lowest quartile (9.6 ± 2.9 μg/L). The differences were 9.8, 13.9, and -7.0 mg/dL for PFOA, PFNA, and PFHxA. Thus, an opposite result was detected for PFHxS. The authors did not find any correlations between PFC concentrations and BMI, hip measurent, or insulin resistance.

Steenland et al. [266] also detected increased blood-lipid levels in connection with elevated PFOA and PFOS concentrations in the blood. HDL was the only exception to this.

The study comprised 46, 294 persons who had been residents for more than 18 years and who drank water contaminated with PFOA by a chemical factory in West Virginia. The mean PFOA and PFOS serum concentrations were found to be 80 μg/L and 22 μg/L. The cholesterol level increased from 11 to 12 mg/dL, from the lowest to the highest decile for both substances.

Elevated PFOA serum concentrations were also associated with a higher prevalence of hyperuricemia. The uric acid level increased by 0.2 to 0.3 mg/dL, from the lowest to the highest decile of PFOA or PFOS concentration [267].

Anderson-Mahoney et al. [268] examined 566 persons who also had been exposed to PFOA via drinking water[g]. In a questionnaire, the participants were asked about their medical anamnesis. There was evidence that exposed persons more often suffered from angina, myocardial infarctions, chronic bronchitis, shortness of breath, and asthma. Further studies will be necessary to determine whether PFOA is the cause of the increased prevalence of these effects in PFOA-exposed persons [268].

Additionally, Lin et al. [269] showed a correlation between PFCs and glucose homeostasis, as well as other indicators of metabolic syndrome. In the general population, elevated PFNA serum concentrations were associated with hyperglycemia, correlated positively with HDL levels in serum, and correlated inversely with the prevalence of metabolic syndrome. Increasing PFOA serum concentrations increased the β-cell function. Elevated PFOS serum concentrations increased the insulin level in the blood, insulin resistance, and β-cell function and was negatively correlated with serum HDL cholesterol values [269].

McNeil et al. [270] pursued the results of a study that established an increased diabetes mortality rate in PFOA-exposed workers. Based on 22 diabetes deaths, employees of the Washington Works exhibited twice the mortality rate over other non-exposed workers [271]. The study population (n = 54, 468) comprised participants in the C8 health project. A total of 1, 055 people had type II diabetes and before diagnosis, had lived in the area of elevated PFOA contamination, most likely the result of contaminated drinking water. The PFOA serum concentration for these individuals was found to be 0.028 mg/L (mean) in contrast to 0.004 mg/L for the general population of the USA. Table 29 lists the PFOA serum concentrations of the participants in this study.

Diabetes prevalence amounted to 7.8%. A decreasing risk of diabetes was found for the highest PFOA serum concentration compared with the lower values, but

Table 29 PFOA serum concentrations (mg/L) in relation to type II diabetes (from McNeil et al. [270])

PFOA serum concentration (mg/L)	All participants (*n* = 54, 468)	Self-reported type II diabetes (*n* = 4, 278)	Diagnosed type II diabetes (*n* = 3, 539)	Long-standing[a] resident with type II diabetes diagnosed in the last 10 years (*n* = 1, 055)
Mean	0.087	0.093	0.091	0.123
Median	0.028	0.030	0.033	0.049
Geometric mean	0.033	0.034	0.036	0.052

[a]At least 10 years in a region with elevated PFOA contamination.

without showing a consistent negative correlation. Taking age into account, however, this correlation could be reversed. A consistent pattern was not apparent for fasting serum glucose levels in connection with PFOA serum concentrations. Consequently, an unambiguous trend for diabetes risk in connection with PFOA serum concentrations did not emerge. However, a correlation cannot be dismissed on the basis of the results of this study since the data was limited due to the cross-sectional design, and a causal relationship cannot be excluded. For these reasons, the authors are planning further studies with an improved design [270]. The studies listed in the 'Studies on biochemical parameters and mortality rates' section are summarized in Table 30.

The US EPA also discussed existing epidemiological studies in 2005. Nevertheless, a consistent correlation between PFOS, PFOA, or APFO serum concentrations in humans and adverse health effects could not be observed.

According to the German BfR [17], only a few individual studies have shown statistically significant relationships between the concentration of liver enzymes, cholesterol level, HDL concentration, and triglyceride concentration or concentration of individual hormones in the human blood and the exposure to PFCs. In addition, the correlations could generally not be confirmed in subsequent studies and even contradicted the effects of the compounds observed in animal experiments [17]. More recent studies indicate correlations between PFC concentrations and diabetes and thyroid disease, as well as blood lipid and uric acid levels; however, these cannot be considered proven and will need to be confirmed in further studies.

Studies on reproductive and developmental toxicity

Grice et al. [248], in a written survey on exposure and the course of pregnancy, comprising 421 women who had been occupationally exposed to PFOS, did not find a correlation between the degree of PFOS exposure and birth weight of the children. One of the first studies published that analyzed the PFC concentration in the maternal blood and cord blood as parameters for the exposure of the fetus and/or newborn stemmed from Inoue et al. [54]. The authors examined 15 mother/child pairs in Japan and did not find a correlation between PFOS in the cord blood and birth weight or concentration of thyroid hormones in the blood of the newborn. This may well be the consequence of the concentrations to be expected in cord blood and the relatively high limit of detection, 0.5 μg/L [54].

In the study by Fei et al. [57], 1, 400 selected mother/child pairs from the Danish 'National Birth Cohort' were examined in an attempt to find possible correlations between the concentration of PFOS and PFOA in the maternal blood during the first and second trimesters of pregnancy and the birth weight and risk of premature birth. A correlation between PFC concentration in the maternal plasma and birth weight of children from mothers of normal weight could only be shown for PFOA. The length of gestation was unrelated to PFOS or PFOA concentrations in the maternal blood [57].

Monroy et al. [56] also measured the PFC concentration in the blood of pregnant women in the 24th to the 28th week, at birth, and in the cord blood. PFOS and PFOA were detected in all samples. Also in this study, the PFOS serum concentrations were higher during gestation (18 ± 11 μg/L) than at the time of birth (16 ± 10 μg/L) and higher than in the cord blood (7.3 ± 5.8 μg/L). The differences were smaller for PFOA (24th to the 28th week of gestation 2.5 ± 1.7 μg/L; birth 2.2 ± 1.6 μg/L; cord blood 1.9 ± 1.5 μg/L). PFHxS was detected in 45.5% of the maternal samples and in 20% of the cord blood samples [56].

On the other hand, in a cross-sectional study, Apelberg et al. [55] found a weak inverse correlation between the concentration of PFOS and PFOA in the cord blood and birth weight, the ponderal index, and head circumference of 293 newborns. No correlation was found between the concentration of the substances and the birth length or gestation time. The results for birth weight were statistically corrected for influence factors such as the mother's smoking habits, diabetes, and hypertension. The authors recommend exercising caution when interpreting these results since the association of head circumference was only for vaginal births; the newborns were all healthy, and the variations in head circumference and birth weight were within the normal range [55]. Washino et al. [272] could also see a negative correlation between the *in utero* PFOS exposure and the birth weight of baby girls. A correlation between the PFOA concentration and the birth weight was not

Table 30 Epidemiological studies on biochemical parameters and mortality rates of humans related to PFC exposure

Effects	Study population	Results	Serum concentration (mg/L)	Reference
Death rates and incidence of cancer	2, 083 Workers in POSF production (USA); minimal time of employment is one year	Heavy-exposure group: deaths resulting from bladder cancer, 3; SMR, 12.8; and no increase in liver disease	PFOS, *ca.* 0.6 to 2 (GM)	Alexander et al. [246]
	Workers in POSF production (USA), 1, 400 questionnaires, and 188 death certificates	11 Cases of bladder cancer and 8 expected	PFOS, *ca.* 1.3 to 1.97	Alexander and Olsen [247]
	Workers in POSF production (USA); 1, 400 questionnaires	No association between PFOS and various forms of cancer, and no correlation between PFOS contamination and state of health, course of pregnancy, or birth weight	PFOS, *ca.* 0.1 to 1.97	Grice et al. [248]
	3, 537 Workers in POSF production (USA)	Elevated SMR for prostate cancer (2.03) and no significant correlation with other cancer or heart diseases	n.r.	Gilliland and Mandel [249]
	3, 992 Workers	All workers: elevated SMR for bladder cancer, 1.31; group with certain exposure: elevated SMR for colon, pancreas, and prostate cancers	n.r.	Alexander [250]
	4, 747 Workers	No clear evidence of increased risk of death that resulted from ischemic heart disease	n.r.	Sakr et al. [259]
	3, 993 Workers of the 3 M plant (USA)	No association with liver, pancreas, and testicular cancer and liver cirrhosis; elevated SMR for prostate cancer, cerebrovascular diseases, and diabetes	Certain APFO exposure is 2.5 to 5.2; possible APFO exposure is 0.3 to 1.5	Lundin et al. [251]
Endocrine effects	191 Workers, 111 in 1993 and 80 in 1995 (USA)	Increase (10%) in estradiol level at > 0.03 μg/mL PFOA (BMI as cofactor); for other hormones: no association with PFOA serum concentration	PFOA, 0 to 26; mean, 3.27	Olsen et al. [257]
Biochemical parameters	115 male workers (USA)	As related to enzymes in the liver, lipoproteins, and cholesterol, no significant indication of liver toxicity or dysfunction	Total fluorine concentration is 0 to 26; mean is 3.3	Gilliland and Mandel [254]
	178 Male workers in 1995 and 149 workers in 1997	No dramatic changes in liver enzymes, cholesterol, or lipoproteins in serum	PFOS, < 6	Olsen et al. [68]
	111 Male workers (1993), 80 male workers (1995), and 74 male workers (1997) in APFO-production	No changes in hepatic enzymes, cholesterol, or liporotein levels	PFOA 5 (1993), 6.8 (1995), and 6.4 (1997)	Olsen et al. [255]
	263 Workers of the 3 M factory in Decatur (USA) and 255 workers from the plant in Antwerp (Belgium)	No conspicuous changes in blood, liver, thyroid, or urinary parameters after correcting for possible interfering factors	Decatur: PFOS, 1.32 and PFOA, 1.78; Antwerp *ca.* 50% lower	Olsen et al. [69]
	506 Workers in the three 3 M factories in Antwerp, Minnesota, and Alabama	No significant correlation of PFOA with total cholesterol or LDL concentrations, liver enzymes, TSH, and T_4; inconsistent results for HDL and triglyceride values; FT_4 was negatively correlated with PFOA; and T_3 elevation with increasing PFOA concentrations → within reference values	PFOA is 0.007 to 92.03; mean is 2.21	Olsen and Zobel [256]
	454 Workers with APFO exposure (USA)	Elevation of total cholesterol and AST levels; no correlation with triglycerides or lipoproteins	n.r.	Skar et al. [258]
	53 Male workers, from 1978 to 2007	No clinical evidence of dysfunction or disease; biochemical parameters for liver, kidneys, and hormonal functionality within reference values; and significant correlation between PFOA serum concentration and total cholesterol and uric acid levels	PFOA is 0.2 to 47.04 (2007); median value is 5.71	Costa et al. [260]
	371 Persons of the general public that were exposed to PFOA via drinking water	No significant correlation of PFOA concentration with liver or kidney function tests, cholesterol levels, TSH hormone level, or values for various blood cells	PFOA median value is 0.354	Emmet et al. [264]
	Participants in the NHANES study 2003/2004 between 12 to 80 years of age	Positive association between PFOS, PFOA, and PFNA and total cholesterol, LDL, and VLDL levels for PFHxS, a negative correlation	PFOA, 0.007; PFOS, 0.038; PFNA, 0.002; PFHxS, 0.005 (median)	Nelson et al. [265]

Table 30 Epidemiological studies on biochemical parameters and mortality rates of humans related to PFC exposure *(Continued)*

	46, 294 Residents for more than 18 years that drank water contaminated with PFOA by a chemical factory in West Virginia	Rising blood lipid values with increasing PFOA and PFOS concentrations in the blood	PFOA, 0.080; PFOS, 0.022	Steenland et al. [266]
	54, 951 Adult residents from Ohio and West Virginia exposed via drinking water	Elevated uric acid concentrations in the highest decile of PFOA or PFOS concentrations compared with the lowest	PFOA, 0.189; PFOS, 0.041	Steenland et al. [267]
Disease	566 Persons exposed to PFOA via drinking water	Increased occurrence of angina, myocardial infarction, chronic bronchitis, shortness of breath, and asthma	n.r.	Anderson-Mahoney et al. [268]
	General public comprising 474 adults and 969 juveniles in Taiwan	Correlation of PFCs with glucose homeostasis and other indicators of the metabolic syndrome	n.r.	Lin et al. [269]
	54, 468 Persons exposed to PFOA via drinking water, 1, 055 with type II diabetes	Reduced risk of diabetes mortality at high PFOA values; not consistent	PFOA, 0.028	McNeil et al. [270]
	28 Patients who had had thyroid operations	No correlation between intrathyroidal PFC concentrations and occurrence of thyroid disease	n.r.	Pirali et al. [262]
	3, 974 Adults of the NHANES study	High PFOA and PFOS serum concentrations associated with thyroid disease	PFOA, ≥0.0057 (women); PFOS, ≥0.0368 (men)	Melzer et al. [263]

n.r., Not reported; GM, geometric mean.

found. Hamm et al. [273] were unable to establish an association between the birth weight or gestation time and the maternal serum concentrations (mean values: PFOA 1.5 μg/L, PFHxS 0.97 μg/L, PFOS 7.8 μg/L). Nolan et al. [274] investigated the relationship between a PFOA-contaminated drinking water supply and the birth weight and gestation time. They did not find any indication for low birth weight or premature birth related to the water supply. The risk group comprised mothers from Washington County, Ohio. The drinking water there had a PFOA concentration 80 times higher (6.8 μg/L) than can be assumed for the general US population [274]. More recent studies by the authors, also dealing with the connections between the PFOA contamination of drinking water (customers of the Little Hocking Water Association) and the pregnancy complications, indicate an association of PFOA exposure, the incidence of anemia, and dysfunction of labor contractions [275].

Stein et al. [276] also described a connection between PFOA and PFOS serum concentrations of residents of the Mid-Ohio Valley (200 to 2, 006) who were exposed to PFOA in drinking water with self-reported courses-of-pregnancy descriptions. PFOA measurements for 1, 845 pregnant women and PFOS values for 5, 262 pregnant women were available. The mean PFOA concentration in serum was 49 μg/L, and the mean PFOS concentration was 15 μg/L. Neither the PFOS nor PFOA serum concentrations could be statistically correlated with miscarriage or premature birth. However, a weak association of PFOA with the development of preeclampsia and congenital defects[h] was noted. A weak connection was also shown for PFOS and development of preeclampsia. Mean PFOS concentrations exceeding 12.8 μg/L were seen to increase the risk of reduced birth weight of newborns. It must be noted, however, that these associations were weak and imprecise and were based solely on reports made by the pregnant women [276].

Fei et al. [277] investigated the development of infants with the help of questionnaires that the mothers were to fill out when their children were between 6 and 18 months of age. These data were examined in connection with prenatal exposure to PFOA and PFOS. The authors could not find any differences in the development of the infants from mothers with high PFOA and PFOS blood concentrations (PFOA 7 to 42 μg/L; PFOS 43 to 107 μg/L) and children of mothers with low PFOA and PFOS blood concentrations (PFOA < 1 to 4 μg/L; PFOS 6 to 26 μg/L). The Apgar scores and point in time of developmental progress were similar for children of mothers with high PFOS and PFOA concentrations in blood as for children of mothers with low PFOS and PFOA blood concentrations. There was only a weak indication that children of mothers with high blood concentrations of PFOS began to sit without support at a later time point [277].

A further study by Fei et al. [278] suggests a possible impairment of fertility as seen in the PFOS and PFOA blood concentrations measured in the general population. The mean PFOS and PFOA plasma concentrations of women who planned to get pregnant were 33.7 μg/L and 5.3 μg/L, respectively. The time until pregnancy was longer in the proband group with higher PFOA and PFOS contaminations [278].

Since PFOA was seen to cause impairment of lactation in mice, Fei et al. [279] examined PFOS and PFOA

concentrations in connection with duration of nursing in 1, 400 nursing women. They discovered a decrease in duration of nursing in correlation with increasing PFC concentrations by women who had had repeated births. Whether PFOS or PFOA really reduces the ability to nurse or whether the correlation is reversed remains questionable.

Hoffman et al. [280] discovered an increasing risk of attention deficit hyperactivity disorder [ADHD] in children with elevated PFC serum concentrations. Data from the NHANES study from 1999 to 2000 and from 2003 to 2004 on ADHD and PFC blood concentrations from 571 children between the ages of 12 and 17 were evaluated in this study. Forty eight children were reported to suffer from ADHD. The corrected odds ratios for an increase of 1 μg/L serum concentration of PFOS, PFOA, PFHxA, and PFNA were 1.03, 1.12, 1.06, and 1.32, respectively. The results of epidemiological studies on the correlation between PFC blood concentration and reproduction and developmental toxic effects are summarized in Table 31.

Olsen et al. [281] published a review article on epidemiological studies that dealt with the influence of PFCs

Table 31 Results of studies on reproduction toxicological effects of PFCs on humans

Effects	Study population	Results	Reference
Birth weight	Occupationally exposed women, USA	No correlation between extent of PFOS exposure and birth weight	Grice et al. [248]
	General population, Japan	No correlation between PFOS concentration in cord blood and birth weight	Inoue et al. [54]
	General population, Danish National Cohort	Correlation between the PFOA concentration in mother's plasma and birth weight; not detectable for PFOS	Fei et al. [57]
	General population, USA	Weak inverse correlation between concentrations of PFOS and PFOA in cord blood and birth weight	Apelberg et al. [55]
	General population, Canada	No correlation of PFC serum concentrations and birth weight	Monroy et al. [56]
	General population, Japan, 2002 to 2005	Negative correlation of *in utero* exposure to PFOS birth weight; not detectable for PFOA	Washino et al. [272]
	General population, USA	No indication of a connection between low birth weight and PFOA-contaminated drinking water	Nolan et al. [274]
	General population, USA, 2000 to 2006	Correlation between PFOS contamination and the risk of reduced birth weight	Stein et al. [276]
	General population, Canada	No correlation between PFOA, PFHxS, PFOS serum concentrations and birth weight	Hamm et al. [273]
Gestation time	General population, Danish National Cohort	No correlation of PFOA and PFOS concentrations in mother's plasma with time of gestation	Fei et al. [57]
	General population, USA	No indication of premature birth as a result of PFOA contamination via drinking water	Nolan et al. [274]
	General population, USA, 2000 to 2006	No connection of PFOS or PFOA serum concentration with miscarriage or premature birth	Stein et al. [276]
	General population, Canada	No correlation between PFOA, PFHxS, PFOS serum concentrations and gestation time	Hamm et al. [273]
Development	General population, Danish National Cohort	No difference in the development of newborns from mothers with high PFOA and PFOS concentrations and children of mothers with low PFOA and PFOS concentrations; sitting without support possibly delayed in children of mothers with high PFOS concentrations	Fei et al. [277]
Other aspects	General population, USA	Weak inverse correlation between concentrations of PFOS and PFOA in cord blood and the ponderal index or head circumference	Apelberg et al. [55]
	General population, Japan	No correlation between PFOS concentration in cord blood and concentration of thyroid hormones	Inoue et al. [54]
	General population, USA, 2000 to 2006	Weak correlation of PFOA concentrations and occurrence of miscarriages	Stein et al. [276]
	General population, USA, 2000 to 2006	Weak association of PFOA and PFOS serum concentrations with the occurrence of preeclampsia	Stein et al. [276]
	General population, 571 children between 12 to 15 years from the NHANES study, 1999 to 2000 and 2003 to 2004	Increased risk of ADHD for children with elevated PFOS, PFOA, PFHxA, and PFNA serum concentrations	Hoffman et al. [280]
Fertility	General population, Danish National Birth Cohort, 1996 to 2002	Fertility disorders related to elevated PFOA and PFOS plasma concentrations	Fei et al. [278]

on human fetal development. The authors compared the published results, listed the strengths and weaknesses, listed alternative possible explanations for published results, and suggested future studies. They came to the conclusion that future research activities on this subject will need to consider more carefully the physiology of pregnant mothers and the increased maternal plasma volume during pregnancy. In addition, participants from the general population with the highest PFOS and PFOA concentrations should be included in order to better recognize possible toxic effects [281].

Steenland et al. [282] also published a review of epidemiological studies on PFOA. The authors concluded that available studies provide consistent evidence of a weakly positive association of the PFOA concentration in serum with cholesterol and the uric acid levels, whereby the magnitude of the effect on cholesterol level is not correlated with the extent of exposure.

Apart from that, there are a few but inconsistent indications of a weakly positive correlation with the activities of liver enzymes. A majority of the results stem from cross-sectional analyses from which it is not possible to draw conclusions of causality. Two cohort studies of occupationally exposed workers do not provide unequivocal evidence of chronic disease; however, as a result of the small numbers of participants, the information they provide is limited.

There is a recent upturn in the number of publications on reproductive effects. The results from these studies are, however, inconsistent, and the observed adverse effects are weak. It is therefore concluded that the information provided by the results from previous epidemiological studies is limited and the data is inadequate to allow unambiguous conclusions to be drawn about the role of PFOA in the development of particular diseases [282].

Summary

The present general opinion is that the main route of PFC uptake is dietary with contaminated fish and game, constituting a majority of the exposure. Nonetheless, the representative data that would allow an estimation of dietary exposure is not available. Therefore, in 2009, the EU launched the research project, PERFOOD, with the aim of improving the level of awareness about PFCs in foods. Among other things, the migration of PFCs from packaging material into foodstuffs is also to be studied. Additionally, other less studied pathways such as skin contact with PFC-treated utensils and inhalation of indoor air in particular should also be further studied. Comparatively, little data is, however, presently available on these paths of exposure. According to previous studies, the total daily PFC uptake is in the range of 2 to 200 ng/kg BW/day for PFOS and 3 to 14 ng/kg BW/day for PFOA. Admittedly, other PFCs such as FTOH or FOSE/FOSA may contribute to the internal contamination of humans. As a result of their lower body mass and increased hand-to-mouth contact, it may be assumed that the internal PFC contamination per kilogram body mass of children is greater than that of adults. In addition, PFOS and PFOA can cross the placental barrier and can pass into breast milk.

The quantitatively dominant component of PFCs in the human blood is PFOS. The PFOA concentrations are generally somewhat lower in the blood than PFOS concentrations. The linear forms of both so-called reference components are most commonly identifiable in blood samples. Geographic differences have been found for PFC serum concentrations in humans. Individual studies show a possible influence of diet on the degree of contamination with PFCs. An unequivocal correlation between age and blood PFC concentration is not evident. Gender-dependent differences are, however, probable. Men generally show a higher contamination with PFCs than women. The serum concentrations of these compounds appear to have risen over the last decades. Whether this trend will continue is presently unknown.

Animal experiments suggest that PFCs are relatively well taken up by the organism both orally and by inhalation. They accumulate primarily in the liver, and after increasing exposure, also in the blood, and other organs such as the kidneys. An explanation of the mechanism by which this distribution takes place involves the preferred binding of PFCs to serum albumin, L-FABP, and membrane structures in the liver. There is presently no evidence for metabolism of PFOS or PFOA. In contrast, there is increasing evidence for metabolism of FTOH. Differences in the excretion of PFCs have been found for different compounds and different species. PFOS is excreted more slowly than PFOA so that the latter has a shorter elimination half-life and higher rate of excretion. It can be assumed that branched chain molecules are more rapidly excreted than the linear isomers, which therefore tend to accumulate more. According to current knowledge, short-chain PFCs such as PFBS are also excreted more rapidly than long-chain PFCs. An active and sex-hormone-regulated mechanism for renal excretion of PFOA has been demonstrated in rats. Enterohepatic circulation appears to reduce the excretion rate of PFOS and PFOA as also shown in an experiment on rats.

Based on the results of animal experiments, the acute toxicity is considered modest. Diverse toxic effects were observed in longer-term animal tests. Hepatotoxic effects have often been described. In addition, lipid metabolism was often affected in experimental animals. Epidemiological studies have indicated effects of PFCs on glucose, urea, and/or uric acid metabolism; therefore,

it would seem that further studies on PFCs and metabolic processes are necessary. Tumor growth has been observed in experimental animals after chronic exposure. Most commonly the liver, Leydig cells, and mammary gland tissue have been involved. Evidence of the occurrence of particular cancer diseases, most often urinary bladder and prostrate cancers, have been observed in individual epidemiological studies. The target organs of animals and humans appear to differ, aside from the pancreas that was seen to be prone to cancerous growth both in humans and in animals. In regard to carcinogenesis, a genotoxic mechanism cannot be assumed for PFOS and PFOA, but rather a tumor promoting effect and/or epigenetic process come into question. Animal studies show unmistakable reproductive and developmental toxic effects that were only partially found in epidemiological studies. There is presently no evidence of teratogenic effects. To more thoroughly understand the influence on human fertility and the development of newborn children, the results on reproductive toxicity from animal studies should be taken into consideration. Considering the results of animal experiments, neuro- and immunotoxic effects will have to be examined in future epidemiological studies. The trigger for hepato- and immuno-reproductive, reproductive, and developmental effects as well as carcinogenesis of PFCs may be partially or completely attributed to the activation of the PPARα. Correspondingly, a change in expression of the genes that control lipid metabolism, energy homeostasis, cell differentiation, and peroxisome proliferation might be involved.

Apparently, different PFCs exhibit different toxicities. PFOS and linear isomers appear to be more toxic than PFOA and branched chain compounds, i.e., in comparison, PFOS and linear isomers exhibit a longer half-life than do PFOA and branched chain compounds and cause adverse effects at lower dosages. The data presently available regarding toxicology of PFCs other than PFOS and PFOA is in comparison meager, inhomogeneous, and fragmentary, particularly in light of the diversity of PFCs occurring in biological matrices.

Conclusions

There are a number of pathways by which PFC contamination of humans can take place including diet, food contact materials, non-food personal items, and indoor and outdoor air. Although a number of authors have attempted to calculate the contribution of these individual pathways to total contamination, the available data records are not presently adequate to allow sound conclusions to be drawn. In addition, the various authors have, to some extent, taken diverse approaches to the subject of dietary contamination. Some have assumed that consumption of fish and seafood are the main source of PFC contamination in humans, whereas others have postulated that, particularly in regions in which only small amounts of fish are eaten, meat and vegetables are the primary source. There is, however, a general consensus that dietary uptake represents the largest contribution. Nonetheless, it will be necessary to establish the contribution of various foodstuffs to total exposure by comparing the data from studies that systematically determine PFC contamination of a large number of edible products. Studies that are limited to local or regional products are not adequate to describe the global exposure scenario. Toxicokinetic and toxicodynamic data of PFCs, aside from those for the so-called reference substances, PFOA and PFOS, are not consistent enough to allow a conclusive toxicological evaluation.

In recent years, numerous publications have appeared in which biological properties of PFCs are described; however, these are generally limited to PFOA and PFOS. These two substances are, to the best of our knowledge, the only PFCs that have been toxicologically examined in animal studies that would allow conclusions to be drawn about potential human toxicity. Data on short-chain PFCs that are apparently being substituted for longer chain molecules in industrial processes are, if available at all, only of a fragmentary nature. Because of their solubility in water and the increasingly wide spectrum and volume of their use, these short-chain PFCs deserve considerable study. This is particularly evident since they appear to be ubiquitously distributed throughout the water pathway and can thus lead to an increased background contamination of the environment. Additionally, PFCs are being used in mixtures with varying compositions, making toxicological evaluations much more difficult. For this reason, standardized *in vitro* and *in vivo* methods should be used and further developed in order to allow reliable conclusions to be drawn concerning the toxicity of the individual substances as well as of various PFC mixtures. Consequently, an adequate toxicological evaluation of the total situation is presently not possible.

Endnotes

[a]The LOD was not specified in the publication. [b]The type of average was not specified. [c]Whether the dose was based on a kg BW is not evident from the description of the study. [d]Calculated according to the following formula: bile clearance (mL/hr/kg BW) = PFOA in the $bile_{0\text{-}300\ min}$ (nmol)/$AUC_{\ 0\text{-}300\ min}$ (nmol·hr/mL)/kg BW. [e]The age of the animals was not listed. [f]Concentrations and locations were not listed. [g]More details were not presented. [h]The types of congenital defects were not described.