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Perfluorinated compounds: biomonitoring and study of health effects

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1. Perfluorinated compounds

1.1. Definition

Perfluorinated compounds [PFCs] are organic substances in which all of the hydrogens of the hydrocarbon backbones are substituted with fluorine atoms. The fluorine-carbon bonds are extremely stabile conferring these substances with very high thermal and chemical stability. PFCs are persistent, and some of the substances bioaccumulate in the environment.

They can be divided into the groups of perfluorinated sulfonic acids, perfluorinated carboxylic acids [PFCA], fluorotelomer alcohols, high-molecular weight fluoropolymers and low-molecular weight perfluoroalkanamides. Perfluorooctanesulfonic acid [PFOS] and perfluorooctanoic acid [PFOA], often referred to as reference or key substances for the first two groups, have been most intensively studied from a toxicological standpoint.

PFCs have been synthesized for more than 50 years and are used in numerous industrial and consumer products. These compounds are intermediates or additives in the synthesis of certain fluorine compounds or their decomposition products. These fluorine compounds are commonly used in consumer products as stain/water/grease repellents in carpets and clothing or in cooking utensils as nonstick coatings (Fricke & Lahl, 2005; Fromme *et al.*, 2006).

The potentially toxic effects of these substances are presently being studied with increasing intensity. The relevance of this topic is also clearly reflected by the number of publications that have appeared in recent years. This increasing interest is the result of reports of toxic effects of PFCs in connection with the ubiquitous detection of this substance in the environment and in sundry matrices, i.e., bodies of water, wild animals, human blood, and breast milk samples, all of which have come to the attention of the public.

An estimate was published in 2008 by the German Federal Institute for Risk Assessment [BfR] and the European Food Safety Authority [EFSA] regarding the potential risks of PFCs in food stuffs for human health. In this document, it was reasoned that adverse effects for the general population were unlikely,

based on the known PFC concentrations in food stuffs and serum samples and the present state of scientific knowledge. However, uncertainty was noted in the risk evaluation, and available data are inadequate in regard to the diversity of foodstuffs. In addition, only PFOS and PFOA were considered in the risk evaluation, but according to the Organisation for Economic Co-operation and Development [OECD], 853 different poly- and perfluorinated compounds exist (Umweltbundeamt, 2007; Umweltbundeamt, 2009). In a European Union [EU]-supported research project, which began in August 2009 and was called Perfluorinated Organic compounds in our Food [PERFOOD], efforts are being made to estimate the dietary exposure to PFCs.

1.2. Exposure

1.2.1. Exposure via the food chain

1.2.1.1. Dietary uptake

One of the pathways by which PFCs can be taken up is through the ingestion of contaminated foodstuffs and/or drinking water. PFCs have been detected in fish, meat, milk products, and plants, e.g., grains. Plants can apparently take up PFCs from contaminated soil. This hypothesis was examined by Weinfurtner et al. (2008), showing that the transfer of PFCs from the soil to the plants for potatoes, silage corn, and wheat was so marginal that no health danger for humans would be expected by this path of uptake.

Stahl et al. (2009) described for the first time a significant, concentration-dependent transfer ('carry over') of PFCs from the soil to the plant. The higher the concentration of PFOA and PFOS in the soil, the higher the concentration that could be detected in the plants. The uptake and storage of these substances in the vegetative parts of the plants appear to be more significant than the transfer to the storage organs within the plants. In this study, the uptake, distribution, and storage of PFOA and PFOS were seen to be dependent upon the type of plant. The uptake of PFOA and PFOS from contaminated soil by plants enables the entrance of PFCs into the food chain of humans and may provide an

explanation for the presence of these compounds in, for example, foodstuffs of animal origin, human blood samples, and human breast milk (Stahl *et al.*, 2009).

Trudel et al. (2008) reported that oral ingestion of contaminated foodstuffs and drinking water accounts for the largest proportion of PFOA and PFOS exposures for adults. Tittlemier et al. (2007) and Haug et al. (2011a;2011b) also expressed the opinion that foodstuffs are the most important uptake path. Within the framework of the 'Canadian Total Diet Study, ' the authors calculated that Canadians ingest on an average of 250 ng of PFCA and PFOS per day. Scheringer et al. (2007) also had come to the conclusion that 90% of all PFOS and PFOA exposures is derived from food. Similarly, Vestergren and Cousins (Vestergren *et al., 2009)* are convinced that the main exposure of humans to PFOA is through dietary uptake.

Fromme et al. (2007) quantified PFC dietary exposure in Germany. The authors collected and analyzed 214 duplicate meals and beverages from 31 volunteers aged 16 to 45 years old on 7 days in a row. The samples were tested for content of numerous PFCs. Perfluorohexane sulfonate [PFHxS] and perfluorohexane acid [PFHxA] levels above the limit of detection [LOD] of 0.1 or 0.2 µg/kg fresh weight, respectively, were detected in only a few samples (3% and 9% of the 214 samples, respectively), whereas perfluorooctane sulfonamide FOSA] was not detected (LOD = 0.2 µg/kg fresh weight). These authors also assume that dietary uptake represents the main source of PFC exposure for humans (Fromme *et al.,* 2007).

Numerous foodstuffs were tested for the presence of PFOS, PFOA, and other PFCs within the framework of the 'UK Total Diet Study' in 2004. PFOS concentrations above the LODa were detected in potatoes, canned vegetables, eggs, sugar, and preserves. Particularly striking was the group of potato products, where in addition to PFOD, PFOA and 10 other PFCs were detected (UK FSA; European FSA, 2008).

Inhabitants of reputedly remote regions are by no means exempt from the uptake of PFCs in their food. In a recent study, Ostertag et al. (2009) examined the dietary exposure of Inuit in Nunavut (Canada) to

these substances. The authors calculated an average daily exposure of 210 to 610 ng/person. The traditional foods such as caribou meat contributed to a higher PFC exposure for this population group. Caribou meat contributed 43% to 75% of the daily exposure (Ostertag *et al.*, 2009).

In 2008, an exposure assessment was made on dietary uptake of PFOS and PFOA in connection with possible health effects. The report was based on published data concerning concentrations of PFOS and PFOA in various foods in Europe and on the amount of the individual foods consumed according to the 'Concise European Food Consumption Database' (UK FSA). Since the data for other foods were inadequate to make an exposure assessment, it was based solely on the presence of PFOS and PFOA in fish and drinking water. The results of the exposure assessment for PFOS suggest a daily exposure of 60 ng/kg body weight [BW] for persons who consume average amounts of fish or 200 ng/kg BW those who consume large amounts of fish. For PFOA, the daily uptake was estimated at 2 ng/kg BW/day, and for those who eat larger amounts of fish and fish products, the estimate was 6 ng/kg BW/day (UK FSA). The estimated consumption of drinking water was 2 L/person/day. The uptake from drinking water of PFOS and PFOA were *ca.* 0.5% and 18%, respectively, of the average amount taken up by consumption of fish and fish products.

The German BfR (2008) also undertook an assessment of dietary exposure of the general population to PFOS and PFOA. As a basis for the calculations, the Federal Office of Consumer Protection and Food Safety provided data on PFC concentrations in foods from 2006 to 2008. The data were, for the most part, derived from the Federal Control Plan (2007) 'Perfluorinated surfactants in specific foods' and encompassed 3, 983 test results on contents of PFOS (1993 data sets) and PFOA (1990 data sets) in foodstuffs. Concentrations of the substances were measured in chicken eggs, beef and poultry liver, pork, game and fish offal, poultry and game meat, salt water and fresh water fish, French fries, honey, and drinking water. In addition, the records contained data on the consumption of food and food products by the German population derived from a survey made in 1998. Since one must assume that for over a longer period of time, some foods that have a higher PFC concentration and others with a

lower concentration will be consumed, the statistical calculations were made using an average[b] value. In addition, the possibility had to be considered that foods that have exceptionally high concentrations may be consumed perhaps because of unusual local paths of entry. Therefore, exposure through particularly heavily contaminated foods was quantified for both average and above average consumers. The following scenarios were assumed for exposure assessment:

- Average concentration of PFOS and/or PFOA and average amounts consumed
- High concentration of PFOS and/or PFOA and average amounts consumed
- Average concentration of PFOS and/or PFOA and large amounts consumed
- High concentrations of PFOS and/or PFOA and large amounts consumed (worst case).

In this exposure assessment, drinking water played a relatively small role in the total exposure to PFOS. The average PFOS uptake from drinking water by an average consumer amounted from 0.02 to 0.08 ng/kg BW/day. The average PFOA uptake from drinking water, however, amounted from 0.32 to 0.40 ng/kg BW/day. Thus, the total PFOA uptake, including drinking water, amounted from 1.03 to 1.34 ng/kg BW/day for an average consumer (BfR, 2008). If, however, the water is contaminated by an unusual source of PFCs, the role of drinking water in exposure to these substances may be considerable. This was the case, for example, in Arnsberg, Germany where the source of drinking water in 2006 was the PFC-contaminated river, Skutlarek (2006). Hölzer et al. (2008) measured a PFOA concentration 4.5 to 8.3 times higher in the blood plasma of residents than in the plasma of a reference population from the neighboring towns, Siegen and Brilon. The highest PFC concentration detected in the contaminated drinking water was for PFOA (Hölzer *et al.,* 2008).

In a follow-up study, it was shown that elimination of PFCs from humans occurs slowly. The geometric mean of the PFOA concentrations in plasma decreased on an average of 10% per year for men, 17% per year for women, and 20% per year for children (Hölzer *et al.,* 2009).

Another study showed that there was no increased PFC exposure in this region in 2006 before contamination of the drinking water. Samples of blood from 30 residents that had been drawn between

1997 and 2004 contained PFOS and PFOA concentrations comparable with those of the general population in Germany (Wilhelm *et al.*, 2009).

After concentrations as high as 0.64 µg/L were measured in drinking water in Arnsberg in 2006, the German Drinking Water Commission derived a critical limit of 0.3 µg/L for a health-based, lifelong exposure to PFOS and PFOA in drinking water. PFOS and PFOA concentrations in drinking water can be reduced by active charcoal filtration. Use and manufacture of PFOS are strictly limited by legal regulation, and a voluntary reduction of PFOA is being sought. Therefore, the focus of a study by Wilhelm et al. (2010) was placed on short-chain C4-C7 compounds that are presently finding use as substitutes for PFOS and PFOA. In a new approach to evaluate short-chain PFCs, based on their half-life in humans, the following preliminary health-related indication values were considered safe for a lifelong exposure via drinking water: 7 µg/L for perfluorobutanoic acid [PFBA], 3 µg/L for perfluoro-n-pentanoic acid [PFPeA], 1 µg/L for PFHxA, 0.3 µg/L for perfluoroheptanoic acid [PFHpA], 3 µg/L for perfluorobutanesulfonic acid [PFBS], 1 µg/L for perfluoropentane-1-sulfonic acid [PFPeS], 0.3 µg/L for PFHxS, and 0.3 µg/L for perfluoroheptane sulfonic acid [PFHpS]. A long-range minimum quality goal or general precautionary value for all PFCs in drinking water was set at ≤ 0.1 µg/L (Wilhelm *et al.*, 2010).

A study by Mak et al. (2009) compared PFC concentrations in tap water from China with that from Japan, India, the USA, and Canada. Samples were collected between 2006 and 2008. Tap water from Shanghai, China contained the highest concentration of PFCs (arithmetic mean sum PFCs 0.13 µg/L; PFOA 0.078 µg/L). The lowest values were obtained from Toyama, Japan (0.00062 µg/L). In addition to PFOS and PFOA, drinking water appears to also contain short-chain PFCs such as PFHxS, PFBS, PFHxA, and PFBA. In relation to the guidelines set down by the United States Environmental Protection Agency [US EPA] and the Minnesota Department of Health (PFOS 0.2 µg/L, PFOA 0.4 µg/L, PFBA 1.0 µg/L, PFHxS 0.6 µg/L, PFBS 0.6 µg/L, PFHxA 1.0 µg/L, PFPeA 1.0 µg/L), tap

water from these countries should not present a health risk for consumers, in respect to PFC contamination (Mak *et al.*, 2009).

In a review article from Rumsby et al. (2009) on PFOS and PFOA in drinking water and in diverse environmental bodies of water, the authors also conclude that PFOS and PFOA are detectable worldwide. Aside from situations in which there are unusual sources of contamination, the concentrations measured are, however, below existing health-based guidelines specified by various international bodies (0.3 to 0.5 µg/L). Nonetheless, further studies of short-chain PFCs such as PFBS must be undertaken. This substance has a shorter half-life, is less toxic, and is not bioaccumulative, but it is nonetheless persistent, and its possible degradation products remain unknown (Rumsby *et al.*, 2009).

D'Eon et al. (2009) point out that perfluorinated phosphonic acids [PFPAs] should also be measured in future environmental monitoring studies. These substances were detected in 80% of all surface water samples and in six out of seven sewage treatment plant outflow samples in Canada. C8-PFPA was detected in concentrations from 0.088 ± 0.033 to 3.4 ± 0.9 ng/L in surface water and from 0.76 ± 0.27 to 2.5 ± 0.32 ng/L in sewage treatment plant outflow samples. Since they are structurally similar, one can assume that just like perfluorocarboxilic acids and perfluorosulfonic acids, PFPAs are also persistent (D'Eon *et al.*,2009).

1.2.1.2. Human exposure via fish consumption

In addition to drinking water, PFC accumulation in fish is also of particular importance for the internal contamination of humans. According to the exposure assessment of the German BfR consumption of salt water and fresh water, fish accounts for approximately 90% of the total dietary exposure to PFOS (BfR, 2008).

The fact that fish are often highly contaminated is a result of the pronounced biomagnification of these substances via the aquatic food chain. The role of fish consumption is apparent in a model calculation by Stahl et al. (2007). Based on the recommendation of the BfR of 0.1 µg PFOS/kg BW/day as a preliminary daily tolerable uptake, a 70-kg adult should not exceed 7 µg of PFOS (Stahl *et al.*, 2007).

Eating reasonable amounts of fish with high levels of contamination, i.e., from bodies of water with unusual sources of PFCs, may in itself result in reaching or exceeding this limit for the short term (Stahl *et al.*, 2007). For example, eating 8 g of eel from Belgium with a concentration of 857 µg PFOS/kg fresh weight or eating 0.6 g of trout from the upper Sauerland region of Germany with a measured maximum level of 1, 118 µg/kg fresh weight, is already adequate. Consumption of a normal portion (300 g) of these trout would result in exceeding the limit by a factor of 57 (Stahl *et al.*, 2007). PFC contamination of fish was also dealt within the following studies.

As an example, analysis was made from a total of 51 eels, 44 bream, 5 herring, 5 mackerel, 3 carp, and 4 trout from various bodies of water in Germany (North Sea, Baltic Sea, Lake Storko in Brandenburg, rivers in Lower Saxony, rivers and lakes within the city limits of Berlin). None of the fish fillet samples had PFOA levels above the limit of detection (0.27 µg/kg); however, PFOS concentrations of 8.2 to 225 µg/kg fresh weight were measured in fish from densely populated regions. With regard to the TDI of 150 µg/kg BW/day (European FSA, 2008) and assuming the consumption of fish on a regular basis, the PFC concentrations in 33 of the 112 fish examined represent a potential health risk to heavy consumers of fish (Schuetze *et al.*, 2010).

In a Swedish study, the authors also came to the conclusion that consumption of fish from fishing grounds with high concentrations of PFCs in the water can play an important role in dietary PFOS exposure (Berger *et al.*, 2009). Fish from Lake Vättern (mean 2.9 to 12 µg/kg fresh weight) had higher PFOS concentrations in the muscle tissue than fish from the brackish water of the Baltic Sea (mean 1.0 to 2.5 µg/kg fresh weight). A PFOS uptake of 0.15 ng/kg BW/day was estimated for a moderate consumption (two portions of 125 g/month) and 0.62 ng/kg BW/day for a higher consumption (eight portions per month) of fish from the Baltic Sea. A PFOS uptake of 2.7 ng/kg BW/day was calculated for people who eat large amounts of fish from Lake Vättern.

No foods that have been examined to date other than fish were found to have a level of contamination great enough to result in reaching the TDI for PFOS or PFOA, assuming realistic consumed amounts. By

way of example, according to the model calculations shown above, an adult in the USA would have to consume 12 kg of beef (0.587 µg PFOS/kg) or 12 L of milk (0.693 µg PFOS/L) per day (at the measured levels of contamination in the USA) in order to reach the TDI (Stahl *et al.*, 2007).

Furthermore, offal from game contained the highest concentrations of PFOS and PFOA of all foods. The PFOS concentrations in offal from game were 100-fold higher than those in muscle tissues (BfR, 2008).

A detailed, up-to-date survey on the presence of PFCs in foods was also recently published by the EFSA (2011) with the title 'Results of the monitoring of perfluoroalkylated substances in food in the period 2000 to 2009.'

When making an exposure assessment, it is important to take into account the fact that many different foods are generally consumed. Studies with the aim of representing the total dietary intake are both quantitatively and qualitatively inadequate. For example, in the various studies including those of the EFSA and the BfR, only a selection of foods were included. In addition, the number of samples was, in part, too small to provide a representative value. For these reasons, the exposure assessments presently available should be considered exploratory. Specific regional sources of contamination can increase PFC levels in foods and drinking water. Furthermore, individual dietary habits, i.e., a predilection for fish or offal from game, must be considered, and additionally, perfluorinated compounds other than PFOS and PFOA must be monitored. Since most studies have examined fresh and unpackaged foods, the effects of migration of PFCs from packaging and cooking utensils on the food products have not been taken into consideration.

1.2.1.3. Exposure of food to food contact materials

When coming into contact with foods, paper and cardboard packaging are protected from softening by treatment with, among other things, water- and oil-resistant perfluoro chemicals. Fluorotelomer alcohols [FTOH] may be present as contaminants in the coatings. About 1% of the FTOH can be converted to PFOA in the body (Martin *et al.*, 2005; Kudo *et al.*, 2005). Furthermore, PFOA is used in the production of polytetrafluoroethylene [PTFE] nonstick surface coatings for cooking utensils or paper

coatings and may therefore be present in residual amounts (BfR, 2005). A migration of < 6 µg/kg (< 1 µg/dm2) FTOH into food has been calculated as the sum of 6:2 FTOH, 8:2 FTOH, and 10:2 FTOH in an acetone extract of treated paper under the assumption of complete migration (European FSA, 2008; BfR, 2005). Powley et al. (2005), using liquid chromatography coupled with tandem mass spectrometry were unable to detect a migration of PFOA from PFTE-coated cooking utensils (LOD 0.1 ng/cm2).

Begley et al. (2005) showed that nonstick cooking utensils contribute less to PFC exposure to food than coated papers or cardboard boxes. Residual amounts of PFOA in the range of a few micrograms per kilogram or nanograms per gram were all that could be detected in PTFE cooking utensils. Of the total amount of PFOA in a PTFE strip, 17% (30 ng/dm^2) migrated into the food simulant heated to 175°C for 2 h. In contrast, some paper and cardboard surface coatings contained large amounts of PFCs. For example, microwave popcorn bags were found to contain 3 to 4 mg/kg (11 µg/dm^2).

After heating, the PFOA concentration in the popcorn itself was about 300 µg/kg. PFOA migrated into the oil that coated the popcorn. Migration was enhanced by a temperature of 200°C (Begley *et al.*, 2005).

Sinclair et al. (2007) examined the emission of residual PFOA and FTOH from nonstick cooking utensils and microwave popcorn bags upon heating to normal cooking temperatures (179°C to 233°C surface temperature). Heating nonstick frying pans released 7 ng to 337 ng (0.11 to 5.03 ng/dm^2) PFOA in the gas phase. Furthermore, concentrations of 6:2 FTOH and 8:2 FTOH of < 0.15 to 2.04 ng/dm^2 and 0.42 to 6.25 ng/dm^2 were detected. Repeated use of some frying pans was observed to result in a reduction in PFOA concentrations emitted in the gas phase. However, this was not the case for all frying pans from all of the manufacturers tested. In addition, 5 to 34 ng PFOA and 223 ± 37 ng (6:2 FTOH) as well as 258 ± 36 ng (8:2 FTOH) per bag were detected in the emitted vapor from microwave popcorn bags (Sinclair *et al.*, 2007).

Tittlemier et al. (2006), in the Canadian Total Diet Study, examined food samples between 1992 and 2004 for contamination with *N*-ethylperfluorooctyl sulfonamide [*N*-EtFOSA], FOSA, *N*,*N*-diethyl-

perfluorooctanesulfonamide, *N*-methylperfluorooctyl sulfonamide, and *N*,*N*-dimethyl-perfluorooctanesulfonamide. FOSA, in ng/kg and a few µg/kg amounts, was detected in all food products tested (pastries, candies, milk products, eggs, fast-food products, fish, meat, and convenience foods). The highest concentrations (maximum 27.3 µg/kg) were found in fast-food products (French fries, sandwiches, pizza), which are foods that are commonly packaged in grease-proof paper. Dietary FOSA uptake in Canada was estimated to be 73 ng/person/day. The *N*-EtFOSA concentrations in the samples seem to drop throughout the time period of sampling. This is possibly the result of fact that manufacturing of perfluoro octylsulfonyl compounds was discontinued (Tittlemier *et al.*, 2006; Fromme *et al.*, 2009).

In studies of packaged food products carried out by Ericson Jogsten et al. (2009), PFHxS, PFOS, PFHxA, and PFOA were detected at levels above the LOD (PFHxS 0.001 µg/kg, PFOS 0.008 µg/kg, PFHxA 0.001 µg/kg, PFOA 0.063 µg/kg) in at least one mixed-food sample. Among the packaged foods tested were goose liver paté, deep-fried chicken nuggets, frankfurters, marinated salmon, and head lettuce (Ericson Jogsten *et al.*, 2009).

Similar to the results of Begley et al. (2005), the US Food and Drug Administration [FDA] named coated paper as the largest possible source of fluorochemicals. According to the FDA, nonstick frying pans are, by comparison, an insignificant source of PFCs (European FSA, 2008). In the ninth list of substances for food contact materials, the EFSA Panel on food additives, flavourings, processing aids and materials in contact with food [AFC] recommends limiting the use of ammonium perfluorooctanoate [APFO] for articles with repeated use to those on which the coating is baked at a high temperature. According to the analytical data, APFO, as auxiliary material in the production of PTFE, could not be detected at levels above the LOD of 20 µg/kg in the finished product. In the worst case, the AFC determined an APFO migration of 17 µg/kg food (European FSA, 2008). As a result of advances in food technology, contamination of foodstuffs during manufacturing, packaging, or cooking only plays a minor role in the total exposure of humans to PFCs (European FSA, 2008).

The German Federal Environment Agency has rated the uptake of PFCs through the use of nonstick pots and pans as low. The available data are, however, not yet adequate for a reliable assessment of PFC exposure through food contact materials (UBA, 209).

Several studies point out the possibility of underestimation of PFC exposure through food contact materials. Mixtures of perfluorooctanesulfonamide esters are often used in the manufacture of water- and greaseproof papers and cardboards. These perfluorooctylsulfonyl compounds have yet to be studied. They may remain as residues in the coatings and migrate into the food.

D'Eon et al. (2010) examined the formation of PFCA through the biotransformation of polyfluoroalkyl phosphate surfactants [PAPS]. The authors showed that, in spite of their large molecular size, these substances are bioavailable and that PFOA and other PFCs may be formed by their biotransformation. PAPS can probably be degraded by dephosphorylating enzymes in organisms because of the phosphate-ester bond between the fluorinated part and the acidic head group. However, it should be noted that the rats in this study were fed high oral doses of 200 mg/kg PAPS. Renner raises concerns of the fact that PAPS may migrate much more effectively into emulsions such as butter, margarine, or lecithin additives than into food simulants such as oil or water (D'Eon *et al.*, 2010; Renner, 2007).

The fact that studies using conventional food simulants do not accurately reflect the actual migration of fluorochemicals into food was confirmed by Begley et al. (2008). They recommend an emulsion containing oil as simulant for greasy food products. The authors measured the migration of three PAPS from the paper packing material, finding 3.2 mg/kg in popcorn after preparation and 0.1 mg/kg in packaged butter after a 40-day storage by 4°C (Begley et al., 2008).

Lv et al. (2009) determined the contents of PFOA and PFOS in packing materials and textiles by means of liquid extraction under pressure and subsequent gas chromatography coupled with mass spectroscopy analysis. PFOA concentrations of 17.5 to 45.9 µg/kg and PFOS concentrations of 17.5 to 45.9 µg/kg were found in the packing materials and textiles tested (Lv *et al.*, 2009).

Given the present state of knowledge, it is not possible to say whether the use of nonstick-coated cooking utensils or packaging materials with PFC-based coating lead to a significant increase in dietary internal PFC contamination of humans.

1.2.2. Additional potential pathways of exposure

PFCs may also enter the body by ingestion of dust and dirt particles and by contact with products that have been treated with substances that contain PFCs or its precursor compounds (Haug *et al.*, 2001b; Goosey *et al.*, 2011). These may include carpets, upholstered furniture, or textiles. These routes of entry may be of particular importance in regard to children because contact can occur indirectly by hand-to-mouth transfer or directly if an infant sucks on the product. Another route that must be considered is inhalation of PFCs in indoor or outdoor air (Haug *et al.*, 2001a; Langer *et al.*, 2011; Huber *et al.*, 2011) as well as the inhalation of waterproofing sprays. Dermal exposure may also occur by skin contact with PFC-treated products (BfR, 2008).

1.2.2.1. Exposure via non-food personal items

An estimate of exposure via non-food products is difficult because of the large number of possible applications of PFCs such as for jackets, trousers, shoes, carpets, upholstered furniture, and as cleaning agents. In addition, only data are available concerning possible PFCs exposure via non-food products. In order to make an estimation of exposure, research groups such as Washburn et al. (2005) have resorted to the use of models.

In this study, the concentrations of deprotonated PFOA [PFO] (the anion of PFOA) were determined by extraction tests and information about the composition of the products.

Age-specific behavior was taken into account in order to assess the PFO exposure of consumers through contact with these products. A one-compartment model was chosen to determine the contribution of PFC-treated non-food products to the concentration of PFO in serum, and a dermal absorption

coefficient of 1.0×10^{-5} per hour was adopted. The values obtained are hypothetical and are categorized as more typical exposure [MTE] or reasonable maximum exposure [RME] scenarios. An assumable daily total PFOA exposure via non-food articles for adults was estimated at 0.09 ng/kg BW (MTE). The maximum uptake of PFOA was estimated at 3.1 ng/kg BW (RME). According to this assessment, the exposure would drop by one or two orders of magnitude upon reaching adulthood because of the low frequency of hand-to-mouth transfer (European FSA, 2008; Washburn *et al.*, 2005).

1.2.2.2. Exposure via indoor and outdoor air

Based on studies in Japan (Moriwaki *et al.*, 2003) and Canada (Kubwabo *et al.*, 2005), the EFSA determined the lifetime average daily dose [LADD] via ingestion, inhalation, and skin contact with contaminated house dust in interior rooms. These calculations by the EFSA are based on mean PFC concentrations of 0.440 ng PFOS/kg and 0.380 ng PFOA/kg in house dust. The exposure to PFOS and PFOA through inhalation was estimated at 0.022 ng/m^3 and 0.019 ng/m^3, respectively (European FSA, 2008). In a recent study by Kato et al. (2009), 39 samples of house dust that had been collected in diverse countries worldwide in 2004 were tested for concentrations of 17 PFCs. Six of the compounds were detected in 70% of the samples tested. The highest mean values measured were for PFOS, PFBS, PFHxS, perfluorooctanesulfonamide ethanol [FOSE], 2-(*N*-ethyl-perfluorooctanesulfonamido) acetic acid (Et-PFOSA-AcOH), and 2-(*N*-Methyl-perfluorooctanesulfonamide) ethanol [Me-FOSE] (Kato *et al.*, 2009).

Data have been published on the inhalation exposure to PFOS and PFOA for Norway, the UK, Japan, and North America. As a result of the large variability of the PFC concentrations in outdoor air, the EFSA calculated LADD values for 'high' and for 'low' PFC exposures via inhalation of outdoor air. Consequently, the uptake of PFOS and/or PFOA from outdoor air, even assuming a high concentration of PFCs, amounts to less than 0.5% or 17%, respectively, of the contamination via indoor air and, in comparison to dietary uptake, would therefore appear to be negligible (European FSA, 2008).

Fromme et al. (2009) summarized human exposure to PFCs via outdoor and indoor air in western countries. A comparison of the various PFCs in outdoor air shows that the levels of FOSE or FOSA, PFOS, and PFOA concentrations decrease according to the sequence city, country, and outlying regions. Furthermore, there appears to be a north-south gradient since the maximum 8:2 FTOH concentrations were 0.19 ng/m^3 in the northern hemisphere and 0.014 ng/m^3 in the southern hemisphere. In addition, it must be assumed that there are seasonal variations in PFOS and PFOA concentrations in outdoor air. Samples taken in the spring contained higher concentrations of PFCs than samples from the winter (Fromme *et al.*, 2009).

1.2.3. Total exposure

The calculated total exposure according to the data of the EFSA (2008) and Fromme et al. (2009) are of the same order of magnitude for PFOA. For PFOS, the total exposure derived from the data of the EFSA (2008) is significantly higher than the result obtained using the data from Fromme et al. (2009). This resulted from the higher values for dietary exposure according to the EFSA (2008). According to this assessment, exposure via drinking water and outdoor air appear to be insignificant, barring special sources of contamination.

Fromme et al. (2007) initiated a study, the Integrated Exposure Assessment Survey [INES] in which PFC concentrations in foods, indoor air, and house dust were correlated with concentrations in blood. The blood concentrations of the 48 INES participants varied between 4.9 to 55.0 µg/L for PFOS and 2.7 to 19.1 µg/L for PFOA. Further details have not yet been published since the study is ongoing.

Zhang et al. (2010) took a different approach. The daily uptake, calculated from blood concentrations using a one-compartment model, was found to agree closely with the daily PFOS uptake via food and house dust (0.74 vs. 1.19 ng/kg BW for men and 1.2 vs. 1.15 ng/kg BW for women) (Zhang *et al.*, 2010).

1.2.4. Pre- and postnatal exposures

PFC exposure of the fetus (prenatal) and nursing infants (postnatal) has also been shown in studies of mother-child pairs.

1.2.4.1. Prenatal exposure

PFOS was detected in cord blood samples in studies from Northern Canada, Germany, Japan, the USA, Canada, and Denmark (Tittlemier *et al.*, 2006; Midash *et al.*, 2007; Inoue *et al.*, 2004; Apelberg *et al.*, 2007; Monroy *et al.*, 2008; DFei *et al.*, 2007) . This also applies to PFOA, with the exception of the Japanese study (Inoue *et al.*, 2004). Thus, PFCs are considered to cross the placental barrier. This was also shown in animal studies (Hinderliter *et al.*, 2005).

In the northern Canadian study, the mean PFOS- and PFOA-cord blood concentrations in humans were 17 µg/L and 3.4 µg/L, respectively. In the other studies, the values were from 3 to 7 µg/L for PFOS and 1.6 to 3.4 µg/L for PFOA. In the German study, PFOS concentrations in cord blood were reported to be lower than the mother's blood by a factor of 0.6 (7.3 µg/L vs. 13 µg/L). By contrast, however, the PFOA concentrations were a factor of 1.26 higher in cord blood than in the mother's blood (3.4 µg/L vs. 2.6 µg/L) (Midash *et al.*, 2007).

Inoue et al. (2004) also compared PFOS concentrations in the mother's blood with the cord blood of the fetus. The concentration in the maternal blood varied from 4.9 to 17.6 µg/L, whereas the cord blood concentration had a PFOS level of 1.6 to 5.3 µg/L. A strong correlation was found between the PFOS concentration in the mother's blood and in cord blood ($r2 = 0.876$). In this study, PFOA was only found in the mother's blood (Inoue *et al.*, 2004).

Monroy et al. (2008) also made comparative measurements of PFC concentrations in mother's blood ($n = 101$) in the 24th to 28th week of gestation and at the time of birth as well as in cord blood ($n = 105$). These authors established higher PFOS concentrations in the mother's blood during pregnancy than at the time of birth. PFOS concentrations in cord blood were lower than those in the mother's blood samples.

Fei et al. (2007) also examined PFOS and PFOA concentrations in the blood of women during the first trimester (n = 1, 400) and during the second trimester (n = 200) of pregnancy. They also analyzed cord blood (n = 50) after birth.

1.2.4.2. Postnatal exposure

The presence of PFOS and PFOA in human breast milk was demonstrated in studies from Sweden (Kärman *et al.*, 2007) and China (So *et al.*, 2006), among others. The PFC concentrations measured in these studies were similar. In another study by Völkel et al. (2007), PFOS and PFOA concentrations were also determined in 57 human milk samples from Germany and 13 samples from Hungary. The PFOA concentrations measured in this study (0.201 to 0.46 µg/L) were similar to those reported by So et al. (2006) and Kärrman et al. (2007). Only 11 PFOA values were greater than the LOD of 0.2 µg/L. In the Swedish study, the same problem emerged, whereby only one sample contained concentrations greater than the blank level of 0.209 µg/L.

In 24 pooled samples of human milk (1, 237 individual samples) obtained in the year 2007 from 12 provinces of China, Liu et al. (2010) measured PFOS concentrations of 0.049 µg/L (mean) and for PFOA, 0.035 µg/L. The concentrations of PFCs varied greatly between different geographic regions. High concentrations of PFOA were measured in Shanghai (0.814 µg/L in rural areas and 0.616 µg/L in urban areas) (Liu *et al.*, 2010).

Using the data from the Swedish study, for example, an infant who weighs 5 kg and drinks 800 mL human milk per day would have a daily uptake of 0.048 to 0.38 µg PFOS and 0.17 to 0.39 µg PFOA (European FSA, 2008). If the data from Shanghai are used, the infant would ingest more PFOA (consumed volume = 742 mL/day, BW = 6 kg) amounting to 0.088 µg/kg BW (Liu *et al.*, 2010), thereby nearly reaching the TDI of 0.1 µg/kg BW/day recommended by the German Drinking Water Commission.

It can be seen in the study by Kärrman et al. (2007) that the mean PFOS concentration of 0.201 µg/L in human milk is correlated with the serum PFOS concentration of 20.7 µg/L (r^2 = 0.7), reaching a level

of about 1% of the serum concentration. A similar and even stronger correlation ($r^2 = 0.8$) was also determined for PFHxS (milk 0.085 µg/L, serum 4.7 µg/L). The total concentration of PFCs was 32 µg/L in serum and 0.34 µg/L in milk. The authors calculated a PFC uptake of about 0.2 µg/day for infants. The PFOS and/or PFHxS concentrations in human milk samples that had been obtained between 1996 and 2004 showed little variation throughout that time period, providing no evidence of a possible temporal trend (Kärman *et al.*, 2007).

Tao et al. (2008) analyzed PFC concentrations in human milk samples from various Asian countries. The PFOS concentration varied between 0.039 µg/L in India and 0.196 µg/L in Japan. The mean PFHxS concentrations ranged from 0.006 µg/L (Malaysia) to 0.016 µg/L (Philippines). The mean PFOA concentration in Japan was 0.078 µg/L. In addition, the average PFC uptake of nursing infants from seven Asian countries was compared to the dietary uptake values from adults in Germany, Canada, and Spain. The PFOS uptake of nursing infants (11.8 ± 10.6 ng/kg BW/day) was 7 to 12 times higher, and the PFOA uptake (9.6 ± 4.9 ng/kg BW/day) was 3 to 10 time higher than the dietary exposure of adults to these substances (Tao *et al.*, 2008).

Llorca et al. (2010) also analyzed human milk samples for PFC contamination. The milk samples, from donors living in Barcelona, Spain, were all from at least 40 days after birth. PFOS and perfluoro-7-methyloctanoic acid were detected in 95% of all samples. Concentrations of 0.021 to 0.907 µg/L PFOA were measured in 8 out of 20 human milk samples. According to this study, infants ingest 0.3 µg PFCs/day while nursing (Llorca *et al.*, 2010).

According to the results of these studies, nursing contributes to PFC exposure of infants. The mechanism by which these compounds pass from the mother's blood to the milk is not fully understood. Bonding to proteins would appear likely (Fromme *et al.*, 2009; Jones *et al.*, 2003).

PFC contaminations of infant formulas were examined in two studies. Tao et al. (2008) detected PFC concentrations above the LOD in only a few cases. Llorca et al. (2010) found six PFCs in all baby formulas of various brands as well as in baby cereals. Elevated concentrations (as high as 1.29 µg/kg) of

perfluorodecanoic acid [PFDA], PFOS, PFOA, and perfluor-7-methyloctanoic acid were detected. Contamination of baby food is likely the result of migration of the compounds from the packaging or containers used during production (Llorca *et al.*, 2010).

1.2.5. Human internal contamination

Taves (1968) and Shen & Taves (1974) were the first to show the presence of organic fluorides in human blood. Until the 1990s, however, the presence of these compounds was not considered of importance. Only since 1993 have PFC concentrations in the serum of exposed workers been the subject of study. The PFOS concentrations in the serum were found to be between 1, 000 and 2, 000 μg/L. Data on serum concentrations in the general population have only been available since 1998. These values were approximately 100 times lower than in occupationally exposed workers (European FSA, 2008; Olsen *et al.*, 2008; Olsen *et al.*, 2003.

The plasma to serum ratio for PFHxS, PFOS, and PFOA is 1:1, independent of the concentration, whereas the ratio of serum or plasma to whole blood was stated to be 2:1. This indicates that the PFC concentration in whole blood is only 50% of the concentration in plasma and/or serum. The difference is the result of the distribution volume of red blood cells in the samples since fluorochemicals are neither found intracellularly nor bound to the red blood cells (Ehresman *et al.*, 2007).

Kannan et al. (2004) examined 473 blood/serum/plasma samples from people of various countries. Of the four PFCs measured (PFOS, PFHxS, PFOA, FOSA), PFOS was quantitatively the dominant component in blood. The highest PFOS concentrations were detected in samples from the USA and Poland (>30 μg/L). In Korea, Belgium, Malaysia, Brazil, Italy, and Colombia, blood PFOS concentrations were in the range of 3 to 29 μg/L. The lowest PFOS concentrations were measured in samples from India (< 3 μg/L). In this study, the PFOA concentrations were lower than the values for PFOS, except in India and Korea. The joint occurrence of the four PFCs varied according to the country

of origin of the samples. This suggests differences in the exposure pattern in the individual countries (Kannan *et al.*, 2004).

Kärrman et al. (2007) measured plasma PFOS concentrations from residents of Australia, Sweden, and the UK with levels of 23.4 μg/L, 33.4 μg/L, and 14.2 μg/L, respectively. Ericson et al. [73] determined average values of 7.64 μg PFOS/L and 1.8 μg PFOA/L in blood samples from the Spanish population (European FSA, 2008).

Calafat et al. (2007), within the framework of the National Health and Nutrition Examination Surveys [NHANES] from 1999 to 2000, also examined serum samples from the US population for concentrations of 11 different PFCs. The group of 1, 562 participants in the study was made up of male and female subjects, three ethnic groups, and four age categories (12 to 19 years, 20 to 39 years, 40 to 59 years, 60 years and older). Consequently, these data are representative of the exposure of the US population to PFCs. PFOS, PFOA, PFHxS, and FOSA were detected in all serum samples (Kärrman *et al.*, 2007).

Wilhelm et al. (2009) took three biomonitoring studies as a basis to arrive at a reference value for PFOA and PFOS in the blood plasma of the general population in Germany. Two studies were carried out in southern Germany (Fromme et al., 2007; Kärrman *et al.*, 2006) and one in North Rhine Westphalia (Hölzer *et al.*, 2008). Although these studies are not representative of the general population of Germany, they present the best basis for deriving a reference value for internal contamination with PFOS and PFOA. Based on the 95th percentile, the following reference values were suggested: for PFOA, 10 μg/L for all groups and for PFOS, 10 μg/L for children of school age, 15 μg/L for adult women, and 25 μg/L for men (Wilhelm *et al.*, 2009).

The mean PFOA concentration in the blood for the European population is within the region of 4 to 20 μg/L; their mean PFOS serum concentration is within the range of 4 μg/L (Italy) and 55 μg/L (Poland). PFOS is the quantitatively dominant component of PFCs in all of the blood samples measured

worldwide. In general, PFOA concentrations in serum are lower than concentrations of PFOS (European FSA, 2008).

Olsen et al. (2003) determined the PFOS concentrations in serum to be 6.1 to 58.3 µg/L and in human liver, 4.5-57 µg/kg (n = 31). The mean liver to serum ratio for PFOS concentration was 1.3:1. Liver to serum ratios could not be established for PFOA, PFHxS, and FOSA because 90% of the concentrations of these substances were below the LOD (Olsen *et al.*, 2003).

Kärmann et al. (2006) analyzed blood samples from 66 Swedish study participants. Concentrations of 12 PFCs were determined (PFBS, PFHxS, PFOS, perfluorooctanesulfonamido acid, FOSA, PFHxA, PFOA, perfluorononanoic acid [PFNA], PFDA, perfluoroundecanoic acid [PFUnA], perfluorododecanoic acid [PFDoA], perfluorotetradecanoic acid [PFTDA]) along with the concentrations of other 'traditional' persistent organic pollutants [POPs]. The mean concentrations of PFCs in whole blood were 20 to 50 times higher than the total concentrations of polychlorinated biphenyls [PCB] and p, p'-dichlorodiphenyldichloroethylene. Similarly, the PFC concentrations were 300 to 450 times greater than for hexachlorbenzene and the sum of the six chlordanes and the three polybrominated diphenyl ethers. However, the PFCs and the POP that were measured behaved differently in regard to their distribution in the body, making an additional comparison of total body contamination necessary. PFCs are mainly found in the blood and the liver, whereas polychlorinated and polybrominated POPs are chiefly present in the fat tissue and blood lipids. The reason for these differences appears to be related to the different basic structures and the binding behavior in blood of these substances (D'Eon *et al.*, 2010; Bischel *et al.*, 2010; Chen *et al.*, 2009). Whole blood contains about 0.5% blood lipids, and thus represents only a small part of the total body contamination of PCB for example. The total body contamination was calculated using the proportionate weights of the main distribution tissues. This analysis showed a similar total body contamination for PFCs and for the POP that had been analyzed to be about 1.6 mg PFOS and 1.7 mg for PCB153, one of the most abundant individual PCB congeners (Kärrman *et al.*, 2007).

1.2.6. Gender and age-dependent differences

No correlation between the PFOS concentration and age or gender were found in studies by Olsen et al. (2003) on US citizens or in the studies by Kannan et al. (2004). Data of Calafat et al. (2007; 2006) show significantly higher PFOS and PFOA concentrations in men than in women; however, an age-related difference was not found. Harada et al. (2004) reported higher PFC serum concentrations in Japanese men than in women, and in addition, they also reported a rise in PFC serum concentrations in women with increasing age so that by age 60, the concentrations in women were comparable to those in men. The situation was similar for PFOA (Harada *et al.*, 2004).

Kärrman et al. (2006) determined a rise in PFOS serum concentrations with increasing age. PFOS, PFOA, and PFHxS concentrations in blood were also higher in men than in women. Ericson et al. (2007) confirmed higher PFHxS and PFOA concentrations in blood of male subjects. Concentrations were significantly different between age groups 25 $\pm$ 5 years (18 participants) and 55 $\pm$ 5 years (30 participants) only for PFHxS and FOSA ($p < 0.05$ and $p < 0.001$, respectively). The group of younger participants (25 $\pm$ 5 years) presented higher PFHxS values and lower FOSA values than did the older participants (Ericson *et al.*, 2007).

Rylander et al. (2010) 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 (Zhang *et al.*, 2010).

Fromme et al. (2007) 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 (Fromme *et al.* 2007). 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 (Hölzer *et al.*, 2008). 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 (Olsen *et al.*, 2005).

According to the EFSA (European FSA, 2008), 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. (2009) 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 (European FSA, 2008) 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, some research groups (Liu J *et al.*, 2010; Liu C *et al., 2010; Liu W et al., 2010)* 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.

1.2.7. 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. (2004) reported differences in the occurrence of PFOS and PFOA among blood donors in nine different countries. Harada et al. (2004) 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 (Midasch *et al.*,2006).

Fromme et al. (2009) came to the conclusion that serum concentrations of the US population are higher than those of inhabitants of Europe, Asia, or Australia.

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 (Rylander et al., 2010a). 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 (Rylander et al., 2010b).

Kärrman et al. (2006) 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 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 (Hanssen *et al.*, 2010).

Hemat et al. (2010) 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 study of Kannan et al. (2004) 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 (Calafat *et al.*, 2005) 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 (European FSA, 2008).

1.3. Toxicology

1.3.1. Toxicokinetics

1.3.1.1. Uptake

Data from animal experiments show that PFC uptake can occur by oral, inhalation, or dermal exposure (Gibson & Johson, 1979; Johson *et al.*, 1979; Kennedy, 1986; Kudo & Kawashima, 2003; Fasano *et al.*, 2006).

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 (1979) and were determined using 14C-labeled PFOS and PFOA (BfR, 2008).

After 10 inhalations of 84 mg/m3 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 (Kennedy, 1986).

Uptake via dermal exposition appears to be somewhat weaker (Kudo & Kawashima, 2003). A study by Kennedy (1986) 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/kgc in 0.5% methyl cellulose was applied. The 8:2 FTOH was labeled with 14C (3-14C 8:2 FTOH) and applied to the shaved area of skin (10 µL/cm2) (Fasano *et al.*, 2006).

1.3.1.2. Distribution

PFOS and PFOA are weakly lipophilic, very water soluble, and bind preferentially to proteins. The principle binding partner is albumin (Völkel *et al.*, 2007; Han *et al.*, 2003); however, it also binds to β-lipoproteins or fatty acid binding proteins in the liver [L-FABP] (Lübker *et al., 2002).*

Approximately 90% to 99% of the perfluoridated carboxylic acids in the blood are bound to serum albumin (Han *et al.*, 2003; Ylinen *et al.*, 1990. The chain length and the functional group of the PFCs have an influence on the preferential binding site and binding affinity (Chen & Guo, 2009). PFCs have the same binding site and a similar affinity to serum albumin as fatty acids (Chen & Guo, 2009).

Qin et al. (2010) 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 (Qin *et al.*, 2010). Bischel et al. (2010) 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. (2003) and Seacat et al. (2003), 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 (Yoo *et al.*, 2009) and Han et al. (2008) 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. (2007) 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. (2007) 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 (Kudo *et al.*, 2007).

Tan et al. (2008) 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 (Tan *et al.*, 2008).

In addition, Liu et al. (2009) 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 (Fasano *et al.*, 2006). 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 (Thibodeaux *et al.*, 2003). 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 (Hinderliter *et al.*, 2005; Lau *et al.*, 2007). Peng et al. (2010) 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 (Martin *et al.*, 2005; Kudo *et al.*, 2005). 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 (Henderson & Smith, 2007).

1.3.1.3. 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 (Kudo & Kawashima, 2003). According to Fromme et al. (2006), only FTOH comes into question regarding metabolism.

For example, Fasano et al. (2006) 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 (Martin et al., 2005; BfR, 2005; Henderson & Smith, 2007). 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. (2010) 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 (Butt *et al.*, 2010).

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. (2009). 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 (Martin *et al.*, 2009).

1.3.1.4. 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 (BfR,2008).

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 (Lau *et al.*,2007).

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 (Kudo *et al.*, 2002). 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 (Van den Heuvel *et al.*, 1991).

Weaver et al. (2010) 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. (1984) 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 (Johnson *et al.*, 1984).

Cui et al. (2010) 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 (Cui *et al.*,2010).

In experiments on chickens, Yoo et al. (2009) 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.

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. Urine was the principle path of excretion for PFOA in monkeys (Butenhoff *et al.*, 2004).

In contrast, the half-life of PFOA in Japanese macaques is notably shorter (2.7 to 5.6 days) (Harada *et al.*, 2005). A half-life of 110 to 130 days was determined for nonhuman primates after a single, intravenous application (COT, 2006).

The elimination half-time for PFOS in male cynomolgus monkeys was found to be about 200 days (Seacat *et al.*, 2002). In addition to species-specific differences, the structure of the PFCs can also influence excretion.

Benskin et al. (2008) 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 (Benskin *et al.*, 2008).

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 (De Silva *et al.*, 2008).

Olsen et al. (2009) 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. 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 (Olsen *et al.*, 2009).

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 (Olsen *et al.*, 2009).

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 (Harada *et al.*, 2005).

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 (Harada *et al.*, 2005). 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 (Hundley *et al.*, 2006). Protein binding and the formation of transporters are decisive factors in the distribution and excretion of PFCs (Lau *et al.*, 2007).

1.3.2. Toxicodynamics

1.3.2.1. 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 (European EFS, 2008). 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. (1986) 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 (1995) determined a dermal LC_{50} of 2, 000 mg PFOA/kg BW in rabbits. Rats and rabbits were tested in another study on the dermal toxicity of APFO by Kennedy (1985). 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 (Biesemeier & Harris, 1974). 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. (2008) 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 (Ji *et al.*, 2008).

1.3.2.2. 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 (COT, 2007). 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 (Christopher & Martin, 1977; Metrick & Marias, 1977; So *et al.*, 2007).

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 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 '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 (Perkins *et al.*, 2004).

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 (COT, 2006).

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 (Loveless *et al.*, 2006). These observations are in agreement with the conclusion drawn above that branched chain isomers are generally excreted more rapidly than the linear forms (Benskin *et al.*, 2008; De Silva *et al.*, 2008).

Seacat et al. (2003) 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 (Seacat *et al.*, 2003).

Curran et al. (2008) 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 (Curran *et al.*, 2008).

In a study on the subacute toxicity of PFCs in rats, Cui et al. (2009) 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 $\pm$ 37 mg/kg at a dosage of 5 mg/kg/day). The highest PFOS concentrations were 648 $\pm$ 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 (Cui *et al.*, 2009).

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 (Goldenthal *et al.*, 1978).

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 (Butenhoff *et al.*, 2002).

In a study by Seacat et al. (2002), 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 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 (Seacat *et al.*, 2002).

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 (Perkins et al., 2004).

Subacute toxic effects of PFC exposure were also observed in fish. Yang (2010) 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 (Langer *et al.*, 2011).

Fang et al. (2010) 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.

1.3.2.3. 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 (Thomford, 2002).

A study by Sibinski (1987) 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 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. 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 & Frame (2004), 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.

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 from the study of Sibinski (1987), they came to the conclusion that the incidence of mammary gland tumors was not changed by chronic exposure to APFO. Feeding female rats as much as 300 mg/kg APFO did not result in an increase in proliferative damage to breast tissue (Hardisty et al., 2010).

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 (Biegel *et al.*, 2001). 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 (Frame & McConnell, 2003).

Sibinski (1987) and Biegel et al. (2001) 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 (Abdellatif *et al.*, 1991; Nilsson *et al.*, 1991).

1.3.2.4. 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 (BfR, 2008).

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 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 (European FSA, 2008).

Murli et al. (1996) 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 (European FSA, 2008).

In the study by Yao & Zhong (2005), 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 (1991) also detected significantly increased 8-dG concentrations.

1.3.2.5. 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 (Lau et al., 2007; Lau et al., 2006; Lau et al., 2003).

For example, in a study by Lau et al. (2003), 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 (Lau et al., 2003).

In a two-generation study on rats, Lübker et al. (2005) 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 (Christian et al., 1999).

Unaltered fertility parameters were found in yet another two-generation study of PFOA-exposed rats by Buttenhoff (2004). 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 (BfR, 2008).

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. (2010) 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 (York et al., 2010).

Lau et al. (2006) 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 (Lau et al., 2006).

Grasty et al. (2003) 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 day 19 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. (2005) 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. Lau et al. (2007) 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 (Wolf et al., 2007).

In a cross-fostering study, Lübker et al. (2005) 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 (Lau et al., 2007).

Yu et al. (2009), 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. (2009) 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 (Liu et al., 2009).

Abbott et al. (2007) 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. 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 (Abbot et al., 2007). Abbott et al. (2009) 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 (Abbot et al., 2009).

The influences on reproduction by PFOS and PFOA are not limited to mammals but have, for example, also shown to affect chickens (Yanai et al., 2008; Penden-Adams et al., 2009; Molina et al., 2006), quail, mallard duck (Newsted et al., 2007), frogs, and fish (Ankley et al., 2004; Ankley et al., 2005).

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 (Lübker et al., 2005; Christian et al., 1999). 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 (Hinderliter et al., 2005). PFBS did not elicit a verifiable developmental effect in rats (Lau et al., 2007). 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 (Das et al., 2008). 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 (York, 2003). Perfluorodecanoic acid, like other PFCs, did not induce deformations and also did not elicit any other developmental toxic effects (Harris & Birnbaum, 1989).

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 (Feng et al., 2009).

1.3.2.6. Neurotoxicity

A study by Austin et al (2003) 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. (2006) 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 (Kovarova & Svobodova, 2008).

Slotkin et al. (2008) 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 (Slotkin et al., 2008).

In their study, Liao et al. (2009) 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 (Liao et al., 2009).

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 (Johansson et al., 2008). In a subsequent study on mice, Johansson et al. (2009) 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 (Löffler et al., 2007). Altered concentrations of these proteins could possibly explain the behavioral changes described above (Johansson et al., 2009).

According to the results of Sato et al. (2009), 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 (Sato et al., 2009).

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 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 (Liu et al., 2009).

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 (Liu et al., 2010).

Pinkas et al. (2010) 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 (Pinkas et al., 2010).

1.3.2.7. Effects on the endocrine system

The first reports of the effect of PFCs on thyroid hormones were from Langley & Pilcher (1985) and Gutshall et al. (2010).

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 (Lau et al., 2006).

Weiss et al. (2009) 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. (2009) 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. (2008) 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 (Kovarova & Svobodova, 2008).

Benninghoff et al. (2007) 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 (Lau et al., 2006).

Wei et al. (2014) 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. (2010) 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 (Zhao et al., 2010). Maras et al. (2006) 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. (2010) 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 (Liu et al., 2010).

It was shown in a study by Shi et al. (2007) 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 (Shi et al., 2009).

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.

1.3.2.8. Immunotoxicity

Yang et al. (2000; 2002a; 2002b) 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 (Yang et al., 2000).

Yang et al. (2002b) 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* (Yang et al., 2002b).

Fang et al. (2008) 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 (Fang et al., 2008).

Peden-Adams et al. (2008) 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 (Peden-Adams et al.,2008).

Keil et al. (2008) 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 (Keil et al., 2008).

Qazi et al. (2009) 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 (Qazi et al., 2009).

Guruge et al. (2009) 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).

DeWitt et al. (2009a) 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 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. (2009b) 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 (DeWitt et al., 2009a).

1.3.2.9. 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 (Lau et al., 2007). 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 (Kudo et al., 2002). 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 (Yang et al., 2002; Fang et al., 2008).

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 (Hu et al., 2009).

In the study by Eriksen et al. (2010) 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 (Eriksen et al., 2010).

Qian et al. (2010) 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 (Qian et al., 2010; Vanden Heuvel et al., 2006) and led to peroxisome proliferation, as had been previously shown only in studies on rodents (Seacat et al., 2003; Ikead et al., 1985; Sohlenius et al., 1992). The hepatotoxic effects of PFOA in studies on rodents may also have resulted from the activation of peroxisome proliferation (Ikead et al., 1985; Sohlenius et al., 1992; Pastoor et al., 1987). 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 (Perkins et al., 2004). The activation of PPARα leads to the expression of genes that are involved in lipid metabolism, energy homeostasis, cell differentiation, and peroxisome proliferation (Shipley et al., 2004). 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. (2006). 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 (Vanden Heuvel et al., 2006).

Shipley et al. (2004) 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 (1999), 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 (Takacs & Abbott, 2007). In studies using transgenic mice, Nakamura et al. (2009) 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. (2002) 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* (Lau et al., 2006). 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 (Seacat et al.m, 2002).

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 (Haughom & Spydevold, 1992). 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 (Martin et al., 2007).

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 (Butenhoff et al., 2002). 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 (Martin et al., 2007). 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 (Martin et al., 2007; Yin et al., 2005; Guruge et al., 2006; Rosen et al., 2007). 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 (Peters et al., 1997).

In the study by Minata et al. (2010), 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 (Minata et al., 2010).

A further study by Elcombe et al. (2007) 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] (Elcombe et al., 2007). Ren et al. (2009) 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 (Hu et al., 2002). 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. (2009) 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 (Upham et al., 2009). The meaning of this widespread and reversible mechanism in carcinogenesis of PFOS or PFOA is, however, still unclear. 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 (Ehresman et al., 2007; Kudo et al., 2006; Permadi et al., 1993).

1.3.2.10. Combination effects

Wei et al. (2009) 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 (Wei et al., 2009). 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 (Hu et al., 2009).

Using a micronuclear test, Jernbro et al. (2007) 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 (Jernbro et al., 2007).

Watanabe et al. (2009) 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 (Watanabe et al., 2009).

1.3.3. 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 (Lau et al., 2007).

1.3.3.1. 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 (Alexander et al., 2003).

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 (Alexander et al., 2007).

Grice et al. (2007) 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. (2003), encompassed 2, 083 workers of a POSF-processing factory in Decatur (Grice et al., 2007).

The first retrospective cohort study on mortality of employees of the PFOA-producing factory of 3 M was carried out by Gilliland and Mandel (1993). 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 (Gilliland & Mandel, 1993).

An update of this study was undertaken by Alexander (2001) 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 (European FSA, 2008).

A recent epidemiological study was undertaken by Lundin et al. (2009). 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 (1993) 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 (Lundin et al., 2009).

Studies by DuPont (2006) 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 (Lau et al., 2007). 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 (DuPont, 2006). No significant liver toxicity was found in a further study by Gilliland and Mandel (1996) 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 (Gilliland & Mandel, 1996).

Olsen et al. (1999) 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 (Olsen et al., 1999).

One year later, a study was published by Olsen et al. (2000) 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 (Olsen et al., 2000).

Olsen et al. (2003) 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 (Olsen et al., 2003).

Possible weak points in the study of Olsen et al. (2003) were taken into consideration in a following study by Olsen and Zobel (2007). 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 (Olsen et al, 2007).

Olsen et al. (1998) 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 (Olsen et al., 1998).

In regard to blood lipids, liver enzymes, and occupational exposure to APFO, Sakr et al. (2007) 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. (2009) 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 (Sakr et al., 2009).

Costa et al. (2009) 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. (2007). This is suggestive of an effect of PFOA on purine metabolism in the liver (Costa et al., 2009).

Very few epidemiological studies exist with data from the general population. In a study by Bloom et al. (2010), 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. (2009) 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. (2010) 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 (Melzer et al., 2010).

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 (Emmett et al., 2006).

Nelson et al. (2010) 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. (2009) 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 (Steenland et al., 2010).

Anderson-Mahoney et al. (2008) examined 566 persons who also had been exposed to PFOA via drinking water. 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 (Lin et al., 2008).

Additionally, Lin et al. (2009) 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 (Lin et al.,2009).

McNeil et al. (2009) 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 (Leonard et al., 2008). 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.

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 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 (MacNeil et al., 2009).

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 (2008), 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 (BfR, 2008). 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.

1.3.3.2. Studies on reproductive and developmental toxicity

Grice et al. (2007), 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. (2004). 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 (Inoue et al., 2004).

In the study by Fei et al. (2007), 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 (Fei et al., 2007).

Monroy et al. (2008) 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 (Monroy et al., 2008).

On the other hand, in a cross-sectional study, Apelberg et al. (2007) 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 (Apelberg et al., 2007). Washino et al. (2009) 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 found. Hamm et al. (2009) 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. (2009) 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 (Nolan et al., 2009). 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 (Nolan et al., 2010).

Stein et al. (2009) 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 (Stein et al., 2009).

Fei et al. (2008) 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 (Fei et al., 2008).

A further study by Fei et al. (2009) 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 (Fei et al., 2008).

Since PFOA was seen to cause impairment of lactation in mice, Fei et al. (2010) 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. (2010) 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.

Olsen et al. (2009) published a review article on epidemiological studies that dealt with the influence of PFCs 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 (Olsen et al., 2009).

Steenland et al. (2010) 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 (Steenland et al., 2010).

2. Material and Methods

2.1. Study population

This study involved 175 subjects. 100 healthly adult, resident in Catania city, were included in this biomonitoring in a framework of regular occupational medical visits in 2016 by random sampling.

Still throughout 2016, 75 children (6 to 11 years of age) living in the city of Catania were contacted randomly by a primary care pediatrician after request to parents.

All subject included were given a general medical/pediatric visit and blood and urine routinary tests. Inclusion criterion was being in good health and not suffering from acute or chronic diseases at the time of collection.

The study was carried out in accordance with the guidelines of the Declaration of Helsinki and the procedures were approved by the ethical board of the University Hospital of Catania (Italy) (N.771/2014). The subjects/parents signed an informed consent to participate in the study.

A questionnaire was administred to partecipants to obtain information on anthropometric, familiar features, occupational exposure and socio-demographic characteristics. For each child, gender, age, height, weight, maternal productive age and maternal parity were recorded.

2.2. Sampling and laboratory analysis

Venous blood (6 mL) was collected from all subjects in the morning following an overnight fasting using test tubes for the serum (Vacuette, Greiner Bio-One, Kremsmünster, Austria). After the collection, the serum tubes serum were left in an upright position for at least 30 min at room temperature but no longer than 60 min. These tubes were then centrifuged at 3500 rpm for 10 min, and then the serum was isolated. Serum was divided in two aliquots in test tubes that guaranteed the non-release of PFCs and stored at -20°C until analysis.

Samples were analyzed for 16 PFCs: perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), perfluoroheptanesulfonate (PFHpS), PFOS, perfluorodecanesulfonate (PFDS).

Reference materials were purchased from Wellington Laboratories Inc. (Ontario, Canada) and Sigma-Aldrich (St.Louis, MO, USA).

Quality assurance was assured using isotope-labeled standards (ISs) perfluoro-n-(1,2,3,4-$^{13}C_4$) octanoic acid ($^{13}C_4$-PFOA) and perfluoro-n-(1,2,3,4--$^{13}C_4$) octane sulfonate($^{13}C_4$-PFOS) -obtained from Restek Corporation (Bellefonte, PA, USA). ISs were prepared and aliquots of both were stored at 4 °C before use. The identity of the PFCs was confirmed by matching their retention time with the peak produced by the coeluting ISs .

Calibration curves were performed with seven points ranging from 0.1 to 50 ng/mL for 16 PFCs.

A solid-phase extraction has preceded high-performance liquid chromatography-isotope dilution tandem mass spectrometry determination as reported by Lien et al. (2011). Briefly, 500 µL of all serum samples and spiked with 40 µL of 0.1 µg/mL ISs and mixed using vortex for 30s; after 1.5 mL of acetonitrile (Merck Chemicals, Darmstadt, Germany) and 1 mL of 2% formic acid (Merck Chemicals, Darmstadt, Germany) were added and vortexed for 30s and finally the mixture was centrifuged at 4000 rpm for 10 min. The samples were extracted by Oasis WAX SPE cartridge (200mg, 6 cm^3 and preconditioned with 4 mL of 2% ammonia in a 1:9 v:v of methanol and MTBE and 4 mL of 2% formic acid) provided by Waters Corporation (Milford, MA, USA). SPE cartridges were eluted with 8 mL of 2%ammonia (Merck Chemicals, Darmstadt, Germany) in 1:9 v:v mixture of methanol (Merck Chemicals, Darmstadt, Germany) and MTBE (Merck Chemicals, Darmstadt, Germany) and after concentrated until about 500 µL using nitrogen stream and transferred to an auto-sampler vial.

A replicate sample was extracted in each batch (10 samples), for internal quality purpose.

The extract was analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS, Agilent 1200 HPLC system with 6460 electro-spray triple quadrupole mass spectrometry). The analytical separation column was an Accucore C18 (2.6 μm, 100×4.6 mm; Thermo Scientific, Pennsylvania, USA) solid core preceded in line with a guard column (2.6 μm, 10×4.6 mm) at 40 °C. The injection volume was 5 μL for all experiments. The mobile phase consisted of methanol (Merck Chemicals, Darmstadt, Germany) and an aqueous solution containing 5 mM ammonium acetate (Merck Chemicals, Darmstadt, Germany) (solvent B). The elution was performed at a flow rate of 0.8 mL/min using the following gradient program 0-1.5 min, 70% B; 1.5-2.5min, 30% B; 2.5-4.5min, 10% B; 4.5-6.5min, 10% B; then back to 70% B for 0.5 min and re-equilibrated at 70% B for 3min.

The ESI source was operated in negative ion mode using a multiple reaction monitoring scan with a spray voltage of -3.5 kV, a source temperature of 650 °C, a curtain gas 30 psi, ion source gas 1 at 50 psi and gas 2 at 60 psi.

The calibration curve linearity coefficients (R^2) were >0.998 for all the PFCs.

In addition to the calibration standards, blanks and quality control were analyzed along with each batch of samples to ensure the data accuracy and reliability.

All chemical and organic solvent used were for LC-MS analysis or at the highest level of purity.

Also limits of detection (LOD) and limits of quantification (LOQ) were determined. LOD was defined as the concentration giving a signal to noise ratio of 3:1; LOQ was set as concentrations giving a peak with a signal-to-noise ratio of 9:1.

In table 1, the method characteristics are summarized.

Table 1. Analytes' characteristics and limit of quantification and detection for the LC-MS/MS analysis.

Compound	Chain length	Retention time (min)	Internal standard	Transitions (*m/z*)	Declustering potential (V)	Collision energy (eV)	Quantification limit (ng/mL)	Detection limit (ng/mL)
PFBA	C4	3.07	$^{13}C_4$-PFOA	212.9 →168.9	29.34	10.79	0.1	0.126
PFPeA	C5	4.03	$^{13}C_4$-PFOA	262.6 → 18.7	32.28	12.48	0.1	0.097
PFHxA	C6	4.32	$^{13}C_4$-PFOA	312.4 → 268.8	29.35	12.48	0.2	0.219
PFHpA	C7	4.54	$^{13}C_4$-PFOA	362.5 → 319.0	14.19	13.91	0.1	0.167
PFOA	C8	4.83	$^{13}C_4$-PFOA	412.4 → 368.9	10.80	14.93	0.3	0.374
PFNA	C9	5.21	$^{13}C_4$-PFOA	462.4 → 418.8	8.99	14.73	0.1	0.194
PFDA	C10	6.04	$^{13}C_4$-PFOA	512.3 → 468.9	17.12	14.50	0.2	0.296
PFUnDA	C11	6.11	$^{13}C_4$-PFOA	562.6 → 519.0	46.20	16.35	0.1	0.087
PFDoDA	C12	6.56	$^{13}C_4$-PFOA	612.6 → 568.9	58.90	17.67	0.1	0.178
PFTrDA	C13	6.99	$^{13}C_4$-PFOA	662.6 → 618.8	50.37	17.89	0.1	0.136
PFTeDA	C14	7.38	$^{13}C_4$-PFOA	712.9 → 668.8	56.99	17.02	0.2	0.224
PFBS	C4	4.07	$^{13}C_4$-PFOA	298.4 → 79.7	34.99	49.07	0.2	0.207
PFHxS	C6	4.54	$^{13}C_4$-PFOS	398.3 → 79.8	51.67	78.85	0.1	0.167
PFHpS	C7	6.23	$^{13}C_4$-PFOS	449.0 → 79.3	21.37	74.12	0.1	0.142
PFOS	C8	5.19	$^{13}C_4$-PFOS	498.5 → 79.7	68.11	92.21	0.2	0.236
PFDS	C10	6.47	$^{13}C_4$-PFOS	598.9 → 79.5	26.70	94.47	0.2	0.284

2.3. Statistical analysis

SPSS software was used for the statistical analysis (Ver. 24.0; IBM, USA). Descriptive analysis of participants' characteristics was used to estimate the mean and standard deviation or frequency and percentage. PFCs concentrations were described using frequency and percentage for detention frequency and median and the quartiles for the analyte concentration. Normality was checked by Kolmogorov-Smirnov test. The Spearmen's rank correlation analysis was used to assess the correlation between PFCs concentrations which were compared through Mann-Whitney test.

Finally, a multivariate linear regression approach was used to investigate the associations between PFCs levels and BMI, maternal productive age, maternal parity and breastfeeding duration, with estimated beta coefficients (p-values). The models were built for each PFC, using the backward elimination procedure. The cut-off level of p for entering the models were 0.10. The goodness of fit of the models was estimated using the R^2. The significance level was set at $p<0.05$.

3. Results

3.1. Adult

Of the 100 (100%) subject invited, 100 (100%) they agreed to participate in the study.

The characteristics of partecipants (sex, age, BMI) together with anthropometric, familiar features, occupational exposure and socio-demographic characteristics are shown in table 2.

Table 2. Adult's main characteristics.

No.	100 (100%)
Gender	
Males	50 (50%)
Female	50 (50%)
Age (years)	41.42 ± 5.53
Body Mass Index	23.86 ± 1.87
Consume of bottled water	100 (100%)
n. child	
0	34 (34%)
1	30 (30%)
2	27 (27%)
3	9 (9%)
>3	0 (0%)
Age first child	34.67 ± 5.02
Lactation duration last child (months)	7.38 ± 5.56
Oscillation weight last year	0.15 ± 5.42
Occupational exposure to PFCs	16 (16%)

Subjects were given a general medical visit and blood and urine routinary tests. Inclusion criterion was being in good health and not suffering from acute or chronic diseases at the time of collection.

All of 100 (100%) adult under exam were used to assuming bottle water instead of tap water in their homes.

In 66% (n=66) of cases had son and/or daughter, the average age of first child was 34.67 ± 5.02. For the female partecipants, the mean of last lactation duration was 7.38 ± 5.56 months. The average of oscillation weitht of subject involved in this study was 0.15 ± 5.42.

16 (16%) workers were exposed to PFCs.

The median concentration of all PFCs (∑PFCs) was 6.743 ng/mL (min-max:4.120-10.078 ng/mL). The concentrations of the 16 compounds are reported in table 3.

Table 3. PFCs concentrations (ng/mL) in 100 adult serum.

Analyte	Detection Frequency (%)	Min	P_{25}	Median	P_{75}	Maximum
PFBA	28 (28%)	<LOQ	<LOQ	<LOQ	<LOQ	0.214
PFPeA	22 (22%)	<LOQ	<LOQ	<LOQ	<LOQ	0.194
PFHxA	0 (0%)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFHpA	16 (16%)	<LOQ	<LOQ	<LOQ	<LOQ	0.241
PFOA	100 (100%)	0.423	0.998	1.546	1.946	2.941
PFNA	55 (55%)	<LOQ	<LOQ	0.266	0.465	0.841
PFDA	59 (59%)	<LOQ	<LOQ	0.301	0.366	0.514
PFUnDA	32 (32%)	<LOQ	<LOQ	<LOQ	0.103	0.170
PFDoDA	7 (7%)	<LOQ	<LOQ	<LOQ	<LOQ	0.248
PFTrDA	13 (13%)	<LOQ	<LOQ	<LOQ	<LOQ	0.182
PFTeDA	0 (0%)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFBS	6 (6%)	<LOQ	<LOQ	<LOQ	<LOQ	0.314
PFHxS	23 (23%)	<LOQ	<LOQ	<LOQ	<LOQ	0.628
PFHpS	14 (14%)	<LOQ	<LOQ	<LOQ	<LOQ	0.405
PFOS	100 (100%)	2.975	3.679	4.481	5.280	6.975
PFDS	0 (0%)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
∑PFCs	100 (100%)	4.120	5.922	6.742	7.903	10.078

PFOS and PFOA were detected and quantified in 100% (n=100) of samples. PFOS showed a median of 6.975 ng/mL (min-max: 2.975-5.280 ng/mL); whereas PFOA had a median of 2.941 ng/mL (range 0.423- 1.946 ng/mL).

In the same way, PFNA and PFDA were detected and quantified in 55% (n=55) and 59% (n=59) of samples, respectively.

Lower than 50% measure of the sample, the authors detected: PFBA (28%); PFPeA (22%); PFHpA (16%); PFUnDA (32%); PFDoDA (7%); PFTeDA (13%); PFBS (6%); PFHxS (23%); PFHpS (14%). Instead, PFHxA; PFTeDA; PFDS were not detected.

The serum concentrations of all PFCs within the subjects were statistically correlated (Table 4).

Table 4. Correlation of serum PFCs concentration.

	PFBA	PFPeA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFBS	PFHxS	PFHpS	PFOS
PFBA													
PFPeA	**0,481****												
PFHpA	**0.571****	**0.374****											
PFOA	0.017	0.052	0.129										
PFNA	-0.009	0.006	-0.046	0.055									
PFDA	0.021	0.102	-0.057	0.195	0.042								
PFUnDA	**0.483****	**0.289****	**0.364****	0.095	**0.210***	0.196							
PFDoDA	-0.012	-0.057	-0.025	-0.073	-0.055	-0.087	0.058						
PFTrDA	0.122	-0.007	**0.244***	**0.365****	0.117	0.165	**0.318****	0.141					
PFBS	-0.049	0.053	-0.015	0.128	0.082	-0.093	0.087	0.100	**0.247***				
PFHxS	0.0130	-0.011	0.095	0.115	0.020	0.052	0.110	0.166	0.135	-0.031			
PFHpS	0.147	0.110	0.046	0.000	0.155	-0.020	0.075	0.029	0.168	**0.326****	**0.256***		
PFOS	**0.232***	0.047	0.110	0.032	0.185	-0.043	0.163	-0.179	0.123	-0.080	-0.117	0.134	

*$p<0.05$; **$p<0.001$.

Moderate, directly proportioned correlations ($p<0.001$), were observed among: PFBA-PFPeA (r=0.481), PFBA-PFHpA (r=0.571), PFBA-PFUnDA (r=483), PFPeA-PFHpA (r=0.374), PFPeA-PFUnDA (r=0.289), PFHpA-PFUnDA (r=0.364), PFOA-PFTrDA (r=0.365), PFUnDA-PFTrDA (r=0.318), PFBS-PFHpS (r=0.326).

Slight, directly proportioned correlations ($p<0.05$), were observed among: PFBA-PFOS (r=0.232), PFHpA-PFTrDA (r=0.244), PFNA-PFUnDA (r=0.210), PFTrDA-PFBS (r=0.247), PFHxS-PFHpS (r=0.256).

3.2. Children

Of the 75 (100%) children invited, 14 (19%) did not partake in the study. In detail, 9 (64%) children were afraid to undergo blood test, in 5 (36%) cases parents gave no consent to blood testing.

The characteristics of study children (sex, age, BMI) together with information on lifestyle in relation to water use and consumption and maternal features are shown in table 5.

Table 5. Children's main characteristics.

No.	61 (100%)
Gender	
Males	32 (53%)
Female	29 (47%)
Age (years)	8.36 ± 1.64
Body Mass Index	19.72 ± 1.93
Consume of bottled water	61 (100%)
Maternal productive age (years)	
≤30	10 (16%)
31-39	45 (74%)
≥40	6 (10%)
Maternal parity	
1	30 (49%)
2	28 (46%)
3	3 (5%)
Maternal lactation duration (months)	6.85 ± 7.60

Children were given a general pediatric visit and blood and urine routinary tests. Inclusion criterion was being in good health and not suffering from acute or chronic diseases at the time of collection.

All of 61 (100%) children under exam were used to assuming bottle water instead of tap water in their homes.

In 74% (n=45) of cases, the average mothers' age ranged between 31 and 39 yrs, the overall mean was 34.3±4.3 yrs. In 49% (n=30) of cases, it was their first children, while in 46% (n=28) their second ones. In 92% (n=56) of cases, children had been breast fed for a period of 6.85 ± 7.60 months (range: 1 to 54 months). The median concentration of all PFCs (∑PFCs) was 5.888 ng/mL (min-max:1.035-12.94 ng/mL). The concentrations of the 16 compounds are reported in table 6.

Table 6. PFCs concentrations (ng/mL) in 61 children serum.

Analyte	Detection frequency n(%)	Min	P_{25}	Median	P_{75}	Maximum
PFBA	13 (22%)	<LOQ	<LOQ	<LOQ	<LOQ	0.219
PFPeA	2 (3%)	<LOQ	<LOQ	<LOQ	<LOQ	0.289
PFHxA	0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFHpA	5 (8%)	<LOQ	<LOQ	<LOQ	<LOQ	0.287
PFOA	52 (87%)	<LOQ	1.016	2.002	2.641	6.148
PFNA	24 (40%)	<LOQ	<LOQ	<LOQ	0.866	1.846
PFDA	43 (73%)	<LOQ	<LOQ	0.361	0.406	0.517
PFUnDA	26 (43%)	<LOQ	<LOQ	<LOQ	0.251	0.512
PFDoDA	0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFTrDA	18 (30%)	<LOQ	<LOQ	<LOQ	0.154	0.303
PFTeDA	0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFBS	0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFHxS	48 (80%)	<LOQ	0.205	0.419	0.582	0.974
PFHpS	30 (50%)	<LOQ	<LOQ	<LOQ	0.196	0.394
PFOS	61 (100%)	0.994	1.795	2.364	2.972	4.397
PFDS	0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
∑PFCs	61 (100%)	1.035	4.248	5.888	7.086	12.94

PFOS and PFOA were detected and quantified in 100% (n=61) and 87% (n=52) of samples, respectively. PFOS showed a median of 2.364 ng/mL (min-max: 0.994-4.397 ng/mL); whereas PFOA had a median of 2.002 ng/mL (range <LOQ-6.148 ng/mL).

In the same way, PFHxS and PFDA were detected and quantified in 80% (n=48) and 73% (n=43) of samples, respectively. PFHxS showed a median of 0.419 ng/mL (range <LOQ-0.974 ng/mL); whereas PFDA had a median of 0.361 ng/mL (range <LOQ-0.517 ng/mL).

In equal and/or lower than 50% measure of the sample, the authors detected: PFHpS (50%); PFUnDA(43%); PFNA (40%); PFTrDA(30%); PFBA (22%); PFPeA (3%); PFHpA(8%). Instead, PFHxA; PFDoDA; PFTeDA; PFBS; PFDS were not detected.

The serum concentrations of all PFCs within the children were statistically correlated (Table 7).

Table 7. Correlation of serum PFCs concentration.

	PFBA	PFPeA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFTrDA	PFHxS	PFHpS	PFOS
PFBA											
PFPeA	**0,442****										
PFHpA	0,704	0,526									
PFOA	**0,457****	**0,265***	**0,375***								
PFNA	**0,396****	**0,274***	0,233	0,219							
PFDA	0,144	0,101	0,137	0,197	**0,413****						
PFUnDA	-0,087	-0,140	-0,091	0,166	0,081	0,451					
PFTrDA	0,040	-0,112	-0,102	**0,286***	0,074	0,165	0,071				
PFHxS	**-0,284***	**-0,275***	-0,239	-0,048	-0,070	0,207	0,239	-0,041			
PFHpS	-0,060	-0,169	-0,133	0,190	0,170	0,193	0,235	**0,401****	0,119		
PFOS	0,250	-0,086	0,123	**0,289***	0,158	0,168	**0,371***	0,089	0,060	0,122	

*p<0.05; **p<0.001.

Moderate, directly proportioned correlations (p<0.001), were observed among: PFBA-PFPeA (r=0.442), PFOA-PFBA (r=0.457), PFNA-PFBA (r=0.396), PFPeA-PFHpA (r=0.526), PFHpA-PFOA (r=0.375), PFDA-PFNA (r=0.413), PFHpS-PFTrDA (r=0.401), PFOS-PFUnDA (r=0.371).

Slight, directly proportioned correlations (p<0.05), were observed among: PFPeA-PFOA (r=0.265), PFNA-PFPeA (r=0.274), PFTrDA-PFOA (r=0.286), PFOS-PFOA (r=0.289).

Slight, inversely proportioned correlations (p<0.05) were observed among: PFBA-PFHxS (r=-0,284), PFPeA-PFHxS (r=-0.275).

From a gender analysis of data, it is possible to observe that PFBA and PFUnDA levels were significantly (p<0.05) higher in boys than girls; whereas PFPeA concentration is significantly (p<0.05) higher in girls than boys (data not shown). As to PFHpA, PFOA, PFNA, PFDA, PFTrDA, PFHxS, PFHpS and PFOS, no statistically significant difference was detected in serum concentrations of both boys and girls.

No statistically significant correlation was detected between BMI and PFCs concentrations.

The results of the multivariate analyses showed that maternal parity is significantly associated with PFDA (beta=-0.114) and PFHxS (beta=0.003), while the variable "months of breastfeeding" is significantly correlated with PFUnDA (Beta=0.008). Finally, female gender is negatively associated with PFOS (beta=-0.41) (see table 8).

Table 5. Results of the Multivariate linear regression analyses.

	BMI	Maternal parity	Months of breastfeeding	Age	Maternal productive age	Gender "F"	R^2
	beta (p)	beta (p)	beta (p)	beta (p)	beta (p)	beta (p)	
PFNA	-0.064 (0.094)						0.047
PFDA		**-0,114 (0.004)**					0.130
PFUnDA			**0.008 (0.006)**			0.079 (0.056)	0,171
PFTrDA				0.013 (0.096)			0.046
PFHxS	0.032 (0.06)	**-0.139 (0.013)**					0.146
PFHpS			0.003 (0.078)	0.015 (0.065)			0.114
PFOS					-0.045 (0.065)	**-0,41 (0.049)**	0.113
∑PFCs		-0.859 (0.073)					0.053

4. Discussion

This study updates the existing knowledge on human exposure levels to PFCs. In particular, it provides valuable data on adult and on 6-11 year-aged children living in Southern Italy (Sicily) exposed to PFCs.

We provide the first exposure data for PFCs in Sicily. They also fall within the ranges found in other countries with similar sampling date being reported in Greece (Vassiliadou et al., 2010), Italy (Ingelido et al., 2010), Germany (Schroeter-Kermani et al., 2013), Australia (Toms et al., 2014), Korea (Cho et al., 2015), Canada (Haines and Murray, 2012) and the United States (Kato et al., 2011).

Information for PFHxS, and PFNA is available from Norway (Rylander et al., 2009), Australia (Toms et al., 2014), Korea (Cho et al., 2015) and the United States (Kato et al., 2011), with PFHxS levels also being available for Germany (Schroeter-Kermani et al., 2013). PFDA was only reported in Australian study (Toms et al., 2014) with a mean value of 0.3 μg/L. Our values reported on our study fall within these ranges. We can therefore conclude that the Sicilian population has the similar exposure patterns to PFCs than other European populations but lower PFCs levels than Australia, Canada and United States, and slightly higher than Korea.

PFCs were detected in all (n=61, 100%) children, the mean concentration of all PFCs being 5.888 ng/mL (range 1.035-12.94 ng/mL). Similar outcome was observed in other studies conducted on young children (Kim et al., 2014; Wu et al., 2015). However, much lower serum concentrations of PFCs were observed in other studies (Wu et al., 2015; Kim et al., 2014; Zhang et al., 2017).

In the present study, PFOS and PFOA were detected and quantified in 100% (n=61) and 87% (n=52) of samples, respectively. PFHxS and PFDA were detected and quantified in 80% (n=48) and 73% (n=43) of samples, respectively. Whereas, in equal and/or lower than 50% measure of the sample the authors detected: PFHpS (50%); PFUnDA (43%); PFNA (40%); PFTrDA (30%); PFBA (22%); PFPeA (3%); PFHpA (8%). No PFHxA; PFDoDA; PFTeDA; PFBS; PFDS were detected.

In a recent study, carried out by Zhang et al. (2017) in order to assess exposure to 14 PFCs in a typical, representative, industrial, Chinese city, 100% of samples (476 children aged 0–7 years), showed PFOS

and PFOA. However, the values observed were much higher than those in the present study, especially as to PFOS (83.65 *vs.* 2.364 ng/mL) and lower as to PFOA (6.58 *vs.* 2.002 ng/mL).

Moreover, they detected PFUnDA, PFNA, PFHxS, PFDA and PFHxA in more than 70% of the subjects, while PFDoDA, PFTrDA, PFBS, PFPeA and PFHpA were observed in 28-60% of the samples tested. PFTeDA and PFBA were detected in fewer than 10% of the samples (Zhang et al., 2017).

The higher percentage and concentration distribution of each single PFC in children studied by Zhang et al. (2017) is probably due to several factors, among which firstly the fact that children lived in a highly polluted city like Foshan (China), compared to Catania (Sicily, Italy).

Furthermore, in our study the samples were obtained by older children than theirs. The authors themselves point out that mean and median concentrations of PFCs peaked at age <2 years. In particular, PFOS peaked at age 0–1 years, while PFOA peaked at age 3–4 years; the concentrations of PFOS, PFHxS and PFDA decreased with age among children aged 0–7 years and no gender-related differences were found in the concentrations of PFCs (Zhang et al., 2017).

Similar results to the present one were observed by Timmermann et al. (2017) in a recent study conducted in Faroese children aged 5-13 years, for PFHxS, PFOA, PFNA and PFDA; whereas PFOS was higher than ours (6.7 *vs.* 2.364 ng/mL).

Instead, the PFCs (PFOS, PFOA, PFHxS and PFNA) concentrations observed by Stein et al. (2016) in 587 National Health and Nutrition Examination Survey (NHANES) 12-15 aged teenagers were greater than ours. This was probably due to the children's different age in this study (9-12 yrs).

The relationship between the concentrations of PFCs and age was inconsistent with previous studies. In Australian samples, concentrations of PFOA and PFHxS peaked at ages 3.5-4 and 12-15 years, respectively (Toms et al., 2009). The concentrations of PFOS, PFOA, PFHxS and PFNA were positively correlated with age (0-12 years) in an American study (Schecter et al., 2012) and the concentration of PFOA increased with age from 0 to 15 years in a Chinese study (Fu et al., 2014). In

contrast, a negative association between PFOA concentration and age was found in Chinese (Zhang et al., 2010), German and Norwegian populations (Verner et al., 2016).

Unfortunately, owing to the limited number of subjects, in the present study it was not possible to determine the concentration trend by the age range.

PFCs levels detected in our study children are lower than those of other studies; such an outcome could also be due to EU new restricting policy which has limited PFOS production and use from 2008 onward (Directive 2006/122/EC). In 2009, PFOS was included in Annex B of the Stockholm Convention on Persistent Organic Pollutants (http://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx).

From the analysis of existing correlations between serum concentrations of each PFC detected in this study, with the exception of long-chain compounds PFDoDA (C12), PFTeDA (C14) and PFDS (C10) which were not detected because in <LOQ concentrations, the other long-chain compounds such as PFOS (C8), PFOA (C8), PFNA (C9), PFDA (C10), PFUnDA (C10) and PFTrDA (C13) moderately-slightly correlated with short-chain compounds like PFBA (C4), PFPeA (C5), PFHpA (C7) and PFHpS (C7).

Similar results were observed in other studies whereby the concentrations of long-chain PFCs were correlated with short-chain ones (Fu et al., 2014; Zhang et al., 2017). In these studies, carried out in adults samples, the correlation was stronger, this being connected to the greater serum concentration detected and then due to the longer period of exposure, (Fu et al., 2014; Zhang et al., 2017; Olsen et al., 2011; Pan et al., 2010; Li et al., 2011). These correlations may be explained by an increasing half-life with increasing chain length (Haug et al., 2009).

In the present study, similarly to what pointed out by Zhang et al. (2017), the authors detected strong correlations between PFOS and other PFCs; while in other studies, statistically significant positive correlations were observed between PFOS and PFPeA, PFOS and PFHxA, PFOS and PFDA, PFOS and PFUnDA and PFOS and PFHxS. PFOS was correlated with PFOA in previous reports (Fu et al., 2014;

Schecter et al., 2012; Wu et al., 2015). Currently, short-chain perfluoroalkyl substances, such as PFPeA and PFHxA, are popular in industry. Additionally, they may coexist with other PFCs in various products (Russell et al., 2014), which may partly explain the correlations between PFOS and PFPeA and between PFOS and PFHxA.

Scientific literature clearly demonstrates that exposure of children, specially quite young ones, is caused by PFCs that were transferred from the mother during gestation and through breast feeding. A number of other studies have shown a positive association between PFC concentrations in mothers and fetuses (Lau et al., 2007; Monroy et al., 2008; Roosens et al., 2010; Tao et al., 2008). Children may also be exposed to more PFCs than adults because they drink more water in relation to their body weight than adults do. Drinking water is one of the most important sources of PFCs in the human body (Post et al., 2012) and previous studies have reported a relationship between PFCs in drinking water or drinking contaminated water and serum PFC concentration (Ericson et al., 2008; Holzer et al., 2008).

Additionally, children tend to ingest and inhale more dust than adults because of their behavior (when in close proximity to and frequent touching of carpets and furniture, which are known to be potential PFC sources for humans (Kato et al., 2009).

Household items that contain PFCs such as carpet care liquids, carpets and nonstick cookware, are regarded as important sources of PFCs (Powley et al., 2005; Sinclair et al., 2007). While Begley et al. (2005) reported that fluoropolymer food-contact materials do not appear to be a significant source of perfluorochemicals (e.g. PFOA) relative to paper that will migrate to food and be consumed.

A number of previous studies have shown that seafood, especially shrimps, is an important dietary source of PFCs for humans (Falandysz et al., 2006; Haug et al., 2010; Holzer et al., 2011; Rylander et al., 2010).

In the present study, all children examined consumed bottle water; in contrast there was no information whatsoever as to the dietary habits of these children, this being one of this study drawbacks.

In this study, analysing data by gender, it was possible to observe that PFBA and PFUnDA values are significantly ($p<0.05$) higher in males than females; whereas PFPeA concentration turns out to be significantly ($p<0.05$) higher in the latter.

As to PFHpA, PFOA, PFNA, PFDA, PFTrDA, PFHxS, PFHpS and PFOS, no statistically significant difference was detected in serum concentrations found in boys as compared to girls.

Similarly, no gender differences in the concentrations of PFCs were found in Australian children <12 years (Toms et al., 2009), American children <12 years (Schecter et al., 2012), Chinese children <15 years (Fu et al., 2014), Norwegian children aged 5–14 years (Haug et al., 2009) and Korean children aged 5–13 years (Kim et al., 2014).

Significant differences between males and females were detected only in PFOS (higher in boys), after studies performed in highly polluted industrial regions (Zhang et al., 2017) and/or from previous studies, when PFOS production and use had not yet been restricted (Olsen et al., 2004).

In this study, female gender was negatively associated with PFOS (beta=-0.41) .

Results similar to those of this study were reported in epidemiological studies carried out in south east Queensland (Australia), Nanchang (China) and Texas (USA) that reported no gender difference in the concentrations of PFOS, PFOA, PFNA and PFHxS in blood samples (Toms et al., 2009; Zhang et al., 2010; Schecter et al., 2012).

Research on children in Queensland, Australia (Toms et al., 2009), Texas, USA (Schecter et al., 2012) and Korea (Cho et al., 2015) showed that the concentrations of PFOS and PFOA increased with age; in contrast, a reduction in the concentration of PFOA was found with age in samples of Chinese, German and Norwegian children, especially in younger children (Zhang et al., 2010; Verner et al., 2016). However, the concentrations of PFOA and PFOS were similar, regardless of children in other studies in the USA (Calafat et al., 2007), Germany (Midasch et al., 2006) and China (Yeung et al., 2006).

The results of the multivariate analyses show that maternal parity is significantly associated with PFDA and PFHxS. Unfortunately there are no literature data with which to compare our results.

In the present study, the authors made a statistically significant correlation ($p<0.05$) between a longer breast- feeding period and PFUnDA concentrations.

As regards the relationship between PFCs and breast-feeding duration, similar results were reported by Kim et al. (2014).

The positive correlation between the duration of breast-feeding and PFCs concentrations indicates that PFCs may be retained for a long time in the infant after being transferred from the mother, retaining an influence until the infant becomes a teenager (Haug et al., 2011).

Many studies have shown that PFCs are present in breast milk and that PFCs are transferred from the mother to the infant through breastfeeding (Karrman et al., 2007; Roosens et al., 2010; Tao et al., 2008; Volkel et al., 2008).

In this study, no statistically significant correlation was detected between BMI and PFCs concentrations. Whereas, in studies like that by Kim et al. (2014), unlike this study, BMI correlated with PFCs concentrations. A plausible explanation of such difference can be provided by the presence of children with higher BMI than in the present study and higher PFCs concentration compared to those detected in the children analysed in this study (4.26-29.70 vs. 1.035-12,94 ng/mL, respectively).

As a matter of fact, PFCs bioaccumulation potential is related to chemicals' kinetic behavior, depending on: i) rapid and extensive oral and inhalation absorption (>90% in 0.25–1.5 h); ii) substantial binding to both plasma and liver proteins (mainly -but not exclusively- albumin) which represent the bioaccumulation reservoir (partition coefficient between plasma and the body lipid fraction, including the adipose tissue); iii) lack of biotransformation; iv) very slow urinary excretion with renal resorption (Ingelido et al., 2018).

As regards effects of single PFCs on childrens' and adults' health, very little is known about them. In the FETOTOX project (http://fetotox.au.dk/), it was found that higher maternal PFCs concentrations were associated with a higher risk for cerebral palsy (Liew et al., 2014). The authors did not find consistent evidence to suggest that PFCs exposure increases the risk for a longer time to pregnancy in

women (Bach et al., 2015 a,b) or attention deficit hyperactivity disease (ADHD) (Liew et al., 2015), autism spectrum disorders (ASD) (Liew et al., 2015), or lower birth weight in the newborn (Bach et al., 2016) although high maternal perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) concentrations have been associated with lower birth weight (Betts, 2007). In addition, studies on cell cultures showed that the PFCs might affect the sex hormone receptors (Kjeldsen and Bonefeld-Jorgensen, 2013; Bjerregaard-Olesen et al., 2016), the aryl hydrocarbon receptor (Long et al., 2013), the thyroid hormone levels (Long et al., 2013) and oxidative stress biomarkers (Wielsøe et al., 2015).

The toxicity of PFOS and PFOA has been studied quite extensively and the main toxic effects identified are hepatotoxicity, developmental toxicity, immunotoxicity and changes in the circulating hormones and lipoproteins (White et al., 2011; Barouki et al., 2012; Dewitt et al., 2012). Nevertheless, the toxicological profile of these substances is far from being adequately characterized because of the marked differences in toxicokinetics that have been observed among species, which make it difficult to extrapolate the experimental data in humans (Lau, 2012). Recently, IARC classified PFOA as possibly carcinogenic to humans (Group 2B), on the basis of *limited evidence* in humans and experimental animals for the carcinogenicity; however, positive association was observed for cancers of the testis and kidney (IARC, 2017).

Most studies of PFC concentrations in humans have used adult as subjects, but compared to adults, researches of PFCs exposure in children were not prevalently done relatively.

Most studies on PFC concentrations in humans have used adults as subjects, but compared to adults, studies in children have been relatively limited.

Young children are at a very sensitive stage of organ development (Webster, 2010) and it is reported, from the results of a number of studies, that PFCs exposure in children can be associated with behavioral disorders (Gump et al., 2011; Stein and Savitz, 2011), disruption of thyroid function (Bloom et al., 2010), pubertal timing (Lopez-Espinosa et al., 2011), pediatric atopy (Wang et al., 2011) and asthma (Dong et al., 2013).

Therefore, additional research into human exposure to PFCs, including subjects of all ages, is still needed. As pinpointed by Kim et al. (2014), one obstacle to further research is the difficulty of collecting blood, especially from young children. Accordingly, there have been attempts to assess human exposure by analyzing chemicals in urine instead of serum.

However, the study limitations were: the limited number of samples; the lack of PFC concentrations for different age range (<6 and >11 years); lack of more detailed information on the exposure paths; the inability to detect most PFCs in urine; so, further research should be conducted to confirm the findings of this study.

5. Conclusion

The 16 PFCs were detected in the serum samples of adult and children from Catania (South Italy), showing that they had been exposed to PFCs, especially for PFOS, PFOA, PFHxs and PFDA. Moreover, the exposure to PFOS and PFOA in particular, was similar or lower than that reported in investigation from other Countries of Northern Europe, USA, China, Australia. The low levels of PFCs observed in children reflect the reduction of production and use of PFOS and PFOA in recent years. In addition, long-chained PFCs were found in serum was correlated with short-chained ones.

Therefore, further studies are needed to monitor and address adverse health outcomes of PFCs, especially those living in industrial regions.

Part of result of this Ph.D. Thesis has been published:

- Ledda C, La Torre G, Cinà D, Paravizzini G, Vitale E, Pavone P, Iavicoli I, Rapisarda V. Serum concentrations of perfluorinated compounds among children living in Sicily (Italy). Toxicol Lett 2018 doi: 10.1016/j.toxlet.2018.09.001.

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