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Risk to human health related to the presence of perfluoroalkyl substances in food

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Abstract

The European Commission asked EFSA for a scientific evaluation on the risks to human health related to the presence of perfluoroalkyl substances (PFASs) in food. Based on several similar effects in animals, toxicokinetics and observed concentrations in human blood, the CONTAM Panel decided to perform the assessment for the sum of four PFASs: PFOA, PFNA, PFHxS and PFOS. These made up half of the lower bound (LB) exposure to those PFASs with available occurrence data, the remaining contribution being primarily from PFASs with short half-lives. Equal potencies were assumed for the four PFASs included in the assessment. The mean LB exposure in adolescents and adult age groups ranged from 0.42 to 3.1, the 95th percentile from 1.3 to 9.9 ng/kg bw per day. Toddlers and 'other children' showed a two-fold higher exposure. Upper bound exposure was 4- to 49-fold higher than LB levels, but the latter were considered more reliable. "Fish meat", "Fruit and fruit products" and "Eggs and egg products" contributed most to the exposure. Based on available studies in animals and humans, effects on the immune system were considered the most critical for the risk assessment. From two human studies, NOAECs of 31.9 and 27.0 ng/mL for the sum of the four PFASs in serum were identified for 1- and 5-year-old children, respectively. Using PBPK modelling, the serum level of 31.9 ng/mL in 1-year-old children was estimated to correspond to long-term maternal exposure of 1.16 ng/kg bw per day. Since accumulation over time is important, a tolerable weekly intake (TWI) of 8 ng/kg bw per week was established. This TWI also protects against other potential adverse effects observed in humans. Based on the estimated LB exposure, but also reported serum levels, the CONTAM Panel concluded that parts of the European population exceed this TWI, which is of concern.

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Summary

The European Commission asked EFSA for a scientific evaluation on the risks to human health related to the presence of 27 per- and polyfluoroalkyl substances (PFASs) in food. PFASs evaluated include perfluoroalkyl carboxylates (PFCAs) and sulfonates (PFSAs). These compounds contain a carbon chain with variable chain length (C4 to C18), and either a carboxylic acid or sulfonate group. At neutral pH these compounds have anionic end-groups, which to some extent explains their properties. In addition, a number of PFASs other than PFCAs and PFSAs are assessed, some of them being precursors of PFCAs and PFSAs.

In 2018, EFSA published an Opinion on two of these PFASs, PFOS and PFOA and derived separate tolerable weekly intakes (TWIs) for these compounds based on effects observed in humans. The current Opinion aimed at assessing the risk for other PFASs, but the CONTAM Panel was also asked to review the risk assessment in the previous Opinion, including the possibility to apply a mixture approach.

Based on several similar effects in animals, toxicokinetics and observed levels in human blood, the CONTAM Panel performed the current risk assessment for the sum of four PFASs: PFOA, PFNA, PFHxS and PFOS. These four made up approximately half of the lower bound (LB) exposure to those PFASs for which occurrence data were available, the remaining contribution being primarily from PFBA and PFHxA, two PFASs with a short half-life. Equal, weight based, potency was assumed for the four PFASs, since derivation of relative potency factors was considered not possible.

For the exposure assessment, an initial number of 97,434 results for food samples analysed for PFASs, obtained from 16 European countries, were available for the assessment. Various criteria were used for cleaning the data set, resulting in 69,433 analytical results for 26 PFASs with 92% of left-censored (LC) data (results below LOD/LOQ).

The CONTAM Panel decided not to run the exposure assessment for PFASs with 100% of LC data. As a result, the exposure assessment was limited to 17 PFASs (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFHxS, PFHpS, PFOS, PFDS and FOSA). For these PFASs, 67,839 analytical data were available. Most data were on PFOS (n = 8,498), PFOA (n = 8,197), PFDA (n = 5,770), PFNA (n = 5,594), PFHxA (n = 5,448) and PFHxS (n = 4,745).

High quantified concentrations (P95 > 10 µg/kg) were reported for some of the PFASs in edible offal from game animals and a number of fish species.

For most food categories and PFASs, calculated mean upper bound (UB) levels (based on using LOQ/LOD in case of non-detected levels) were much higher than LB levels (using zero for non-detects). This is due to high proportions of LC results and/or only limited availability of data for many PFASs. Therefore, exposure calculations should be considered only as a rough indication of the range of chronic dietary exposure, and should thus be interpreted with caution. The CONTAM Panel concluded that the calculated LB exposure is likely to be more realistic than the UB exposure.

As mentioned above, the CONTAM Panel decided to limit the risk assessment to the sum of PFOA, PFNA, PFHxS and PFOS. For this sum, the median exposure in adolescents, adults, elderly and very elderly ranged from 0.42 to 3.1 ng/kg bw per day at the LB and from 11.4 to 41.5 at the UB. Toddlers and other children had approximately two-fold higher mean intake than older age groups (adolescents, adults, elderly, very elderly), ranging from 0.84 to 6.5 ng/kg bw per day at the LB and from 38.5 to 112 ng/kg bw per day at the UB. In infants the mean exposure ranges were at the LB 2.4 to 12.2 ng/kg bw per day and at the UB 42.8 to 115 ng/kg bw per day. The 95th percentile exposures ranged across surveys and age groups at the LB from 1.3 (adults) to 27.9 (infants) ng/kg bw per day and at the UB from 21.9 (very elderly) to 229 (toddlers) ng/kg bw per day.

When focussing on some of the individual PFASs, mean PFOA exposure ranged for the LB from 0.1 to 0.6 ng/kg bw per day and for the UB from 3.0 to 29 ng/kg bw per day across surveys and age groups. At high (95th percentile) exposure, the LB ranged from 0.2 to 2.1 ng/kg bw per day and the UB from 5.6 to 59 ng/kg bw per day. Mean PFNA exposure ranged for the LB from 0.02 to 11.7 ng/kg bw per day and for the UB from 2.7 to 29.4 ng/kg bw per day across surveys and age groups. At high (95th percentile) exposure, the LB ranged from 0.05 to 27.9 ng/kg bw per day and the UB from 4.9 to 57.5 ng/kg bw per day. Mean PFHxS exposure ranged for the LB from 0.04 to 0.36 ng/kg bw per day and for the UB from 2.5 to 29.0 ng/kg bw per day across surveys and age groups. At high (95th percentile)

exposure the LB ranged from 0.09 to 0.86 ng/kg bw per day and the UB from 4.6 to 57.6 ng/kg bw per day. Mean PFOS exposure ranged for the LB from 0.23 to 2.6 ng/kg bw per day and for the UB from 3.3 to 31 ng/kg bw per day across surveys and age groups. At high (95th percentile) exposure, the LB ranged from 1.0 to 8.5 ng/kg bw per day and the UB from 6.25 to 62 ng/kg bw per day.

When comparing exposure of adults to these 4 PFASs, PFOA contributed 21%, PFNA 4%, PFHxS 10% and PFOS 66% to the sum, based on the median of the mean LB contributions across surveys. The contributions were similar in the age groups 'Other children', 'Adolescents', 'Elderly' and 'Very elderly'. These four contributed in adults approximately 46% to the sum of all PFASs for which the exposure was calculated, with relative contributions of 9%, 2%, 4% and 30% for, respectively, PFOA, PFNA, PFHxS and PFOS. Other PFASs that contributed more than 5% to this sum were PFBA (16%) and PFHxA (15%) which have short half-lives in humans.

For PFOS and PFOA "Fish and other seafood" was the most important contributor to the mean LB exposure, followed by "Eggs and egg products", "Meat and meat products", and "Fruit and fruit products". For PFOA, "Vegetables and vegetable products" and "Drinking water" were also important contributors. For several of the other PFASs, "Fish and other seafood", "Fruit and fruit products", "Vegetables and vegetable products", "Drinking water", as well as "Starchy roots and tubers" were the most important food groups. Although for infants and children "Food for infants and small children" was a major contributor, this was highly uncertain since this was based on few samples with detected values. For the combined exposure to PFOA, PFNA, PFHxS and PFOS, the main contributing food categories were "Fish meat", "Fruit and fruit products" and "Eggs and egg products", observed for all population groups.

There were some clear differences with the exposure assessments for PFOS and PFOA in the previous opinion from 2018, both in terms of the actual exposure and the relative contribution of food groups. These are due to differences in databases, assumptions and treatment of the occurrence data.

The use of PFAS-containing food contact materials is likely to contribute to human exposure to PFASs, and this may not be included in the reported data. Diet is the major source of PFAS exposure for most of the population, but on an individual basis, other routes such as dust ingestion and indoor air inhalation may also contribute substantially.

Considering toxicokinetics, many of the 27 PFASs considered in this opinion are shown to be readily absorbed through the gastrointestinal tract in mammals, including humans. They distribute predominantly to the plasma and, depending on the PFAS, to the liver, and are excreted in both urine and faeces. Neither PFCAs nor PFSA are metabolised by animals or humans, whereas precursors such as fluorotelomer alcohols (FTOHs) and polyfluoroalkyl phosphate esters (PAPs) are biotransformed to several metabolites, including PFCAs.

Routes and rates of elimination of PFASs vary according to the chemical end-group, the chain length and the species. In rats, but not in humans, sex differences were observed in toxicokinetics for a number of PFASs. In rodents, half-lives vary from few hours to several weeks and are in general much shorter than in humans.

In humans, the estimated half-lives for short-chain PFASs (such as PFBA, PFBS and PFHxA) were found to range from a few days to approximately one month, whereas for compounds having a long perfluoroalkyl chain length (such as PFOA, PFNA, PFDA, PFHxS or PFOS), it can be several years. The long elimination half-lives of these PFASs mainly originate from their interactions with various transporters involved in the reabsorption processes occurring at the hepatic, intestinal and renal level.

The maternal transfer of PFASs to offspring occurs both prenatally (in utero) and postnatally (via breastfeeding).

PFASs are transferred from soil to plants. In general, transfer rates are higher for the short chain PFASs. The transfer rates decrease from roots to leaves to fruits, due to natural barriers within the plants. Nevertheless, PFASs have been detected in fruits. This transfer to plant derived materials is relevant for both food and feed, including straw. In food producing animals, PFASs transfer from feed to animal derived food like milk, eggs and meat, with clear differences between species and the type of PFAS. This is also relevant for soil ingestion by foraging farm animals.

PFASs have been detected in humans. Blood serum and blood plasma are matrices for biomonitoring of PFASs. Generally, after the year 2000, the concentrations of PFOS, PFOA and in some studies PFHxS

have decreased, while the concentrations of PFNA, PFDA and PFUnDA have increased. No clear trends have been reported for other PFASs.

Summary statistics was performed on median levels from the various studies published from 2007 to 2018. Median values for the study medians were determined, referred to as median concentrations. The most prominent PFAS in serum of adults was PFOS (64%), followed by PFOA (16%), PFHxS (5.6%) and PFNA (5.1%). For children, PFOS and PFOA contributed almost the same with 35.0% and 36.6% of the total, followed by PFNA (8.8%) and PFHxS (6.7%). For adults the median concentrations in serum or plasma were 7.7, 1.9, 0.67, 0.61, 0.30 and 0.28 ng/mL for PFOS, PFOA, PFHxS, PFNA, PFDA and PFUnDA, respectively, while the concentrations of the remaining PFASs were below 0.25 ng/mL. For children the median concentrations in plasma were 3.2, 3.3, 0.79, 0.60 and 0.30 ng/mL for PFOS, PFOA, PFNA, PFHxS and PFDA, respectively, while the concentrations of the remaining PFASs were below 0.25 ng/mL.

Considerably higher concentrations have been observed for some individuals, including both occupationally exposed adults, and children and adults, which have experienced elevated exposure from e.g. contaminated drinking water. In these cases the relative abundance of the various PFASs may deviate considerably from what is observed in general populations.

Considering effects in laboratory animals, repeated dose toxicity studies were identified for 11 PFCAs (PFBA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, PFHxDA, PFODA), for 3 PFASs (PFBS, PFHxS, PFOS) and for 2 other PFASs (8:2 FtOH, EtFOSE). The most consistent endpoint was increased liver weight, seen for all PFASs studied but with clear differences in relative potencies. Disturbances in lipid metabolism, including hepatocellular steatosis and hepatotoxic effects, were evident mostly at higher dose levels. Many PFASs decreased the levels of thyroid hormones (both T4 and T3). For some PFASs, increased relative kidney weight, and alterations of the mucosa in the nasal cavity and olfactory epithelium were observed.

The most sensitive developmental effect was impaired development of mammary glands in mice, after exposure of the dams late in gestation or the offspring in utero or via lactation. The impairment persisted beyond sexual maturity. Only PFOA has been investigated in relation to this outcome, with a maternal LOAEC around 66 ng/mL serum. No NOAECs could be identified from the latter studies.

More general developmental toxicity studies in rodents were identified for 10 PFCAs (PFBA, PFHxA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, PFHxDA and PFODA), for 3 PFASs (PFBS, PFHxS and PFOS), and for 2 other PFASs (8:2 FtOH and EtFOSE). The effects most often observed were increased fetal and/or neonatal mortality and reduction in fetal weight and/or postnatal growth. In general, developmental toxicity occurred at similar or slightly lower doses than those exerting maternal toxicity. LOAELs and NOAELs were orders of magnitude higher than those for developmental effects on mammary glands seen with PFOA.

After PFAS exposure no consistent findings were identified for female reproductive parameters. Effects on male reproductive parameters, including atrophy of the testicular interstitium, accompanied by reduced serum testosterone levels and epididymal and testicular weights, as well as degenerative changes and spermatid retention in seminiferous tubules, have been reported for PFNA and PFDA.

PFOS and PFOA exert developmental neurotoxic effects in rodents at doses of 0.1 to 0.3 mg/kg bw per day or higher. PFOS exposure mostly decreased spontaneous activity, while PFOA increased it. Studies with PFDA, PFHxS and PFDoDA indicate developmental neurotoxic effects.

In animal studies, PFOS and PFOA have been shown to cause a reduced response to vaccination (T-cell dependent antibody response) and PFOS also caused a reduced resistance to infection. Effects were noted at doses where no overt toxicity was evident. Whereas effects on the immune system have also been observed for other PFASs, i.e. PFNA and PFDA, the available database for these compounds is more limited and does not include vaccination response. The CONTAM Panel concluded that the immune system is a prime target of PFASs.

For PFOS and PFOA no evidence for a direct genotoxic mode of action was identified. For PFASs other than PFOS and PFOA, the number of studies and data are limited. However, structural similarity for PFHxS and PFOS, as well as for PFNA and PFOA, indicates that also for these PFASs a direct genotoxic mode of action is unlikely.

Available studies indicate that PFOS and PFOA are tumour promoters in rodent liver and that PFOA also induces Leydig cell tumours in rats. The only long-term study available for PFHxA provides no evidence for carcinogenicity. PFNA and PFDA promoted liver tumour formation in a trout two-stage model of hepatocarcinogenesis, while 8:2 FTOH failed to do so.

Many studies have looked into potential effects of PFASs in humans, examining associations between serum levels of PFASs and specific disease incidences or markers of effects. The previous opinion (EFSA CONTAM Panel, 2018) extensively reviewed the existing data on potential associations, but this was restricted to PFOS and PFOA. For some potential critical effects new studies appeared since then.

Epidemiological studies published since the publication of the previous EFSA CONTAM Panel opinion on PFOS and PFOA, provide further support for the conclusion that PFOS and PFOA are associated with reduced antibody response to vaccination, observed in several studies. The evidence for other PFASs is weaker, related to the mixture of PFASs in blood, the lower concentrations compared to PFOS and PFOA, and the small number of studies. Some of the studies suggest that serum levels of PFOS and PFOA are associated with increased propensity for infection. As for PFOS and PFOA, epidemiological studies provide insufficient evidence to conclude on associations between exposure to PFASs and asthma and allergies.

Epidemiological studies provide clear evidence for an association between exposure to PFOS, PFOA and PFNA and increased serum levels of cholesterol. As for PFOS and PFOA, epidemiological studies provide insufficient evidence to conclude on associations between exposure to other PFASs and increased risk of cardiovascular disease. Epidemiological studies provide evidence for an association between exposure to PFASs and increased serum levels of the liver enzyme alanine transferase (ALT). The magnitude of the associations was small (~3%), however, and few studies found associations with ALT outside the reference range, and there were no associations with liver disease. There is insufficient evidence for associations between exposure to PFASs and diabetes, obesity and metabolic syndrome.

Studies on PFOS and PFOA published since 2018 confirm previous conclusions from the PFOS and PFOA opinion (EFSA CONTAM Panel, 2018) that "there may well be a causal association between PFOS and PFOA and birth weight". Maternal serum levels in studies reporting results on other PFASs were generally much lower and those studies provide no evidence for an adverse association for other PFASs and birth weight.

As for PFOS and PFOA, epidemiological studies show no associations between other PFASs and fertility and reproductive outcomes in both males and females.

Epidemiological studies provide insufficient evidence for associations between exposure to PFASs and neurodevelopment outcomes, growth in infancy or childhood, neurobehavioural, neuropsychiatric, cognitive outcomes or thyroid function.

Epidemiological studies provide insufficient evidence for associations between exposure to PFASs and changes in kidney function or serum levels of uric acid, as well as low bone mineral density or osteoporosis.

Epidemiological studies show no evidence for carcinogenicity of PFOS and PFOA in humans as concluded by EFSA (EFSA CONTAM Panel, 2018). Limited information was identified for other PFASs.

Certain effects seem to be associated with binding of PFASs to the PPAR α receptor, but to what extent this mechanism can explain the respective adverse effects is unclear. PPAR α KO mice but also mice expressing human PPAR α have been used to study the role of this receptor, including potential differences between rodents and humans. Like peroxisome proliferators, PFASs seem to cause liver hyperplasia via the murine PPAR α . However, PFASs induce steatosis in rodent hepatocytes in a PPAR α -independent way. Hepatocellular steatosis appears to be causally related to the occurrence of necrotic hepatocytes and the increased serum transaminase levels, but thorough knowledge of the MoA underlying this effect is missing.

There is no clear mode of action behind the strong decrease in thyroid hormone levels (T₄, T₃) observed in rodents exposed to PFASs, which is normally not accompanied by increased TSH levels. PFASs compete with T₄ for binding to transthyretin and can induce glucuronosyltransferase activity in the liver, but it is unclear if this is behind the decrease in hormone levels observed in animals.

No mode of action of immunotoxicity by PFASs has been established. Data from *in vivo* and *in vitro* studies on PFOS and PFOA suggests that immunotoxic effects may originate from modulation of PPARs, NF-κB regulated gene transactivation and/or regulation of apoptosis.

The MOA behind the impaired mammary gland development in mice dosed with PFOA during gestation and neonatally is unknown.

Considering critical effects, in human studies various associations between serum levels and a number of outcomes have been reported. In the previous Opinion (EFSA CONTAM Panel, 2018), four endpoints were selected as potential critical effects for PFOS and/or PFOA. These were (i) increased serum total and LDL cholesterol (risk factor for cardiovascular disease), (ii) increased ALT levels (indicating effects on liver cells), (iii) reduced birth weight, and (iv) effects on the immune system as shown by decreased antibody response to vaccines.

In 2018, the CONTAM Panel used the effects on serum cholesterol levels to derive TWIs for both PFOS and PFOA. Those TWIs were also protecting towards the other potential critical endpoints. Although the association with increased cholesterol was observed in a large number of studies, the CONTAM panel now considers the uncertainty regarding causality to be larger. This is primarily due to a postulated biological process around the enterohepatic cycling of both PFASs and bile acids, the latter affecting serum cholesterol levels. This should be further investigated.

For increased ALT, the CONTAM Panel concluded that more studies are needed to support the causality of the effect. Concerning reduced birth weight, a recent study seems to strengthen the causality. However, as concluded in 2018, the decrease in birth weight after adjusting for confounders is not large and the adversity of such a decrease might be questioned.

The CONTAM Panel concluded that effects on the immune system, which were observed at the lowest serum PFAS levels in both animals and humans, is critical for the risk assessment. The findings of a decreased immune response were considered robust since they were consistently observed for the two studied PFASs in rodents (PFOA, PFOS) and in humans. The CONTAM Panel noted that this is not the case for effects on mammary gland development, which are observed at similar low serum levels in mice but have not been studied in other animal models or humans. Therefore, the CONTAM Panel decided to base the present assessment on PFASs on effects on the immune system.

Based on observations in animals and humans, the CONTAM Panel decided to combine its assessment on the sum of four PFASs, i.e. PFOA, PFNA, PFHxS and PFOS. At present, these four PFASs contribute most to the levels observed in human serum. In humans, these four PFASs share toxicokinetic properties and show similar accumulation and long half-lives. Also, in terms of effects, these compounds in general show the same effects when studied in animals. Current data do not allow the derivation of potency factors. As a pragmatic approach, the CONTAM Panel assumed by default equal potencies for effects of these four PFASs on immune outcomes.

Two critical studies have been identified for the derivation of the Health Based Guidance Value. A study with children on the Faroe Islands showed various associations between the serum levels of individual PFASs, but also the sum of PFOA, PFNA, PFHxS and PFOS, and antibody titres against diphtheria and tetanus. A NOAEC of 27.0 ng/mL was identified for the sum of these four PFASs at 5 years of age and the antibody titres against diphtheria at 7 years. In addition, the CONTAM Panel identified a new study with children from Germany showing an inverse association between serum levels of PFOA, but also the sum of PFOA, PFNA, PFHxS and PFOS, and antibody titres against haemophilus influenzae type b (Hib), diphtheria and tetanus in serum sampled from 1-year-old children, predominantly breastfed. A NOAEC of 31.9 ng/mL at the age of 1 year was derived for the sum of PFOA, PFNA, PFHxS and PFOS, based on the inverse association between serum levels of the sum of these 4 PFASs and antibody titres against haemophilus influenzae type b (Hib).

This NOAEC of 31.9 ng/mL corresponds to a lower intake by the child and thus the mother in her life up to pregnancy, since PFAS serum levels in breastfed children are in general higher at 1 years of age than at 5 years. Therefore, this NOAEC was used to estimate the daily intake by mothers that would result in this critical serum concentration at 1 year of age in breastfed children. This daily intake was subsequently used to derive a maternal reference point for the sum of PFOA, PFNA, PFHxS and PFOS.

Using a PBPK model, and assuming 12 months of breastfeeding, it was estimated that the NOAEC in infants corresponds to an intake by the mother of 1.16 ng/kg bw per day for the sum of the four PFASs. Such intake would result in a serum level in the mother at 35 years of age of 12.6 ng/mL.

The CONTAM Panel decided to use the daily intake of 1.16 ng/kg bw per day as the starting point and established a group tolerable weekly intake (TWI) of $7 \times 1.16 = 8$ ng/kg bw per week for the sum of PFOA, PFNA, PFHxS and PFOS. It was decided that no additional uncertainty factors need to be applied, because the NOAEC is based on infants which are expected to be a sensitive population group, as is true for many immunotoxic chemicals. In addition, the NOAEC is based on risk factors for disease rather than disease.

This TWI should prevent that mothers reach a body burden that results in levels in milk that would lead to serum levels in the infant associated with a decrease in vaccination response. As a result, the higher exposure of breastfed infants is taken into account in the derivation of the TWI, and this TWI is not applicable for infants.

The CONTAM Panel noted that this TWI is protective for the other potential critical endpoints (increase in serum cholesterol, reduced birth weight and high serum levels of ALT) considered in the previous opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018).

Based on the occurrence data obtained from the Member States, the exposure to individual PFASs was estimated for different age groups across different food consumption surveys. Since it was decided to base the risk assessment on the sum of PFOA, PFNA, PFHxS and PFOS, also the combined exposure to these four PFASs was estimated. The CONTAM Panel noted that several factors contribute to the high uncertainty of the present exposure assessment. Large differences between LB and UB concentrations were observed in foods, as a result of analytical methods being used that are not sufficiently sensitive. This results in a large difference between maximum UB and minimum LB chronic dietary exposure estimates for PFASs. The CONTAM Panel considers that the true exposure level for PFOA, PFNA, PFHxS and PFOS is closer to the LB than the UB values. This assumption is based on two facts. First of all, studies performed using the best analytical methods with high sensitivity and high levels of quality control give results with fewer left censored data and confirm occurrence in foods at levels close to the LB estimates. Secondly, LB exposure estimates in this opinion are more consistent with reported median blood serum levels.

Regarding the combined exposure to the four PFASs expressed on a weekly basis, for toddlers and other children, mean LB exposure varied between 6 and 46 ng/kg bw per week. The high (P95) LB exposure ranged from 19 to 96 ng/kg bw per week. UB exposure was much higher, for the mean varying between 270 and 785 ng/kg bw per week, with high (P95) UB exposure between 553 and 1603 ng/kg bw per week. For toddlers, the high exposure from food is influenced by a high contribution from PFNA and associated with high uncertainty. The groups of adolescents, adults, elderly and very elderly showed in general a lower estimated exposure. Mean LB exposure varied between 3 and 22 ng/kg bw per week, as compared to high (P95) LB exposure between 9 and 70 ng/kg bw per week. UB exposure for these age groups was again higher, for the mean varying between 81 and 290 ng/kg bw per week, with P95 exposure between 153 and 294 ng/kg bw per week. The highest mean LB exposure for adolescents and adults exceeds the TWI by a factor of 1.5. At the highest LB P95 exposure this is a factor of 4.5. At the UB exposure the exceedance is much larger. These calculated exceedances of the TWI at LB exposure estimates indicate a health concern.

The CONTAM Panel identified a number of uncertainties and has a number of recommendations to decrease these uncertainties. To improve the exposure assessment, data obtained by more sensitive analytical methods with high levels of quality control (to avoid matrix effects or impact of background contamination) are needed in order to increase the proportion of quantified results and thus reduce uncertainty in the dietary exposure assessment. This is needed for all PFASs and a broad range of widely consumed food products. For the determination of the total amount of PFASs, sensitive and accurate methods, which facilitate determination of the total amount of PFASs in samples of food and drinks are needed. Additional studies on the relative contribution of sources other than food are needed, especially for PFASs which are present in the highest concentrations in indoor air and house dust, such as n:2 FTOHs and PAPs. Also more studies on the effect of cooking and food processing, in particular in relation to transfer to food from food contact materials that contain PFASs are needed, given that most food is consumed after cooking/processing, and the fact that data reported in the scientific literature are

inconsistent regarding the impact this has on exposure. More information is needed on the transfer of PFASs along the food chain.

Concerning potential adverse effects, studies on effects of other PFASs, and in particular PFNA and PFHxS on the immune system should be conducted. Studies for the potential critical effects that allow for a derivation of potency factors for PFASs should be conducted. In addition, studies to characterize the mode of action of immunotoxicity and mammary gland development of PFASs should be performed.

More longitudinal epidemiological studies are needed on human endpoints, in particular prospective vaccination studies covering more varied types of vaccines, different populations, as well as more studies on other immune outcomes including risk of infections. Most epidemiological studies examine associations between health-related outcomes and single PFASs separately in spite of co-exposures. For risk assessment, results for the sum of several PFASs should be reported.

Experimental evidence is needed to understand and quantify the association between PFASs and blood lipids, for example the importance of enterohepatic circulation. PBPK models for PFASs should be further optimised.

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DRAFT

1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

Background

Following the Scientific Opinion on Perfluorooctane sulfonate (PFOS) perfluorooctanoic acid (PFOA) and their salts¹, the European Commission recommended an EU-wide monitoring² of perfluoroalkylated substances in food. The occurrence data generated by this monitoring have been used in the Scientific Report entitled "perfluoroalkylated substances in food: occurrence and dietary exposure"³.

Terms of reference

In accordance with Art 29 (1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to prepare an opinion on the risks to human health related to the presence of perfluoroalkylated substances in food, considering existing hazard assessments and available occurrence data

1.2. Interpretation of the Terms of Reference

Following an agreement reached in June 2017 with the European Commission, the CONTAM Panel decided to address the mandate in two separate opinions, one on perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) (EFSA-Q-2015-00526) and another on other perfluoroalkylated substances (EFSA-Q-2017-00549).

In 2010, EFSA issued a call for data on PFASs in food. Thirteen countries submitted analytical results on 27 PFASs in food covering the sampling period 1998 to 2012. In addition, data on PFASs in food from a three-year EU research project (PERFOOD⁴) were submitted to EFSA. The selection of compounds in this opinion is based on these 27 PFASs (excluding PFOS and PFOA) that were subject of the EFSA 2012 Scientific exposure report (EFSA, 2012).

¹ Opinion of the Scientific Panel on Contaminants in the Food chain on Perfluorooctane sulfonate (PFOS) perfluorooctanoic acid (PFOA) and their salts. The EFSA Journal (2008) Journal number, 653, 1-131.

² Commission Recommendation 2010/161/EU on the monitoring of perfluoroalkylated substances in food (OJ L 68, 18.3.2010, p.22).

³ European Food Safety Authority, perfluoroalkylated substances in food: occurrence and dietary exposure. EFSA Journal 2012, 10(6) 2743

⁴ PERFOOD is a consortium of several European research institutions which collaborate in a FP7 EU project with the aim to improve the analytical tools for the determination of PFASs in food items, to contribute to the understanding of PFASs transfer from the environment into dietary items and to quantify the possible contribution of food/beverage contact materials and food and water processing to the overall PFASs levels in the diet (www.perfood.eu).

1.3. Additional information

1.3.1. Chemistry and Synthesis

In the 2008 Opinion (EFSA, 2008), it was described that poly- and perfluoroalkyl substances (PFASs) is the collective name for a vast group of fluorinated substances, including oligomers and polymers. OECD (2018) has identified 4730 PFAS-related CAS numbers. In the literature, many individual substances as well as groups of substances are described under more than one acronym and also substances or groups are discussed under identical acronyms.

PFASs (R-X) are substances consisting of a hydrophobic alkyl chain, R, of varying length (typically C4 to C16) and a hydrophilic end group, X. The hydrophobic part may be fully $[R=F(CF_2)_n-]$ or partially fluorinated. When fully fluorinated, the molecules are also called perfluoroalkyl substances. Their general structure is given in Figure 1 and some chemical characteristics of individual PFASs considered in this opinion are given in Table 1.

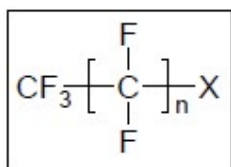


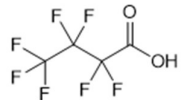
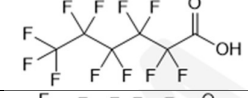
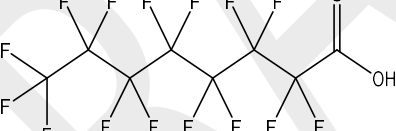
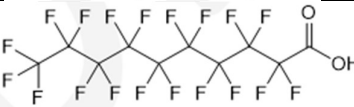
Figure 1: General structure of PFASs

The hydrophilic end group can be neutral, or positively or negatively charged. The resulting substances are non-ionic, cationic or anionic surface-active agents due to their amphiphilic character. Examples of substances with anionic end groups are the perfluoroalkane sulfonic acids (PFASs), which include PFOS, and the perfluoroalkyl carboxylic acids (PFCAs), which include PFOA. In cationic PFASs, the fluorinated hydrophobic part is attached to e.g. a quaternary ammonium group. Examples of neutral end groups X are: -OH, -SO₃NH₂, and include among others the fluoroteleomer alcohols (FTOHs) and perfluoroalkane sulfonamides (FASAs). Due to the strong covalent C-F bond, perfluoroalkyl substances are highly persistent.

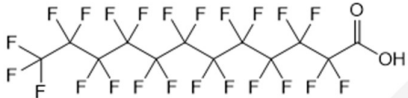
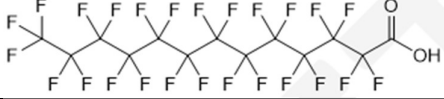
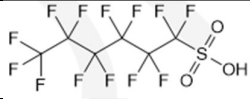
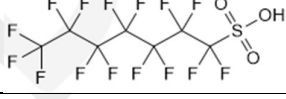
Many PFASs are considered to be potential precursors of other PFASs, which has been described in more detail in EFSA CONTAM Panel, (2018). These precursors are usually not environmentally persistent but may be transformed in the environment among others through biodegradation. For instance, N-ethylperfluoroalkane sulfonamidoethanols (EtFASes) and N-ethyl perfluoroalkane sulfonamides (EtFASAs) can biodegrade to FASAs and further to PFASs. Because the precursors include products that are not fully fluorinated, some of the partially fluorinated substances are also discussed in this opinion. For the partially fluorinated compounds the position and number of fluorines determine their characteristics. Considering the entire family of PFASs, it should be noted that there are interrelationships between individual substances as industrial, environmental, or metabolic precursors or transformation products of one another. This is described in more detail by e.g. Buck et al. (2011) and the Swedish Chemicals Agency (2015).

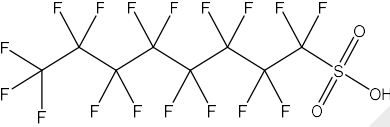
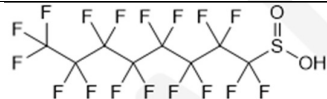
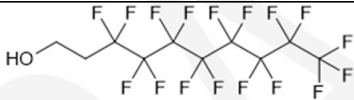
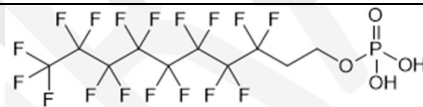
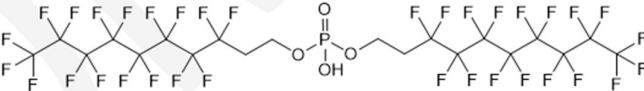
This opinion only considers those partially fluorinated substances that contain a -CH₂CH₂- moiety between the hydrophilic part and the remaining fully fluorinated carbon chain: F(CF₂)_n-CH₂CH₂-X. These partially fluorinated compounds are called telomer substances and derive their name from the telomerisation production process (see Section 1.3.3). The telomerisation process results only in substances consisting of a linear alkyl chain with an even number of carbon atoms', whereas the electrochemical process produces a mixture of branched (br-PFAS) and linear (n-PFAS) isomers (EFSA, 2008; Buck et al., 2011; Löfstedt Gilljam et al., 2016). In environmental and human samples PFASs are found as a mixture of the linear and the branched isomers (De Silva and Mabury, 2006; Riddell et al., 2009; EFSA CONTAM Panel, 2018).

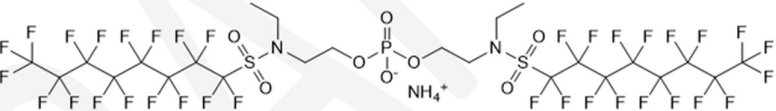
631 Table 1: Chemical characteristics of individual PFASs considered within this opinion and their uses as derived from Buck et al. (2011)

Acronym	Chemical name	CAS number	Structural formula	Molecular weight	Uses
Perfluoroalkyl carboxylic acids (PFCAs)					
PFBA	Perfluorobutanoic acid	375-22-4		214.04	Surfactant
PFPeA	Perfluoropentanoic acid	2706-90-3		264.05	Surfactant
PFHxA	Perfluorohexanoic acid	307-24-4		314.05	Surfactant
PFHpA	Perfluoroheptanoic acid	375-85-9		364.06	Surfactant
PFOA	Perfluorooctanoic acid	335-67-1		414.07	Surfactant
PFNA	Perfluorononanoic acid	375-95-1		464.08	Surfactant
PFDA	Perfluorodecanoic acid	335-76-2		514.08	Surfactant
PFUnDA	Perfluoroundecanoic acid	2058-94-8		564.09	Surfactant

Perfluoroalkyl substances in food

Acronym	Chemical name	CAS number	Structural formula	Molecular weight	Uses
PFDODA	Perfluorododecanoic acid	307-55-1		614.10	Surfactant
PFTrDA	Perfluorotridecanoic acid	72629-94-8		664.11	Surfactant
PFTeDA	Perfluorotetradecanoic acid	376-06-7		714.11	Surfactant
PFPeDA	Perfluoropentadecanoic acid	18024-09-4		764.12	Surfactant
PFHxDA	Perfluorohexadecanoic acid	67905-19-5		814.13	Surfactant
PFODA	Perfluorooctadecanoic acid	16517-11-6		914.15	Surfactant
Perfluoroalkane sulfonic acids (PFSAs)					
PFBS	Perfluorobutane sulfonic acid	375-73-5/ 45187-15-3 [59933-66-3 as hydrate]		300.10/ 299.09 [318.11]	Surfactant
PFHxS	Perfluorohexane sulfonic acid	355-46-4/ 108427-53-8		400.11/ 399.10	Surfactant
PFHpS	Perfluoroheptane sulfonic acid	375-92-8		450.12	Surfactant

Acronym	Chemical name	CAS number	Structural formula	Molecular weight	Uses
PFOS	Perfluorooctane sulfonic acid	2795-39-3 (Potassium salt; 1763-23-1 (Acid))		538.22 (Potassium salt) 500.13 (Acid)	Surfactant
PFDS	Perfluorodecane sulfonic acid	335-77-3		600.14	Surfactant
Perfluoroalkane sulfinic acids (PFSIAs)					
PFOSI	Perfluorooctane sulfinic acid	647-29-0		484.13	Intermediate environmental transformation product
n:2 fluorotelomer alcohols (n:2 FTOHs)					
8:2 FTOH	8:2 Fluorotelomer alcohol	678-39-7		464.12	Major raw material for surfactant and surface production products
n:2 polyfluoroalkyl phosphoric acid esters (PAPs)					
8:2 monoPAP	8:2 Fluorotelomer phosphate monoester	57678-03-2		544.10	Surfactant and surface protection products
8:2 diPAP	8:2 Fluorotelomer phosphate diester	678-41-1		990.20	Surfactant and surface protection products
Perfluoroalkane sulfonamides (FASAs)					
FOSA	Perfluorooctane sulfonamide	754-91-6		499.14	Major raw material for surfactant and surface production products
N-ethyl perfluoroalkane sulfonamides (EtFASAs)					
EtFOSA	N-ethyl perfluorooctane sulfonamide	4151-50-2		527.20	Major raw material for surfactant and surface production products

Acronym	Chemical name	CAS number	Structural formula	Molecular weight	Uses
N-ethylperfluoroalkane sulfonamidoethanols (EtFASEs)					
EtFOSE	N-ethyl perfluorooctane sulfonamidoethanol	1691-99-2		571.25	Major raw material for surfactant and surface production products
Perfluoroalkyl phosphate					
FC-807	Ammonium bis[2-[N-ethyl(heptadecafluorooctane)sulphonylamino]ethyl]phosphate	30381-98-7		1221.49	Surfactant

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1.3.2. Methods of analysis

1.3.2.1 Analytical methods for PFASs

Analytical methods for PFASs were described in the EFSA Opinion on PFOS and PFOA in Food (EFSA CONTAM Panel, 2018). PFASs are normally measured alongside PFOS and PFOA as part of multi-analyte methods. These do not always measure the same PFASs and some methods measure more compounds than others. Methods with best sensitivity and quality control usually involve stable isotope dilution (internal standards) and consist of extraction and clean-up of food samples followed by determination using liquid chromatography (LC) coupled to quadrupole tandem mass spectrometry (LC-MS/MS) with electrospray ionization (ESI).

1.3.2.2. Analytical methods for determination of PFASs in biological samples

The instrumental detection of PFASs in biological samples is usually done in the same way as for food samples (EFSA CONTAM Panel, 2018). However, the sample preparation methods may differ substantially. It is common to make use of liquid-liquid extraction or off-line solid phase extraction, but also quite a few laboratories apply direct analysis or on-line column switching technology after protein precipitation (Salihovic et al., 2013; Jahnke and Berger, 2009). Clean up is often done using dispersed graphitized carbon with glacial acetic acid or by filtration (Salihovic et al., 2013).

1.3.3. Production and use of the substances

PFASs are reported to be, or have been, used in e.g. water- and stain-resistant coatings for clothing, leather and carpets; oil-resistant coatings for food contact paper; aviation hydraulic fluids; fire-fighting foams; paints, adhesives, waxes, polishes; in industrial applications as surfactants, emulsifiers, and coatings and personal care products including cosmetics (see Section 3.2.4.2) (Kissa, 2001; Prevedouros et al., 2006; Lindström et al., 2011; Fujii et al., 2013; Wang et al., 2014a,b). Specific information relating to the volumes of production for each of these uses is not publically available.

Wang et al. (2014a) have further estimated the global cumulative emissions of C₄ – C₁₄ PFCA homologues during 1951-2030. The estimations are presented as the outcome from two scenarios, low and high. For perfluorobutanoic acid (PFBA) global cumulative emissions (low and high) are estimated to 15 and 915 tonnes, whereof 47% and 26% are estimated to come from direct sources. Direct sources are here defined as all sources of individual PFASs through their whole product cycles (production to disposal), whereas indirect sources are those where the same compounds appear as a result of degradation, or other conversion process in the environment, of precursor compounds as described in Section 1.3.1. The corresponding figures for PFHpA, PFOA and PFTeDA are (low and high) 59 and 3,264 tonnes, 26% and 26%; 2,078 and 18,366 tonnes, 100% and 98%; and 0 and 19 tonnes, 0% and 1% respectively. Comprehensive overviews of historic and current synthesis scheme of PFOA-, PFOS- and common fluorotelomer-based products applied by different companies are described by Wang et al. (2014a) and Wang et al. (2014b). Furthermore, estimated use of ammonium perfluorooctanoate/sodium perfluorooctanoate (APFO/NaPFO) for fluorotelomer production is also given for different country groups. The estimates of consumption of APFO/NaPFO volumes for fluorotelomer production are decreasing in one group of countries: Western Europe, Japan and the US whereas it is indicated to increase in another group of countries: China, India, Poland and Russia. However, the total consumption during 2011-2015 is indicated to be in the same range, around 350 tonnes in the two country groups. In Wang et al. (2017a) an overview of the production, use and emissions of PFASs from perfluorooctanesulfonyl fluoride (POSF)-based products is given along with a description of the production pattern of POSF-based substances.

FTOH-based products have been produced since the 1970s and the global production in the early 2000s has been estimated to be between 5,000 and 6,500 tonnes per year (US EPA, 2002) but already in 2004 it was estimated to be around 12,000 tonnes per year. No more recent production volumes have been identified, but it is likely that the production has continued to increase.

According to the Swedish Chemicals Agency (2015) there were at least 3,000 PFASs on the global market and recently OECD (2018) has identified 4,730 PFAS-related CAS numbers. There is however just minor or no information on the production and use for most PFASs on the market, resulting in little or no understanding on how much has been, and will be, released, transformed and accumulated in the environment and biota over time.

1.3.4. Environmental fate

A wide range of PFASs have been used in numerous industrial applications and consumer products due to their unique properties such as the ability to create stable foams, chemical resistance, and surface tension lowering properties (Kissa, 2001; Buck et al., 2011). Among the various PFASs, some groups of compounds are extremely persistent such as PFCAs and PFSA (Kissa, 2001) while others are easily degraded in the environment and in humans (Ellis et al., 2004; Martin et al., 2006). Some of the non-persistent compounds, for example PAPs, FTOHs and EtFASes, are degraded to highly persistent PFASs such as PFCAs and PFSA and are thus contributing to the exposure to these groups of persistent compounds (D'Eon and Mabury, 2007; Nabb et al., 2007; Peng et al., 2014; Lee et al., 2010). Due to their extensive use and high persistency in the environment, a wide range of PFASs have frequently been detected in the environment, as well as in wildlife and humans (Giesy and Kannan, 2001).

1.3.4.1 Release and distribution in the environment

PFASs can be released to the environment during production, use and disposal (Ahrens and Bundschuh, 2014). Municipal wastewater treatment plants and landfill waste sites represent important direct sources of PFASs in aquatic ecosystems, while atmospheric deposition is also a major contributor (Ahrens and Bundschuh, 2014). As many of these compounds are persistent and relatively water soluble, they may be transported long distances in water and also in aerosols (Prevedouros et al., 2006; Ahrens et al., 2011a). In addition, volatile precursors may undergo long-range transport in the atmosphere (Young and Mabury, 2010; Benskin et al., 2011). One or more PFASs have been detected in more than 90% of all European rivers investigated and several PFASs have been detected in drinking water (Ahrens and Bundschuh, 2014).

1.3.4.2 Bioaccumulation in aquatic and terrestrial food chains

The octanol-water equilibrium coefficient is usually reflecting the bioaccumulation potential for fat soluble compounds, while for PFASs the partitioning to serum proteins is likely to be one of the driving mechanisms (Martin et al., 2003a,b; Kelly et al., 2009). Gebbink et al. (2016) reported on field-based sediment-water distribution coefficients (K_D) for PFSA and PFCAs. The K_D for PFSA was found to increase from around 0.3 to 2.5 with increasing chain length between four to eight carbon atoms. For PFCAs, the corresponding increase was reported to be from around 2.5 to 4.7 with increasing chain length between seven to ten carbon atoms. The same authors also reported on bioaccumulation factors (BAFs) from water to Baltic Herring (*Clupea harengus*). For PFSA, the BAF increased from 3.3 to 4.1 with increasing chain length from six to eight carbon atoms, whereas the corresponding increase for PFCAs was reported to be from around 2.0 to 5.3 with increasing chain length between six to ten carbon atoms.

The bioaccumulation processes is more complex for terrestrial food chains. In a study by Stahl et al. (2009), it was demonstrated that PFASs are taken up by the roots from the soil, and Felizeter et al. (2012) reported that PFASs with longer chain lengths have a greater potential to bioaccumulate in hydroponically grown plants. Sorption to organic matter is also known to increase with chain length (Higgins and Luthy, 2006) and so the uptake of long-chain PFASs may become limited by the fraction available in the pore water. Based on the strong bioaccumulation of several PFASs in humans (Olsen et al., 2007) and other mammals (Houde et al., 2006, 2011), it is no surprise that bioaccumulation of PFSA and PFCAs is also important in farm animals. Biomagnification of PFNA, PFDA, PFUnDA and PFDoDA from lichen to caribou to wolf has been reported (Müller et al., 2011), and it has been concluded that the trophic magnification factors are higher in the aquatic food webs compared to terrestrial food webs (Müller et al., 2011).

Biotransformation and fate of PFASs are also described in Section 1.3.1 'Chemistry' and 3.3.1 'Toxicokinetics'.

1.3.4.3 Time trends

Concentrations of PFASs in environmental samples vary amongst others depending on compounds and sampling period. As an example, Table 2 summarises temporal variation of many PFASs from the Baltic Sea area and the Swedish mainland. A comprehensive review published in 2018 (Land et al., 2018) of the effects of phasing out long-chain PFASs on their (and 'possible precursors') occurrence in the environment and how temporal variation of concentrations in abiotic and biological samples could be retrieved and analysed in a systematic way.

Table 2: Temporal trends for a range of PFASs in Grey seal (*Halichoerus grypus*) and Peregrine falcon (*Falco peregrinus*). From Holmström et al. (2010) and Kratzer et al. (2011). These studies are also discussed with respect to temporal variation in Swedish Environmental Agency (2012).

Compound	Species/organ, period			
	Peregrine falcon/egg		Grey seal/liver	
	1974 - 2007	2000 - 2007	1974 - 2008	1998 - 2008
FOSA	N.A.	N.A.	---	---*
PFNA	+++	0	+++	--
PFDA	+++	0	+++	0
PFUnDA	+++	0	+++	0
PFDoDA	+++	0	+++	0
PFTTrDA	+++	-	+++	0
PFTeDA	+++	--	+++	+++
PFPeDA	+++	--	N.A.	N.A.
PFHxS	+++	0	---	--
PFHpS	N.A.	N.A.	+++	--
PFDS	+++	0	N.A.	N.A.
PFOSI	N.A.	N.A.	0	---

* 1987-2008

N.A. = Not available, +++ = increasing trend ($p < 0.02$), --- = decreasing trend ($p < 0.02$), -- = decreasing trend ($p > 0.02$ but < 0.05), - = decreasing trend ($p = 0.09$), 0 = no significant trend. For Peregrine falcon log-linear regression; for Grey seal two-way ANOVA.

1.3.4.4 Contamination of food

Two main processes are thought to lead to contamination of food with PFASs, namely bioaccumulation in aquatic and terrestrial food chains, and transfer from contact materials used in food processing and packaging (for further information on migration from food contact materials, including non-stick coatings used on cookware see Section 3.1.4.1). Terrestrial food ingredients could of course also be contaminated directly via atmospheric deposition but it is likely that this route could only be of importance close to substantial sources. It can be assumed that contamination from packaging and processing reflects current production and use of PFASs, while bioaccumulation is regarded to reflect long-term use. Knowledge on the relative contribution from bioaccumulation and food processing is useful for *i. a.* prediction of possible future dietary exposure.

1.3.4.5 Levels of PFASs in environmental samples useful for human exposure assessment

Information on the occurrence of PFASs in the environment could also be considered in the human exposure assessment, especially when data on occurrence in food are scarce or lacking. Occurrence of individual PFASs in ground- and surface water, plants and wildlife such as mammals, birds, fish and

shellfish, could raise concern as such data indicate that these compounds are also likely to be found in food originating from similar environmental habitats. This concern could be strengthened when environmental monitoring indicates increasing temporal trends of individual PFASs.

In Section 3.2.1 (Current exposure assessment), it is described that in addition to PFOA and PFOS, a dietary chronic exposure assessment has been performed for PFBA, PFPeA, PFHxA, PFHpA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFHxS, PFHpS, PFDS and FOSA. Due to a very high proportion of results below the limit of detection/quantification (LOD/LOQ) (around 99%) and/or only limited availability of data, exposure calculations for PFBA, PFHpA, PFTeDA and PFBS, PFHpS, PFDS should be considered only as a rough indication of the range of chronic dietary exposure. For the remaining PFASs; PFPeDA, PFHxDA, PFODA, PFOSI, 8:2 FTOH, 8:2 monoPAP, 8:2 diPAP, EtFOSA, EtFOSE and FC-807 no exposure assessment could be carried out.

Considering this categorisation, information on the occurrence of PFBA, PFHpA, PFTeDA, PFPeDA, PFHxDA, PFODA, PFBS, PFHpS, PFDS, PFOSI, 8:2 FTOH, 8:2 monoPAP, 8:2 diPAP, EtFOSA, EtFOSE and FC-807 in the environment could be of importance for the recommendations on future actions. The compilation below is therefore restricted to these compounds. Occurrence of PFOS and PFOA in the environment was described in the previous opinion (EFSA CONTAM Panel, 2018).

1.3.4.5.1 Levels and temporal trends of PFASs for which none or only rough exposure assessment was performed

PFBA

PFBA has been found in the environment in Europe and the USA in an array of matrices, such as groundwater, wastewater, plants, limnic biota and birds liver (Campo et al. 2015, Chu et al., 2015; Eschauzier et al., 2013 and Gobelius et al., 2017). In a study by Campo et al. (2014) PFBA was found in wastewater in concentrations up to 20.5 ng/L with an increasing temporal trend. Eschauzier et al. (2013) reported extremely high concentrations in groundwater, up to 1208 ng/L from the Netherlands. Potential point sources in the area are a former landfill and a military camp with an airbase. Möller et al. (2010) reported on PFASs in the Rhine/Meuse delta but also River Rhine from Constance to its estuary. Downstream from Leverkusen, the authors reported a profound increase of the concentration of many PFASs but particularly PFBS and PFBA. This increase became even more obvious upstream from Rees. These increases are probably due to impact from local sources. The maximum concentration of PFBA recorded in the Rhine/Meuse delta was 330 ng/L as estimated from the author's figure.

Zhang et al. (2017) reported on PFBA in food from a contaminated site and a site without known contamination. The concentration in legumes and leafy vegetables from the contaminated site were up to 200 ng/g ww and 1,000 ng/g ww, respectively, whereas the concentrations in the same vegetables from the non-contaminated site were 1 ng/g ww or lower. The relative contribution from different food categories was estimated to be almost 100%, as attributed to leafy vegetables in the contaminated area whereas some 85% was attributed to legumes in the non-contaminated area. Gobelius et al. (2017) analysed sap from birch (*Betula pendula*) sampled in the vicinity of fire training facilities. Concentrations up to 0.17 ng/L, corresponding to bioconcentration factors (related to concentration in ground water) up to 0.03 were reported. In a study by Garcia-Valcarce et al. (2014) on Great Brome (*Anisantha diandra*), the ratio between the concentration in the plant and that in the nutrient solution (0.5 µg/mL) were reported to be in the order of 0.258 to 5.65 depending on exposure time (1 – 20 days, respectively). In a study by Blaine et al. (2014) celery was grown in biosolid-amended soil. Mean concentration in shoots was reported to be 232 ng/g. Different species of fish from Spain, prepared as total homogenates, showed a mean concentration of 0.6 ng/g ww and the maximum concentration was 4.92 ng/g ww (Lorenzo et al., 2016). In liver from Baltic herring (*Clupea harengus*) and flounder (*Platichthus flesus*) PFBA has been reported at concentrations up to 0.23 and 0.45 ng/g ww, respectively (Lilja et al., 2009). Mink (*Neovison vison*), which to a great extent is a fish-eating mammal, sampled in Sweden had mean and maximum concentrations in the liver of 0.6 ng/g ww and 4.7 ng/g ww, respectively (Persson et al., 2013). Plasma samples from another fish-eating species, Bald eagle (*Haliaeetus leucocephalus*), collected in the USA, were analysed to determine PFBA, and mean and maximum concentrations of 0.55 and 78 ng/mL, respectively, were reported (Route et al., 2014).

PFHpA

More than 50 reports on PFHpA levels in the environment have been found in the scientific literature and these reports demonstrate its worldwide occurrence in the environment, eg. groundwater, surfacewater, seawater, sediment but also wastewater. Eschauzier et al. (2013) reported up to 320 ng/L in groundwater from the Netherlands. Potential point sources in the area are a former landfill and a military camp with an airbase.

In a study by Blaine et al. (2014), celery (*Apium graveolens*) was grown in biosolid-amended soil. The mean concentration in shoots was reported to be 20 ng/g ww. Gobelius et al. (2017) analysed sap from birch (*Betula pendula*) sampled in the vicinity of fire training facilities. Concentrations up to 0.80 ng/L, corresponding to a bioconcentration factor (related to concentration in ground water) of 0.072, were reported. Zhang et al. (2017) report on PFHpA in food from a contaminated site and a site without known contamination. The authors calculated that the total daily intake by adults (fish liver not considered due to lack of data) from the contaminated site was 2.94 ng/kg bw per day and 0.003 ng/kg bw per day at the non-contaminated site. The relative contribution from different food categories was estimated, with almost 100% being attributed to leafy vegetables in the contaminated area whereas some 50% was attributed to each leafy vegetables and offal in the non-contaminated area.

PFHpA has also been detected in biological organisms such as decapods, mussels, fish, birds and mammals (Armstrong et al., 2016; Arvaniti and Stasinakis 2015; Blaine et al., 2014; Campo et al., 2015; Gebbink et al., 2016; Fang et al., 2014; Bytingsvik et al., 2013; Baduel et al., 2014; Furdui et al., 2007; Cervený et al., 2016; Butt et al., 2008). Mean concentrations of PFHpA in Baltic Herring (*Clupea harengus*) and Sprat (*Sprattus sprattus*) were reported to be around 0.025 ng/g ww, whereas maximum concentrations in Lake trout (*Salvelinus namaycush*) whole body homogenate from the US and European Chub (*Squalius cephalus*) liver sampled in Poland were reported to be 1.43 and 27.2 pg/g, respectively. There is no obvious temporal trend for the occurrence of PFHpA in the environment, but the concentrations in biosolids in the US and Spain have been reported to decrease during 2005-2013 and 2010-2011 by Armstrong et al. (2016) and Campo et al. (2014), respectively. In a systematic review and compilation of data (including recalculations) performed by Land et al. (2018), most studies of biota and indoor air and dust show decreasing or non-significant temporal trends. However, two out of three Chinese lake sediments show significant increasing temporal trends.

PFTeDA

Around 25 scientific reports have reported on levels of PFTeDA in the environment. These studies contain results from a worldwide study of wastewater and biosolids (maximum concentration 20 ng/L and 50 ng/g, respectively (Arvaniti and Stasinaki, 2015)), as well as a French study (Munoz et al., 2015) of river sediments showing a maximum concentration of 1.3 ng/g dw. Occurrence in biota is reported from Europe including Greenland, North America, Oceania and Asia. In Europe, mean concentrations of PFTeDA in Baltic Herring (*Clupea harengus*) and Sprat (*Sprattus sprattus*), analysed by Gebbink et al. (2016), were 0.0087 and 0.0027 ng/g ww, respectively. Roos et al. (2013) studied Swedish Otters (*Lutra lutra*) and found a maximum concentration in liver of 11 ng/g ww. The same authors reported on a rapid increase in the concentrations of PFTeDA in Otter liver from 1974 onwards. This increase is supported by results from a study by Kratzer et al. (2011) where increasing concentrations were observed between 1974-2008 in Grey Seal (*Halichoerus grypus*) from the Baltic Sea area. The maximum concentration in liver in this study was 0.7 ng/g ww. Eggs (yolk only) from Swedish Cormorants (*Phalacrocorax carbo*), a fish-eating species, were shown to contain, on average, 10.4 ng/g ww with a maximum of 32.9 ng/g ww (Nordén et al. 2013). Holmström et al. (2010) reported on the occurrence of PFTeDA in Peregrine falcon (*Falco peregrinus*) eggs from 1974 to 2007 and identified an increasing trend up to 2004. The increase during this period was from 0.2 to 3.2 ng/g ww, as read from the author's figure.

PFPeDA

PFPeDA is mainly identified in biological samples from Europe, including Greenland, and the US. Furdui et al. (2007) analysed whole fish homogenates of Lake Trout from Lake Huron and found a mean concentration of 1.10 ng/g ww. In Baltic Herring (*Clupea harengus*) and Sprat (*Sprattus sprattus*), analysed by Gebbink et al. (2016), the concentrations were almost three orders of magnitude lower, while egg yolk from Swedish Cormorants (*Phalacrocorax carbo*), a fish-eating species, were shown to

contain on average 3.23 ng/g ww with a maximum of 24.9 ng/g ww (Nordén et al., 2013). There was no evident temporal trend in the occurrence of PFPeDA in the environment.

PFHxDA

Campo et al. (2014) has presented results from analyses of wastewater and WWTP sludge in Spain. The concentrations in 2011 were 0.04 ng/L and 0.13 ng/g dw, respectively. In another study, Campo et al. (2015) reported a much higher maximum concentration in the Llobregat River system (Spain), being 4.25 ng/L.

There are also a few reports on PFHxDA in biota. Chu et al. (2015) studied PFHxDA in liver, muscle and adipose tissue of Black-footed Albatross (*Phoebastria nigripes*) at Midway Atoll. The authors reported concentrations in liver up to 0.14 ng/g ww. Muscle and adipose tissue samples were all below LOQ (0.08 ng/g). Letcher et al. (2015) assessed PFASs in Herring Gull (*Larus argentatus*) eggs at 19 locations around the Great Lakes and found mean concentrations of PFHxDA up to 0.15 ng/g ww (maximum concentration 0.24 ng/g ww). Rubarth et al. (2011) found PFHxDA in Red-throated Divers (*Gavia stellata*), a mainly fish-eating bird, but no numbers were presented due to sparse and irregular detection.

PFODA

Campo et al. (2014) identified PFODA in influent and effluent wastewater at Spanish Sewage treatment plants (maximum concentrations 300 and 160 ng/L respectively) in 2011. In 2010 PFODA was not detected at the same plants, which could indicate an increasing temporal trend in this matrix.

Letcher et al. (2015) studied PFASs in Herring Gull (*Larus argentatus*) eggs at 19 locations at the Great Lakes and found PFODA in only two out of 114 samples. The concentrations were above LOD (0.012 ng/g ww) but below LOQ (0.030 ng/g ww).

PFBS

PFBS has been identified in the environment in more than 30 studies from Europe, North America, Asia and Africa. In a worldwide study on PFASs in wastewater treatment plants, Arvaniti and Stasinakis (2015) reported concentrations as high as 100 ng/L in wastewater and 80 ng/g ww in biosolids. Möller et al. (2009) assessed PFASs in water from the Rhine/Meuse delta but also River Rhine from Constance to its estuary. Downstream Leverkusen, the authors report a profound increase of the concentration of many PFASs but particularly PFBS and PFBA. This increase became even more obvious upstream Rees. These increases are probably due to the impact from local sources. The maximum concentration of PFBS recorded in the Rhine/Meuse delta was 115 ng/L, as estimated from a figure. Even higher concentrations of PFBS, up to 2900 ng/L, were reported from the Rhine tributary Aare (Lange et al., 2007 as cited by Möller et al., 2009). Möller et al. (2009) estimated a yearly mass flow of around 17 tonnes PFASs from the River Rhine into the delta and further into the North Sea, whereof PFBS represented 5.1 tonnes. In a study from the Netherlands, Eschauzier et al. (2013) reported concentrations in groundwater up to 104 ng/L.

PFBS has also been shown to be available to plants via the root system. Blaine et al. (2014) performed a study on the uptake into pea shoots grown in soil amended with biosolids, and the mean concentration in the shoots was 150 ng/g ww. Gobelius et al. (2017) analysed sap from birch (*Betula pendula*) sampled in the vicinity of fire training facilities. Concentrations up to 6.2 ng/L, corresponding to bioconcentration factors (related to concentration in ground water) up to 0.027 were reported.

Different species of fish from Spain, had a mean and maximum concentration of 4.9 ng/g ww and 7.3 ng/g ww, respectively (Lorenzo et al., 2016). In Germany, PFBS has been detected in Harbour Seal (*Phoca vitulina*) liver (maximum concentration 3.1 ng/g ww) (Ahrens et al., 2009). In the same study a decreasing temporal trend has been observed since 2002. In a systematic review and compilation of data (including recalculations) performed by Land et al. (2018), studies on biota sediments, as well as fresh- and seawater samples showed non-significant temporal trends.

PFHpS

There are around ten studies reporting on the environmental occurrence of PFHpS. These studies contain results from worldwide studies of wastewater and biosolids (maximum concentrations 10 ng/L

and 100 ng/g, respectively (Arvaniti and Stasinaki, 2015)), as well as a French study of river water and sediments showing maximum concentrations of 17 ng/L and 0.15 ng/g dw, respectively.

There are also a few studies on the occurrence of PFHpS in biota. Ahrens et al. (2011b) reported decreasing trends in the concentrations of PFHpS in Tawny Owl (*Strix aluco*) eggs sampled between 1986 and 2009. The mean concentration in eggs was 0.08 ng/g ww. A decreasing temporal trend was also reported by Ahrens et al. (2009) in a study from Germany on Harbor Seal (*Phoca vitulina*) liver, but here the maximum concentration was as high as 53 ng/g ww. The reported decreasing temporal trend as described above, is to some extent supported by observations by Kratzer et al. (2011) studying Grey Seal (*Halichoerus grypus*) in the Baltic Sea area during 1974-2008, where a maximum concentration in liver of 1.3 ng/g ww was recorded in 2000.

PFDS

Occurrence of PFDS in the environment has been reported from Europe including Greenland, North America and one worldwide study. The worldwide study by Arvaniti and Stasinakis (2015) covers PFASs in wastewater treatment plants, and they report concentrations up to 150 ng/L in wastewater and 40 ng/g ww in biosolids.

In biota, PFDS has been found in German Harbor seal (*Phoca vitulina*) liver (maximum concentration 4.1 ng/g ww), in a study by Ahrens et al. (2009). In the same study an increasing trend was reported from 2002 onwards. In the US, Lake trout (*Salvelinus namaycush*) from Lake Erie showed a mean concentration of 9.8 ng/g ww (Furdui et al., 2007). Bald eagle plasma samples (*Haliaeetus leucocephalus*) from the USA were analysed for PFDS by Route et al. (2014) and mean and maximum concentrations of 265 ng/mL and 1400 ng/mL were found, respectively. In Sweden, Roos et al. (2013) reported on increasing concentrations in Otter (*Lutra lutra*) liver between 1972 and 2011. On the other hand, Ahrens et al. (2011b) and Ullah et al. (2014) observed decreasing trends in Tawny Owl (*Strix aluco*) eggs and Herring (*Clupea harengus*) liver during 1986-2009 and 1991-2011, respectively. The inconsistent outcome of studies of environmental temporal trends is confirmed by a systematic review and compilation of data (including recalculations) performed by Land et al. (2018).

PFOSI

Perfluorooctane sulfinic acid (and perfluorooctane sulfinate) was considered in a small number of studies. Liver from Harbour seals sampled in Germany was reported to contain up to 1.7 ng/g ww (Ahrens et al. 2009). Kratzer et al. (2011) assessed PFOSI concentrations in Grey Seal (*Halichoerus grypus*) from the Baltic Sea area during 1974-2008 and reported a maximum concentration in liver of 0.5 ng/g ww in the sample from 1998. Statistically significantly increasing concentrations were observed until 1997, while a statistically significant decrease was seen during 1997-2008.

8:2 FTOH

Environmental occurrence of 8:2 FTOH was studied by a number of research groups. Gewurtz et al. (2013) reported concentrations in air sampled in Arctic Canada (Alert, Nunavut) between 1-10 pg/m³, as estimated from the author's figure. Del Vento et al. (2012) analysed a number of PFASs in eight atmospheric samples from western Antarctic Peninsula and found 8:2 FTOH in all samples ranging from 3.9 – 15.4 pg/m³, with a mean of 9.9 pg/m³. Dreyer et al. (2009) collected high volume air samples on-board research vessels in the Atlantic Ocean and the Southern Ocean in 2007 and 2008. The samples were analysed for a set of PFASs. The concentrations of 8:2 FTOH were between 1.8 and 130 pg/m³, and among the compounds studied, 8:2 FTOH was the analyte that usually was observed at the highest concentrations. Furthermore, the highest concentrations were recorded close to European source regions, whereas the lowest concentrations were found in samples from the Southern Ocean.

These concentrations could be compared with concentrations reported from indoor air, e.g. in Shoeib et al. (2011) in Vancouver, where the geometrical mean was 2900 pg/m³.

It should also be noted that despite the presence of 8:2 FTOH in many environmental matrices, it may also undergo degradation in the environment. For instance, Wang et al. (2009) reported that 8:2 FTOH could be biodegraded in different aerobic soils, with a half-life generally less than seven days. Depending on soil characteristics, around 10 to 30% was shown to be degraded into PFOA.

8:2 monoPaP

No relevant information on the occurrence in the environment was identified.

8:2 diPaP

Very few results above LOD/LOQ have been identified in the literature. Eriksson et al. (2016a) reported the maximum concentration of 8:2 diPaP in eggs of Swedish Common kestrels (*Falco tinnunculus*) to be 0.16 ng/g ww, and Gebbink et al. (2016) reported the mean concentration in zooplankton in the Baltic Sea to be 0.057 ng/g ww. No temporal trend was identified.

EtFOSA

EtFOSA has been identified in a number of biological matrices. Taniyasu et al. (2013) studied its occurrence in blood samples from wild rats and mice in Japan (*Rattus rattus*, *R. norvegicus* and *Apodemus speciosus*). EtFOSA (reported as its acetate, N-EtFOSAA) was identified in most samples and the maximum concentration in rats was 102 ng/mL. Swedish Guillemot (*Uria aalge*) eggs and Grey seal (*Halichoerus grypus*) livers were analysed by Löfstrand et al. (2008) and Kratzer et al. (2011), respectively. The highest concentration in Guillemot eggs was 1.1 ng/g ww, while the geometric mean concentration in Grey seal was 0.03 ng/g ww.

EtFOSE

EtFOSE has often been identified in atmospheric samples. One study in Canada by Gewurtz et al. (2013) reported a mean concentration in ambient air to around 3 pg/m³ (as estimated from an author's figure) and another Canadian study by Ahrens et al. (2011c) reported a mean concentration of 20 pg/m³ in air inside a wastewater treatment plant. Shoeib et al. (2006) collected in 2005 twenty high-volume air samples during a crossing of the North Atlantic and Canadian Archipelago. The concentrations ranged from <1 to 8.9 pg/m³. Dreyer et al. (2009) collected high volume air samples on-board research vessels in the Atlantic Ocean and the Southern Ocean in 2007 and 2008. The samples were analyzed for a set of PFASs. The average concentrations of EtFOSE in the gas phase and the particle phase from the Northern hemisphere were 0.7 and 0.5 pg/m³, respectively. The corresponding figures for the Southern hemisphere were 0.6 and 0.3 pg/m³, respectively.

FC-807

No relevant information on the occurrence in the environment was identified.

1.3.4.5.2 Temporal environmental trends of PFASs for which exposure assessment was performed

In a recent review by Land et al. (2018) environmental trends have been reviewed for nine PFASs, as follows:

- PFPeA and PFHxA; No temporal trends for PFPeA or PFHxA were reported. However, Wang et al. (2013) reported that it is likely that emissions of PFHxA could occur as a result of increasing use of polymers based on 6:2 FTOHs in surface treatment products, as this compound could undergo environmental degradation to PFHxA but also to PFBA and PFBS.
- PFNA; concentrations in biota generally show an increasing trend (21 studies) or no significant trend (19 studies), whereas one study on Beluga Whales (*Delphinapterus leucas*) showed a decreasing trend.
- PFDA; concentrations in biota generally show an increasing trend (21 studies) or no significant trend (20 studies), whereas one study on Beluga whales (*Delphinapterus leucas*) showed a decreasing trend.
- PFUnDA; concentrations in biota generally show an increasing trend (22 studies) or no significant trend (9 studies). Just one study Bald eagle nestlings (*Haliaeetus leucocephalus*)

showed a decreasing trend but this study had a short duration.

- PFDoDA; concentrations in biota generally show an increasing trend (13 studies) or no significant trend (13 studies), whereas one study on Beluga Whales (*Delphinapterus leucas*) showed a decreasing trend since 2000.
- PFTrDA; concentrations in biota generally show an increasing trend (14 studies) or no significant trend (13 studies), whereas one study on Beluga Whales (*Delphinapterus leucas*) shows a decreasing trend since 2000 and another study on Harbor seals (*Phoca vitulina*) since 2004.
- PFHxS; concentrations in biota show an increasing trend in 5 studies whereas no significant trends were observed in 23 studies. In one study on Loggerhead sea turtle (*Caretta caretta*), a previously decreasing temporal trend was in 2000 replaced by an increasing temporal trend as high as 40% per year. On the other hand, significant decreasing temporal trends were reported in one study of fish (Swedish west coast) and two studies of seals (German Bight and Greenland) and one study of Harbor porpoise (North Sea). These results illustrate that the temporal trends could vary in a considerable way between different geographical areas.
- FOSA; two studies showed increasing temporal trends, whereas 8 showed decreasing and 14 showed no significant temporal trends. This is similar to what was observed in corresponding analyses for PFOS.

1.3.4.6 Summary

PFASs constitute a vast and complex group with respect to differences in *i.a.* chain-length, molecular weight, degree and pattern of fluorination and polar functional groups. Therefore, it is probably not meaningful to generalize their properties or environmental fate. From the compilations presented above it is clear that most of the substances selected can be found in environmental samples. These matrices are more or less closely related to food. Matrices as fish and fish-eating birds or mammals could to a large extent serve as indicators of food contamination. Reliable information on the occurrence in such matrices are found for PFBA, PFHpA, PFTeDA, PFPeDA, PFBS, PFHpS, PFDS, PFOSI and FOSA and possibly also for EtFOSA, PFHxDA, and 8:2 diPaP. There are consequently good arguments for further investigations of the occurrence of these compounds in food. The presence of PFASs in groundwater and other freshwater bodies could be an indicator for possible occurrence in drinking water. Reliable information on the occurrence in these matrices is found for PFBA, PFHpA, PFBS and PFHpS. Likewise, these findings support further investigations of the occurrence of these compounds in drinking water.

From the compilation of observations of compounds in this opinion, categorised to have sufficient data for conducting a dietary exposure assessment, it can be concluded that the concentrations of PFASs in the environment show a generally increasing temporal trend. This should be considered when estimating human dietary exposure to these compounds.

Regarding the sixteen compounds presented in more detail in this section, an important observation to be further considered is the increasing temporal trends of some compounds in relevant environmental matrices. Matrices considered relevant are those that, with respect to their content and spatial and/or temporal concentration trends of contaminants, could be associated with the occurrence of the same contaminants in food. Such matrices are e.g. biota inhabiting the same or similar environmental matrices as biota used as food. This could be the case for fish, marine vertebrates and wild mammals. Regarding drinking water, surface- and groundwater, and to some extent also freshwater sediments could be relevant matrices.

Unfortunately, very few studies are performed in such a way that reliable temporal trends could be identified. However, increasing temporal trends in relevant matrices have been identified for PFTeDA and EtFOSA. An increasing trend was also identified for PFODA in wastewater.

One further factor that needs attention is the long-range transport properties of the compounds (usually based on their detection in remote areas). This is the case for PFBA, PFHpA, PFTeDA, PFPeDA, PFBS

and PFDS. It is therefore likely that these compounds are able to stay for a long time in the environment, which could increase the probability for finding these compounds in food.

Finally, substances that have been regulated (banned or with reductions of their production volumes and/or restrictions to certain applications only) generally show a reduction in their occurrence in environmental matrices. As mentioned above and seen from Table 2, different environmental matrices show different temporal trends, indicating possible differences in environmental transport but also in kinetics of the compounds in animal species studied that could influence the outcome.

1.3.5. Previous risk assessments

This chapter reviews risk assessments for PFOS and PFOA that have been published after the 2018 EFSA Contam Panel opinion, and previous risk assessments for PFASs other than PFOS and PFOA.

In 2018 the EFSA CONTAM Panel established a tolerable weekly intake (TWI) of 13 ng/kg bw per week for PFOS and 6 ng/kg bw per week for PFOA. The derivation of TWIs was based on human epidemiological studies. For PFOS, the increase in serum total cholesterol in adults, and the decrease in antibody response at vaccination in children were identified as the critical effects. For PFOA, the increase in serum total cholesterol was the critical effect. Also reduced birth weight (for both compounds) and increased prevalence of high serum levels of the liver enzyme alanine aminotransferase (ALT) (for PFOA) were considered.

In 2012 the Swedish Environmental Protection Agency published an Environmental and Health Risk Assessment of 23 PFASs in Sweden. Hepatotoxicity and reproductive toxicity were identified and selected as toxicological endpoints common for PFASs. For PFASs lacking internal dose measurements or toxicity data (PFHxA, PFHpA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, 6:2 FTSA, PFBS, PFDS and FOSA) a read-across was carried out to the closest most potent congener for the respective endpoint. For the general population the risk characterization did not indicate any cause of concern for reproductive toxicity or hepatotoxicity, neither for most assessed individually nor in combination. In the general population, for hepatotoxicity RCRs (risk characterisation ratios) were highest for PFOS (0.17) and PFOA (0.04) contributing in total with 77% to the cumulative RCRs. For PFNA and PFHxS, RCRs of 0.014 and 0.0048 were calculated. For reproductive toxicity, the highest RCR was identified for PFOS (0.14), contributing with 76% to the cumulative RCR. RCRs for PFOA, PFNA and PFHxS were, respectively 0.008, 0.0073 and < 0.0035 (Swedish Environmental Protection Agency, 2012).

In 2015 the Danish Environmental Protection Agency stated that for FOSA no sufficient data were available for derivation of a specific TDI. However, because FOSA is the amide derivate of PFOS and is used as a precursor for PFOS formation, the Danish Environmental Protection Agency concluded that it seems justifiable to apply the TDI for PFOS (0.03 µg/kg bw per day) on FOSA as well (Danish EPA, 2015).

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) published an opinion on PFBA, PFHxA, PFBS and PFHxS (ANSES 2015). Chronic indicative toxicity values (iTV) were established for PFBA (0.024 mg/kg bw per day) and PFHxS (0.004 mg/kg bw per day) based on liver effects (increase in absolute and relative liver weight, hepatocellular hypertrophy) in rodents. Chronic toxicity reference values (TRV) were established for PFHxA (0.32 mg/kg bw per day) and PFBS (0.08 mg/kg bw per day) based on kidney effects in rodents.

In 2017, the German Human Biomonitoring (HBM) Commission established drinking water guide values (TW_{LW}, Trinkwasser-Leitwerte) for PFBA (TW_{LW} 10 µg/L), PFHxA (TW_{LW} 6 µg/L), PFHpA (TW_{LW} 10 µg/L), PFOA (TW_{LW} 0.1 µg/L), PFNA (TW_{LW} 0.06 µg/L), PFBS (TW_{LW} 6 µg/L), PFHxS (TW_{LW} 0.1 µg/L), PFOS (TW_{LW} 0.1 µg/L) or Health-based orientation values (GOWs, gesundheitliche Orientierungswerte) for PFPeA (GOW 3.0 µg/L), PFHpA (GOW 0.3 µg/L), PFDA (GOW 0.1 µg/L), PFHPs (GOW 0.3 µg/L) and FOSA (GOW 0.1 µg/L) (Bundesgesundheitsblatt 2017, 60:350-352).

In 2017, FSANZ (Food Standards Australia New Zealand) published a hazard assessment report for PFOS, PFOA and PFHxS. With respect to PFHxS, FSANZ stated that there was insufficient toxicological and epidemiological information to justify establishing a TDI. They concluded that the Environmental Health Standing Committee (enHealth) 2016 approach of using the TDI for PFOS (0.02 µg/kg bw per

day based on decreased parental and offspring body weight gains in a multi-generation reproductive toxicity study in rats) is likely to be conservative and protective of public health as an interim measure. For PFOA, a TDI 0.16 µg/kg bw per day based on a NOAEL for fetal toxicity in a developmental and reproductive study in mice was recommended (FSANZ, 2017).

In 2017, the Department of Environmental Protection (New Jersey, US) developed a Health-based Maximum Contaminant Level (MCL) for PFOA of 0.014 µg/L using a risk assessment approach intended to protect for chronic (lifetime) drinking water exposure (DEP, 02/2017). For PFOS, an MCL of 0.013 µg/L has been proposed (DEP, 11/2017) and for PFNA an MCL of 0.013 µg/L. The PFNA MCL is based on increased liver weight (BMD10) in pregnant mice exposed to PFNA for 16 days (DEP 10/2017).

In 2018 ATSDR has prepared a draft for public comment on the Toxicological Profile for 14 PFASs (PFOS, PFOA, PFBA, PFHxA, PFHpA, PFNA, PFDA, PFUnDA, PFDoDA, PFBS, PFHxS, FOSA, 2-(N-methyl-perfluorooctanesulfonamido)acetic acid, 2-(N-ethyl-perfluorooctane-sulfon-amido)acetic acid. The document summarizes that sufficient data are available for derivation of provisional intermediate-duration oral MRLs for PFOA, PFOS, PFHxS, and PFNA based on laboratory animal data. The databases were not considered adequate for derivation of MRLs for the other PFASs. Hepatic, immune, and developmental endpoints were the most sensitive targets for PFOA (MRL 0.003 µg/kg per day) and PFOS (MRL 0.002 µg/kg per day), hepatic (Increased liver weight and centrilobular hepatocellular hypertrophy) and thyroid endpoints (thyroid follicular cell damage, hypertrophy, and hyperplasia) for PFHxS (MRL 0.002 µg/kg per day) and decreased body weight gain and developmental delays in the offspring for PFNA (MRL 0.003 µg/kg per day).

In 2018 RIVM published a Relative Potency Factor (RPF) approach for 19 PFASs and selected PFOA as the Index Compound. Liver toxicity in rodents, as revealed by liver hypertrophy (hepatocellular, centrilobular) and accompanying liver enlargement, i.e. absolute and relative liver weight, was selected as the critical effect and the basis to derive RPFs for all 19 PFASs. For PFASs with no experimental animal data available for the selected endpoint a read across approach was applied. The assessed PFAS (with the respective RPF reported in bracket) were: PFBA (0.05), PFPeA (0.01-0.05), PFHxA (0.01), PFHpA (0.01-1), PFOA (1), PFNA (10), PFDA (4-10), PFUnDA (4), PFDoDA (3), PFTrDA (0.3-3), PFTeDA (0.3), PFHxDA (0.02), PFODA (0.02), PFBS (0.001), PFPeS (0.001-0.6), PFHxS (0.6), PFHpS (0.6-2), PFOS (2).

Michigan Science Advisory Workgroup recommended Health-based Drinking Water Values (HBV) for six PFASs (Michigan Science Advisory Workgroup⁷¹, 2019). The recommended HBV for PFHxA (400,000 ng/L), PFOA (8 ng/L), PFNA (6 ng/L), PFBS (420 ng/L), PFHxS (51 ng/L) and PFOS (16 ng/L) were based on experimental animal studies. It is also stated in the document that based on the similarity in toxicity for the long-chain PFASs, the Workgroup recommends the use of the established HBV for PFNA as a screening value for other long-chain PFASs, which are included in the US EPA method 537.1 but for which so far not enough information is available to support HBVs.

1.3.6. Legislation

The legal status for PFOS and PFOA was summarised in the Scientific Opinion on the risk to human health related to the presence of perfluorooctanesulfonic acid and perfluorooctanoic acid in food (EFSA CONTAM Panel, 2018). PFOS, including its salts and perfluorooctane sulfonyl fluoride (PFOSF), is now listed in Annex I of the POP Regulation (Regulation (EU) 2019/1021) and added to Annex B (Restriction) to the Stockholm Convention. These documents are relevant for considerations of whether other PFAS are to be considered as PFOS-related substances e.g. as precursors. The definition of PFOS-related substances is described in "Guidance on PFOS, its salts and PFOSF."⁵

Regarding PFOA, in 2013, the Member State Committee, referred to in Article 76(1)(e) of Regulation (EC) No 1907/2006, identified PFOA as a persistent, bioaccumulative and toxic (PBT) substance, in accordance with Article 57(d) of that Regulation. PFOA was also in 2013 included in the Candidate List of Substances of Very High Concern (SVHC), for possible inclusion into Annex XIV to Regulation (EC) No 1907/2006. By means of Commission Regulation (EU) 2017/1000, PFOA was included in Annex XVII

⁵ UNEP/POPS/POPRC.12/INF/15/Rev.1

to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards PFOA, its salts and PFOA-related substances. In May 2019 the COP (Conference Of Parties) of the Stockholm Convention adopted a corresponding, but slightly more comprehensive, regulation of PFOA. In November 2019 the Commission presented a proposal on how to amend Annex I to Regulation (EU) 2019/1021 of the European Parliament and of the Council as regards the listing of perfluorooctanoic acid (PFOA), its salts and PFOA related compounds. Deadline for responses from memberstates was December 5th 2019. This regulation would open the field for classifying certain PFAS, *e.g.* as precursors, as PFOA-related based on their structural properties. The term PFOA-related compounds has been defined by POPRC (2017)⁶ as:

"(a) PFOA (pentadecafluorooctanoic acid, CAS No: 335-67-1, EC No: 206-397-9) including any of its branched isomers;

(b) Its salts; and

(c) PFOA-related compounds which, for the purposes of this risk management evaluation, are any substances that degrade to PFOA, including any substances (including salts and polymers) having a linear or branched perfluoroheptyl group with the moiety (C7F15)C as one of the structural elements, for example:

(i) Polymers with ≥C8 based perfluoroalkyl side chains;

(ii) 8:2 fluorotelomer compounds;

(iii) 10:2 fluorotelomer compounds."

In practice this makes it possible to classify a number of PFASs as PFOA- or PFOS-related. This is described in more detail below under each substance.

In contrast to PFOS and PFOA, no other PFASs addressed in this opinion have so far been legally restricted in Europe with respect to production, marketing or use. There are however, a number of initiated or on-going activities with the aim to reduce human and environmental risk connected to a number of PFASs discussed in this opinion. C9-C14 PFCA are included in the SVHC candidate list which implies certain terms of their use.

PFBA and PFPeA

No activities identified.

PFHxA

A risk management option analysis (RMOA) performed by Germany and SVHC (Substance of very high concern) classification was proposed but later withdrawn⁷. A restriction proposal was submitted in December 2019⁸.

PFHpA

Substance evaluation is performed by Belgium. A proposal for harmonized classification as Repr. 1B⁹, H360D, and STOT RE 1¹⁰, H372, is under development¹¹.

PFNA, PFDA, PFUnDA, PFDODA, PFTrDA and PFTeDA

⁶ <http://www.pops.int/Default.aspx?tabid=5965> → UNEP/POPS/POPRC.13/7/Add.2

⁷ <https://echa.europa.eu/sv/registry-of-svhc-intentions/-/dislist/details/0b0236e1827be5e2>

⁸ <https://echa.europa.eu/sv/registry-of-restriction-intentions/-/dislist/details/0b0236e18323a25d>

⁹ H360 May damage fertility or the unborn child

¹⁰ H372 Causes damage to organs through prolonged or repeated exposure

¹¹ <https://echa.europa.eu/sv/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e18333861c>

1204 Proposals on restriction of PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA have passed the ECHA
1205 RAC (Risk Assessment Committee) and SEAC (Socioeconomic Assessment Committee) and the proposal
1206 have been submitted to the European Commission.¹²

1207 **PFPeDA, PFHxDA and PFODA**

1208 Under the scope of the PFOA-restriction in the Stockholm Convention (includes related substances in
1209 polymers up to C18).¹³

1210 **PFBS**

1211 A RMOA was developed by Norway.¹⁴ Proposal on SVHC was submitted in August 2019.¹⁵ At ECHA's
1212 67th Memberstates Committee Meeting, in December 2019, PFBS was classified as SVHC with
1213 reference to article 57 f¹⁶. **PFHxS**

1214 A SVHC under REACH based on its vPvB-properties (very Persistent and very Bioaccumulative)¹⁷, passed
1215 screening criteria for inclusion into Annex A of the Stockholm Convention.¹⁸ Proposal on EU-restriction
1216 was submitted by Norway in April 2019.¹⁹ A risk profile was adopted by POPRC²⁰. Risk Management
1217 Evaluation to be discussed at nCOP21²¹.

1218 **PFHpS and PFDS**

1219 No activities identified.

1220 **8:2 FTOH, 8:2 monoPAP and 8:2 diPAP**

1221 All three substances are PFOA related.

1222 **PFOSI, FOSA, EtFOSA, EtFOSE and FC-807**

1223 All five substances are PFOS-related and should therefore fall under the current PFOS regulation.

1224 **2. Data and Methodologies**

1225 **2.1. Data**

1226 **2.1.1. Occurrence in food data**

1227 **2.1.1.1. Data collection and validation**

1228 Following an EC mandate to EFSA, a call for annual collection of chemical contaminant occurrence data
1229 in food, including PFASs, was issued by the former EFSA Dietary and Chemical Monitoring Unit (now
1230 DATA Unit)²² in December 2010 with a closing date of 01 October of each year²³. European national
1231 authorities and similar bodies, research institutions, academia, food business operators and other
1232 stakeholders were invited to submit analytical data on PFASs in food. The data for PFASs were provided

¹² <https://echa.europa.eu/documents/10162/5aabe3cc-a317-4b2f-5446-5fc22c522c31>

¹³ <http://www.pops.int/Default.aspx?tabid=5965> → UNEP/POPS/POPRC.13/7/Add.2

¹⁴ <https://echa.europa.eu/registry-of-restriction-intentions/-/dislist/details/0b0236e1827f87da>

¹⁵ <https://echa.europa.eu/registry-of-svhc-intentions/-/dislist/details/0b0236e182bbccf8>

¹⁶ <https://echa.europa.eu/-/four-new-substances-added-to-candidate-list>

¹⁷ <https://www.echa.europa.eu/documents/10162/20a23653-34b1-bb48-4887-7ea77bedc637>

¹⁸ <http://www.pops.int/Default.aspx?tabid=5965> → POPRC-13/3. Risk profile under development.

¹⁹ <https://echa.europa.eu/registry-of-restriction-intentions/-/dislist/details/0b0236e1827f87da>

²⁰ <http://chm.pops.int/TheConvention/POPsReviewCommittee/Meetings/POPRC14/Overview/tabid/7398/Default.aspx> → UNEP/POPS/POPRC.14/6/Add.1

²¹ <http://chm.pops.int/TheConvention/POPsReviewCommittee/Meetings/POPRC15/Overview/tabid/8052/Default.aspx>

²² From 1 January 2014 onwards, Evidence Management Unit (DATA).

²³ <http://www.efsa.europa.eu/en/data/call/datex101217>

by national authorities from Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Malta, Norway, Slovenia, Spain and the United Kingdom (UK).

The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010a); occurrence data were managed following the EFSA standard operational procedures (SOPs) on 'Data collection and validation' and on 'Data analysis of food consumption and occurrence data'.

By the 16th of May 2018, a total of 97,448 results (representing 11,528 samples) of food and beverages with analytical data on PFASs were available in the EFSA database.

Data received after that date were not included in the dataset used for further evaluation for this opinion.

2.1.1.2. Data analysis

Following the EFSA SOP on 'Data analysis of food consumption and occurrence data' to guarantee an appropriate quality of the data used in the exposure assessment, the initial dataset was carefully evaluated applying several data cleaning and validation steps. Special attention was paid to different parameters such as 'Sampling strategy', 'Sampling method', 'Sampling year', 'Sampling country', 'Analytical methods', 'Reporting unit', 'Limit of detection', and the codification of the different samples under FoodEx classification. The outcome of the data analysis is presented in Section 3.1.1.

In the analysis of PFAS occurrence data, the left-censored data (results below LOD or below LOQ) were treated by the substitution method as recommended in the "Principles and Methods for the Risk Assessment of Chemicals in Food" (WHO, 2009). The same method is indicated in the EFSA scientific report "Management of left-censored data in dietary exposure assessment of chemical substances" (EFSA, 2010b) as an option in the treatment of left-censored data. The guidance suggests that the lower bound (LB) and upper bound (UB) approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins). The LB is obtained by assigning a value of zero (minimum possible value) to all samples reported as lower than the LOD (< LOD) or LOQ (< LOQ). The UB is obtained by assigning the numerical value of LOD to values reported as < LOD and LOQ to values reported as < LOQ (maximum possible value), depending on whether LOD or LOQ is reported by the laboratory.

2.1.2. Consumption data

2.1.2.1. Food consumption data

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) provides a compilation of existing national information on food consumption at individual level. Details on how the Comprehensive Database is used are published in the Guidance of EFSA (EFSA, 2011a). The food consumption data gathered by EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. The latest version of the Comprehensive Database updated in 2018 contains results from a total of 60 different dietary surveys carried out in 25 different Member States covering 119,458 individuals. The age classes considered are the following:

Infants:	< 12 months old
Toddlers:	≥ 12 months to < 36 months old
Other children:	≥ 36 months to < 10 years old
Adolescents:	≥ 10 years to < 18 years old
Adults:	≥ 18 years to < 65 years old
Elderly:	≥ 65 years to < 75 years old
Very elderly:	≥ 75 years old

Four additional surveys provided information on specific population groups: 'Pregnant women' (≥ 15 to ≤ 45 years old for Latvia; 17 to 46 years for Portugal) and 'Lactating women' (≥ 28 to ≤ 39 years old

for Greece; 18 to 45 years for Estonia). When for one country and age class two different dietary surveys were available, only the most recent one was used. Additionally, for chronic exposure assessment, surveys that lasted only 1 day were excluded. This resulted in a total of 38 different dietary surveys carried out in 23 different European countries used for the chronic dietary exposure assessment. In Annex A, Table A1, these dietary surveys and the number of subjects available for the chronic exposure assessment are described.

Overall, the food consumption data gathered by EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. Consumption data were collected using single or repeated 24- or 48-hour dietary recalls or dietary records covering from three to seven days per subject. Owing to the differences in the methods used for data collection, direct country-to-country comparisons can be misleading.

2.1.2.2. Food classification

Consumption data were classified according to the FoodEx classification system (EFSA, 2011b). FoodEx is a food classification system developed by EFSA in 2009 with the objective of simplifying the linkage between occurrence and food consumption data when assessing the exposure to hazardous substances. It contains 20 main food categories (first level), which are further divided into subgroups having 140 items at the second level, 1,261 items at the third level and reaching about 1,800 end-points (food names or generic food names) at the fourth level.

In 2011, a new version of FoodEx, named FoodEx2 has been developed and is described in the scientific document "Report on the development of a Food Classification and Description System for exposure assessment and guidance on its implementation and use" (EFSA, 2011c). The last release of FoodEx2 complements the previous hierarchical classification system of basic codes with more detailed food levels and gives the possibility of reporting additional information through the use of facets and facet descriptors (EFSA, 2015).

2.1.3. Toxicokinetic and Toxicological data

Data were obtained from the scientific literature as described in Section 2.2.2.

2.2. Methodologies

2.2.1. Dietary Exposure assessment

The CONTAM Panel considered it appropriate to estimate only chronic exposure to PFASs (see Section 3.4). As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011a), dietary surveys with only 1 day per subject were not considered as they are not adequate to assess repeated exposure. Similarly, subjects who participated only 1 day in the dietary studies, when the protocol prescribed more reporting days per individual, were also excluded for the chronic exposure assessment. Thus, for chronic exposure assessment, food consumption data were used from 35 different and most recent dietary surveys carried out in 19 different European countries present in the latest version of the Comprehensive Database (Annex A, Table A1). Not all countries provided consumption information for all age groups, and in some cases the same country provided more than one consumption survey.

For calculating chronic dietary exposure to PFASs, food consumption and body weight data at the individual level were accessed in the Comprehensive Database.

Occurrence concentrations were averaged at FoodEx level 1, 2 or 3 depending on the food category. For Meat and meat products, and Milk and dairy products, occurrence was averaged at FoodEx level 2 to allow for a more detailed assessment and for Fish and other seafood at FoodEx level 3 to account for different fish species. When the available number of samples for a certain food category was less than 6 or zero, the mean occurrence value of the parent level was used in the analysis if available. FoodEx 1 level categories with less than 6 samples were retained in the analysis and the mean occurrence value calculated on the available samples.

In cases where the food consumption data are only specified at a higher FoodEx level, like fish meat, the mean occurrence value of certain FoodEx level 1 categories could be strongly affected by high occurrence values in FoodEx level 2 categories that are commonly analysed, but that are rarely consumed (e.g. Meat and Meat products mean affected by occurrence results in Edible offal from game mammals).

To mitigate this effect, all FoodEx level 2 occurrence values for all food categories were weighted against their consumption amount when calculating the mean for FoodEx level 1. The same was done with FoodEx level 3 categories when calculating the mean occurrence value of FoodEx level 2 categories related to Fish and Fish products.

To assess the combined exposure to PFOA, PFNA, PFHxS and PFOS the mean occurrence value of each of the four PFASs for each food category was summed and considered to be the combined occurrence of these PFASs within the food category.

Occurrence data and consumption data were then linked at the appropriate FoodEx level.

The mean and the high (95th percentile) chronic dietary exposures were calculated by combining mean PFAS occurrence values for food samples collected in different countries (pooled European occurrence data) with the average daily consumption for each food at individual level in each dietary survey and age class. Consequently, individual average exposures per day and body weight were obtained for all individuals. On the basis of distributions of individual exposures, the mean and 95th percentile exposure were calculated per survey and per age class. Dietary exposure was assessed using overall European LB and UB mean occurrence values of PFASs.

All analyses were run using the SAS Statistical Software (SAS enterprise guide 5.1).

2.2.2. Literature search and appraisal of studies

EFSA outsourced an extensive literature search related to the oral toxicity of PFASs, their precursors and potential replacements, in experimental animals and humans (contract: RC/EFSA/BIOCONTAM/2012/02). The aim of the assignment was to identify and collect all relevant literature regarding PFASs. The search was performed in March 2013. The methodology and the results are detailed in Bull et al. (2014).

The following areas were covered:

- Area 1: Data on toxicokinetics (absorption, distribution, metabolism and excretion) in *in vitro* studies, experimental animals and humans.
- Area 2: Data on toxicity in experimental animals (i.e acute and repeat dose toxicity, immunotoxicity, developmental and reproductive toxicity, neurotoxicity, carcinogenicity and other effects).
- Area 3: Data on observations in humans, including epidemiology, case reports and biomarkers of exposure and effects.

In addition to the literature search outsourced by EFSA, further literature searches were performed covering up to 16 August 2019, for the above 3 areas, in order to cover peer-reviewed literature published between the beginning of 2013 and August 2019. Further search strategies were designed to identify literature published after 2007, which covered additional areas, including, chemistry, analysis, synthesis, production, use, environmental fate, food occurrence and human exposure. An overview of the search terms is given in Appendix N (Table N.1).

Web of Science²⁴ and PubMed²⁵ were identified as databases appropriate for retrieving literature for the present evaluation. The references resulting from the literature search were imported and saved using a software package (EndNote²⁶), which allows effective management of references and citations.

Reviews, relevant scientific evaluations and assessments by national or international bodies were also considered for the current risk assessment. When relevant papers were identified during the risk assessment process (e.g. from other studies or reviews), they were also considered.

The references obtained were screened using title and abstract to identify relevant literature.

The information retrieved was subsequently reviewed by the CONTAM working group (WG) on PFASs in food and has been used for the present assessment based on expert judgement. Selection of the scientific papers for inclusion or exclusion was based on consideration of the extent to which the study was relevant to the assessment and general study quality considerations.

2.2.3. Methodology applied for risk assessment

The CONTAM Panel applied the general principles of the risk assessment process for chemicals in food as described by WHO (2009), which include hazard identification and characterisation, exposure assessment and risk characterisation. In addition to the principles described by WHO (2009), any EFSA guidance pertaining to risk assessment and relevant for the present assessment has been duly considered (see Appendix O).

3. Assessment

3.1. Occurrence data

3.1.1. Current occurrence data in food

An initial number of 97,434 results for food samples analysed on PFASs from 16 European countries were available for the assessment. The major contributor of data on PFASs in terms of number of results, was Norway which reported 38% of the data, followed by Germany (33%) and France (15%). In terms of number of samples, Germany provided 58% of all data.

The current data were not systematically checked for possible duplicate occurrence with the data reported in Section 3.1.3. This might have resulted in a partial overlap between the data reported in the scientific literature and the data reported to EFSA and used in the current exposure assessment.

Data were reported on samples collected between the years 2000 and 2016 with 74% of the samples collected after 2007.

Approximately 79% of the data were obtained from samples collected within official monitoring programs, 7% from dietary exposure studies, 11% from surveys and 3% from a combination of programs.

Most results were obtained by LC-MS/MS based methods (86%). Very few (<1%) results were reported as obtained using GC based methods. For the remaining data, no information on analytical methods was reported.

All analytical results were expressed on whole weight basis, thus no conversion had to be applied.

A proportion of analytical results (9%) was obtained from pooled samples, meaning that the result represented an average of a number of samples taken in equal parts from different consignments/batches and pooled together for the laboratory analysis. Since the level of aggregation

²⁴ Web of Science (WoS), formerly ISI Web of Knowledge, Thomson Reuters. Available at: <http://thomsonreuters.com/thomson-reuters-web-of-science/>

²⁵ PubMed, Entrez Global Query Cross-Database Search System, National Center for Biotechnology Information (NCBI), National Library of Medicine (NLM), Department of the National Institutes of Health (NIH), United States Department of Health and Human Services. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/>

²⁶ EndNote X5, Thomson Reuters. Available at: <http://endnote.com/>

for pooled samples matched the level of classification of the individual samples (only similar food matrices were pooled together) results from pooled samples were retained for further evaluation. To ensure a proportionate representation of the individual samples and thus an accurate use of occurrence data in assessing the dietary exposure, the mean concentrations per food category were calculated by weighting the reported analytical results for the number of samples pooled.

Quantitative data were available for only 17 PFASs out of the 28 for which results were available. Overall, 93.5% of the results were left-censored (below LOQ or LOD).

The number of reported analytical results for 28 different PFASs and the % of left-censored results for each PFAS are presented in Table 3 (see also Appendix B, Table B1).

Table 3: List of PFASs for which data were reported (before applying exclusion criteria)

PFASs ^a	Acronyms	N of results	% LC ^c
Perfluoroalkyl carboxylic acids (PFCAs)			
Perfluorobutanoic acid	PFBA	3,096	98.6
Perfluoropentanoic acid	PFPeA	4,882	95.7
Perfluorohexanoic acid	PFHxA	7,019	96.4
Perfluoroheptanoic acid	PFHpA	4,882	98.4
Perfluorooctanoic acid	PFOA	11,506	91.3
Perfluorononanoic acid	PFNA	6,970	95.4
Perfluorodecanoic acid	PFDA	6,979	94.2
Perfluoroundecanoic acid	PFUnDA	4,885	93.8
Perfluorododecanoic acid	PFDoDA	5,234	95.0
Perfluorotridecanoic acid	PFTrDA	2,673	95.1
Perfluorotetradecanoic acid	PFTeDA	2,992	98.9
Perfluoropentadecanoic acid	PFPeDA	14	100
Perfluorohexadecanoic acid	PFHxDA	1,886	100
Perfluorooctodecanoic acid	PFODA	1,865	100
Perfluoroalkane sulfonic acids (PFSAAs)			
Perfluorobutane sulfonate	PFBS	6,370	98.4
Perfluorohexane sulfonate	PFHxS	6,010	98.2
Perfluoroheptane sulfonate	PFHpS	1,387	99.9
Perfluorooctane sulfonic acid	PFOS	11,476	75.2
Perfluorodecane sulfonate	PFDS	3,418	99.5
Perfluoroalkane sulfinic acids			
Perfluorooctane sulfinic acid	PFOSi	320	100
n:2 Fluorotelomer alcohols (n:2 FTOHs)			
8:2 fluorotelomer alcohol	8:2 FTOH	36	100
n:2 polyfluoroalkyl phosphoric acid esters (PAPs)			
8:2 fluorotelomer alcohol (8:2 FTOH) mono-phosphate	8:2 monoPAP	22	100
8:2 fluorotelomer alcohol (8:2 FTOH) di-phosphate	8:2 diPAP	12	100
Perfluoroalkane sulfonamides (FASAs)			
Perfluorooctane sulfonamide	FOSA	3,361	93.3
Perfluorooctane sulfonylamide ^b		36	100
N-ethyl perfluoroalkane sulfonamides (EtFASAs)			
N-ethyl perfluorooctane sulfonamide	EtFOSA	46	100
N-ethyl perfluoroalkane sulfonamidoethanol (EtFASEs)			
N-ethyl perfluorooctane sulfonamidoethanol	EtFOSE	45	100
Perfluoroalkyl phosphate			
Perfluoroalkyl phosphate	FC-807	12	100

Total		97,434	93.5
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N: number; LC: left-censored.

(a): PFAS names as reported by data providers. They may differ from those reported in Table 1.

(b): There is no acronym available, and it was not clear to which PFAS the compound refers to, and therefore it was not considered for further evaluation. These data were not used for the assessment.

(c) The % LC was calculated over the number of analytical results and not weighted for the number of samples included in pooled samples.

As stated in Section 2.1.1, the occurrence data were carefully evaluated and a list of validation steps was applied before being used to estimate dietary exposure.

Outdated results may not reflect current levels of contamination. Therefore, only results from samples collected after the beginning of 2007 were retained for the further assessment and samples collected before then (n=2,587) were excluded.

Particular attention was paid to data reported as suspect samples. Suspect samples are samples taken repeatedly from the same site as a consequence of evidence or suspicion of contamination, and are often taken as a follow-up of demonstrated non-compliance. Because inclusion of these data may result in an overestimation of the contamination levels, the CONTAM Panel decided to exclude them (n=486) from further analysis. However, it can not be excluded that some of the remaining samples included in the analysis may also have been collected in a targeted way, including suspect samples not reported as such or samples sampled under other targeted criteria.²⁷

In addition, 16 samples of cow's milk from Germany (144 results) were discarded based on the indication of the data provider that concentrations were determined with a non-validated method. These samples showed unusually high PFOA levels (>1 ng/mL), whereas a study with dairy cows showed poor transfer of PFOA into milk (Kowalczyk et al., 2013). For EFSA, this was a reason to start an inquiry.

The analytical results (n=36) with unclear identification of the PFAS compound, reported as perfluorooctane sulfonylamide, were excluded from further evaluation.

The Commission Recommendation 2010/161/EC²⁸ recommends a LOQ of 1 µg/kg for the monitoring of PFASs in food. The LODs/LOQs of the PFAS data reported to EFSA varied between laboratories, food matrices and substances. A high percentage of results below LOD/LOQ in combination with high LODs/LOQs increases the uncertainty associated with the dietary exposure estimations. In order to reduce the impact of this, the recommended LOQ cut-off of 1 µg/kg was applied to all food categories with two exceptions. One was "Drinking water" for which a cut-off of 0.01 µg/kg was applied, because of the high intake of water and reported low LOQs/LODs. The other one was the food categories of "Edible offal, game animals" and "Fish offal" for which no cut-off was applied to avoid the loss of too many quantitative samples. For those results for which only the LOD was available, the LOQ was calculated by multiplying the LOD by a factor of 3. Quantified results for which neither the LOD nor the LOQ was available, with an occurrence value greater than 1 µg/kg and not belonging to the food categories "Edible offal, game animals" and "Fish offal", were excluded from the final dataset (n=217). Quantified results for which neither the LOD nor the LOQ was available, with an occurrence value below 1 µg/kg (implying a low LOQ), were retained in the final dataset (n=1271).

For FTOH, all results (n=36) were generated using a method with a LOQ > 1 µg/kg, thus all data for this substance were discarded. As all results were left-censored the substance would not have been included anyway in the exposure assessment.

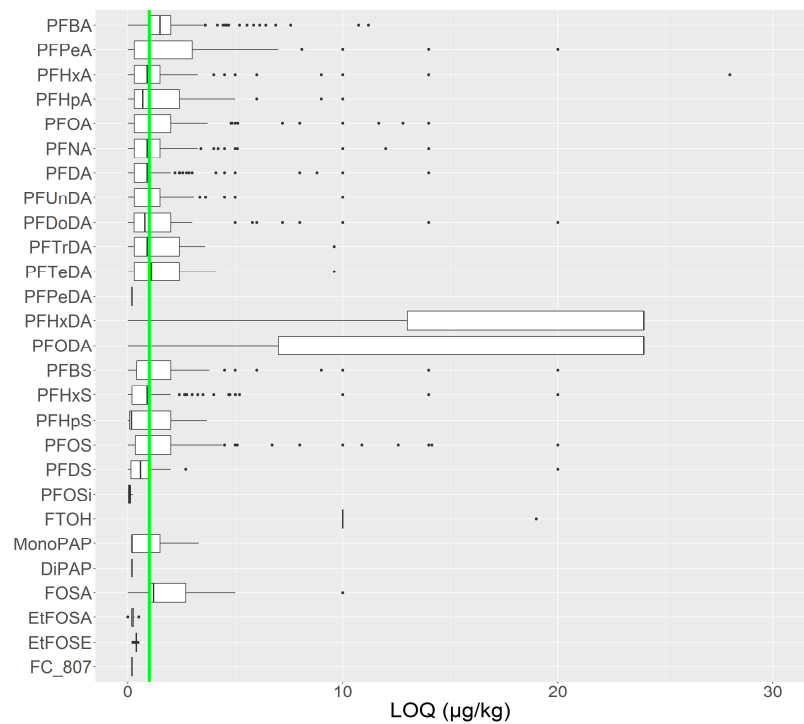
An additional 24,531 results were excluded following the application of the 1 µg/kg cut-off. Out of these, 99% were left-censored. After cut-off application and removal of quantified results with missing LOQ information, 92% of the results were left-censored.

Annex A Table A2 shows the effect of the LOQ cut-off application on data availability and mean occurrence values for each food category.

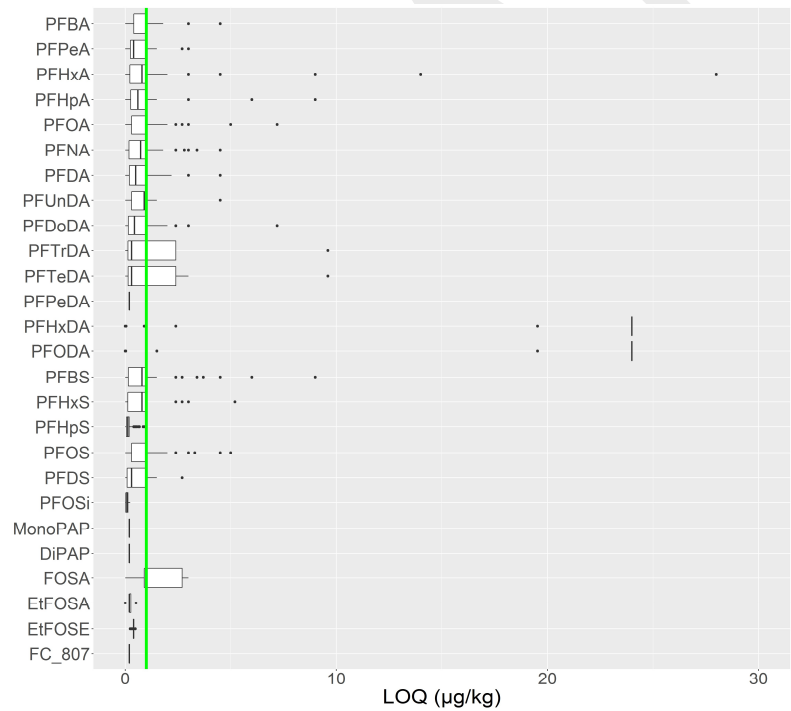
Figure 2 shows the distribution of the reported LOQs before and after applying cut-offs.

²⁷ Targeted samples are based on certain sampling criteria e.g. monitor the background contamination in matrices with high accumulation potential, controlling compliance with the maximum levels laid down in the relevant legislation.

²⁸ Commission Recommendation of 17 March 2010 on the monitoring of perfluoroalkylated substances in food (2010/161/EU).



1462



1463

1464 Box-plot showing whiskers at minimum and maximum, box at P25 and P75 with line at P50, outliers shown
1465 as dots. The vertical green line shows the 1 µg/kg LOQ cut-off, applied to most foods categories except
1466 drinking water (0.01 µg/kg) and "Edible offal, game animals" and "Fish offal" (no cut-off).

1467 **Figure 2:** Distribution of the limits of quantification (LOQs) of PFASs for reported results before
1468 (upper figure) and after (lower figure) applying the LOQ cut-off

As a consequence of the cleaning, a total of 28,001 analytical results were excluded (Annex A Table A3).

The final validated dataset contained 69,433 analytical results for food samples analysed for 26 PFASs.

The analytical results included in the final dataset were as in the original dataset, mostly from Norway (24,869 analytical results), Germany (19,980 analytical results) and France (13,594 analytical results) (Annex B, Table B2). It should be noted that the origin of the data was not always the European country reporting the data, i.e. the dataset also contained samples originating from North and South America, Africa, Asia and Australia, but available on the European market. The distribution of analytical results per sampling country and sampling year are summarised in Figures 3 and 4 and Annex B, Tables B2-B4.

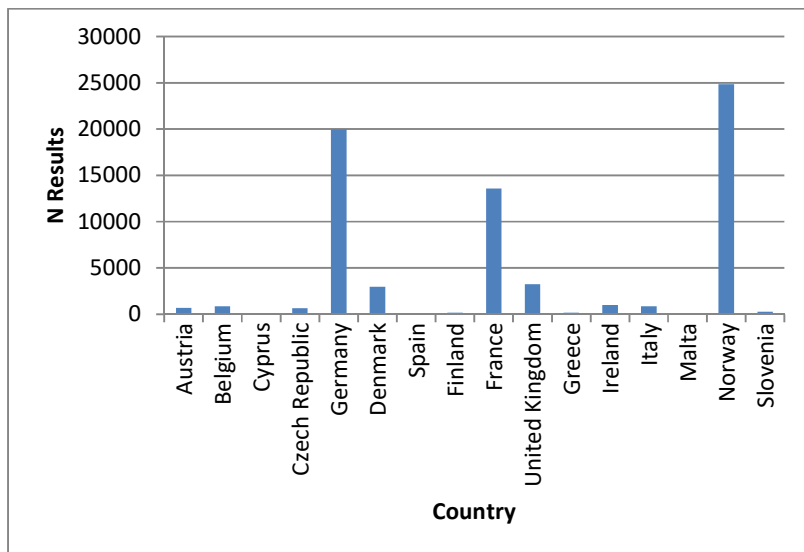


Figure 3: Distribution of analytical results for PFASs divided by sampling country

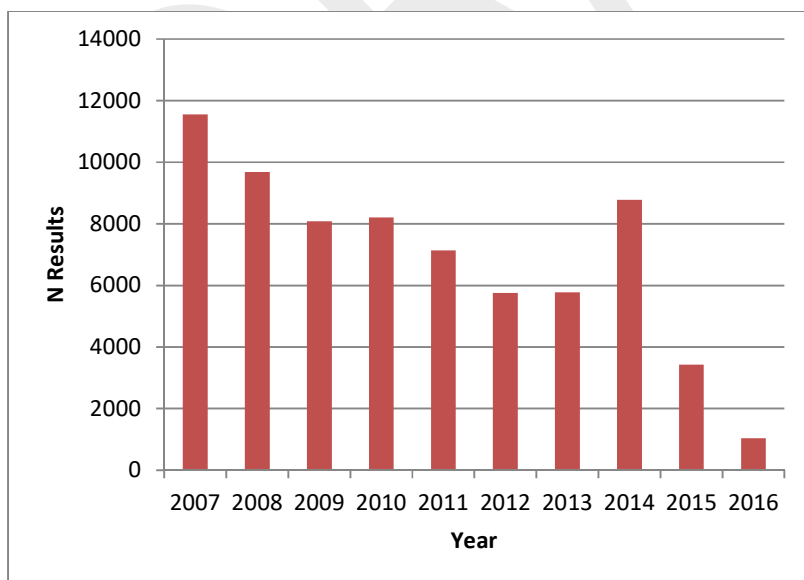


Figure 4: Distribution of analytical results for PFASs divided by sampling year

The analytical results in the final dataset were reported as corrected for recovery, or not in need of correction in approximately 69% of the cases. For results which were reported as not corrected for recovery and for which the recovery rates were available (19%), the results were corrected prior to being used for exposure assessment. For results for which the information about the need for correction or the recovery value was not available were used as provided (12%).

The list of food categories and the number of results available for each of them and for each PFAS is detailed in Annex B, Table B5. The five most frequently analysed food categories were 'Fish meat' (n = 23,572), 'Fish offal' (n = 9,745), 'Livestock meat' (n = 4,475) and 'Drinking water' (n=3,803) and 'Vegetables and vegetable products' (n = 3,697). The number of data available per food category was not evenly distributed across the 26 PFASs. Most data were on levels of PFOS (n = 8,498) followed by PFOA (n = 8,197), PFDA (n = 5,770), PFNA (n = 5,594), PFHxA (n = 5,448) and PFHxS (n = 4,745).

The CONTAM Panel decided not to run the exposure assessment for PFASs for which the fraction of left-censored data was 100%. As a result, the exposure assessment was limited to 17 PFASs. In total, 67,839 analytical results were used for calculating the mean level in food and used in the exposure assessment.

3.1.2. Analytical results

The mean occurrence values calculated on the final dataset for the selected 17 PFASs in each food category and used in the exposure assessment are displayed in Annex A, Table A4. Table 4 shows the mean levels in a number of food categories for the 4 PFASs selected based on biomonitoring data, being PFOA, PFNA, PFHxS and PFOS. Table 5 shows the mean concentrations of these PFASs in a number of fish species (see Appendix A and Annex A, Table A4 for more fish species).

High quantified concentrations (P95 > 10 µg/kg) were reported for PFOS, PFOA, PFDA and PFNA in edible offal from game animals, PFOS in a number of fish species, like sardine and pilchard, sea catfish and wolf-fish (*Anarhichas*), eel, perch, barbel, roach and bream, PFOA in carp and PFDA in perch. The highest mean and P95 LB concentrations were for PFOS in edible offal from game animals, being respectively 214 and 753 µg/kg.

It is relevant to focus on regularly consumed food categories, which contribute most to the exposure to each of the PFASs. For PFOS, highest mean LB levels were observed for various fish species, followed by eggs and egg products, livestock meat and fruit and fruit products. For PFOA, in addition to fish, eggs and egg products and livestock meat, also drinking water, fruit and fruit products and vegetables and vegetable products showed relatively high levels, considering the large consumption of these products. In addition, a relatively high mean level was calculated for alcoholic beverages, but this was based on 1 out of 6 samples with a level above the LOQ. For PFNA, relatively high levels were observed for fruit and fruit products, and for food for infants and small children, the latter due to one sample out of ten with a detectable level. For PFHxS, highest mean levels were reported for fruit and fruit products, but also the level in alcoholic beverages (one sample > LOQ) caused a significant contribution to the exposure to this PFAS. For most food categories and PFASs, UB levels were much higher than LB levels.

In fish, highest mean PFOS levels were reported for carp, eel, roach, perch, bream, barbel and sardine, in addition to fish offal. In general, mean LB levels of PFOA, PFNA and PFHxS in fish are much lower, with the exception of carp.

Concerning drinking water, there were a considerable number of analytical results (206 to 452) for 9 PFASs, varying between 78% and 100% left-censored. To calculate the mean occurrence for drinking water, occurrence values for FoodEx level 2 categories of water (tap, well and bottled) were weighted according to the consumption of these categories. The highest mean LB level was for PFHxA, followed by PFHxS, PFBS and PFOA, being respectively 0.0022, 0.0018, 0.0015, and 0.0013 µg/L. Despite the low LOQ cut-off applied (0.010 µg/L), mean UB levels were a factor of two higher.

For fruit and fruit products, PFOS and PFOA were detected in respectively 23% and 37% of the samples with mean LB levels of 0.0267 and 0.0086 µg/kg. Mean UB levels were 10- and 31-fold higher. Rather high mean LB levels were also detected for PFHxA and PFHxS, being 0.0529 and 0.0224 µg/kg, followed by PFNA with a mean LB level of 0.0111 µg/kg. Detectable levels for PFOS were reported for some

1533 samples of apple, pear, peaches, table grapes and strawberries, and for PFOA in some samples of apple,
1534 pear and strawberries. These results were obtained from the EU-project Perfood²⁹ that applied a very
1535 sensitive method and investigated pooled samples from a number of European countries. PFOS was
1536 detected in 11 out of 24 samples, PFOA in 16 out of 25 samples, PFNA in 9 out of 25 samples and PFHxS
1537 in 6 out of 25 samples.

1538 For pooled samples of vegetables from the Perfood project, the detection rates were lower, PFOS, PFOA,
1539 PFNA and PFHxS being detected in respectively 9, 28, 7 and 3 out of 62 samples. The detected levels
1540 were in general below LOQs reported by other data providers. Regarding the large consumption of fruit
1541 and vegetables these low levels may still be relevant for exposure. The high detection frequency for
1542 fruit and, to a lesser extent, vegetables in the Perfood project was a major reason to assume that all
1543 kinds of fruits and vegetables may contain PFASs.

²⁹ PERFOOD is a consortium of several European research institutions which collaborate in a FP7 EU project with the aim to improve the analytical tools for the determination of PFASs in food items, to contribute to the understanding of PFASs transfer from the environment into dietary items and to quantify the possible contribution of food/beverage contact materials and food and water processing to the overall PFASs levels in the diet (www.perfood.eu).

1544 Table 4: Mean levels (µg/kg) of PFOS, PFOA, PFNA and PFHxS in selected food categories (except fish)

Food category	PFOS				PFOA				PFNA				PFHxS			
	N	%LC	LB	UB	N	%LC	LB	UB	N	%LC	LB	UB	N	%LC	LB	UB
Vegetables and vegetable products*	477	95%	0.003	0.15	489	86%	0.006	0.16	275	96%	0.001	0.12	274	98%	0.000	0.10
Fruit and fruit products	143	77%	0.027	0.25	144	63%	0.009	0.26	98	73%	0.011	0.17	94	84%	0.022	0.16
Livestock meat	461	93%	0.028	0.17	459	96%	0.028	0.17	348	99%	0.000	0.14	222	100%	0.000	0.09
Poultry	169	99%	0.009	0.13	185	98%	0.002	0.15	170	100%	0.000	0.14	130	100%	0.000	0.11
Game mammals (meat)	574	71%	0.94	1.59	572	91%	0.38	1.23	33	100%	0.000	0.67	28	96%	0.015	0.68
Milk and dairy products	13	85%	0.001	0.12	13	85%	0.001	0.13	13	92%	0.000	0.10	13	92%	0.000	0.08
Liquid milk	235	96%	0.001	0.14	236	100%	0.000	0.15	111	100%	0.000	0.11	126	100%	0.000	0.10
Eggs and egg products	174	92%	0.27	0.35	177	92%	0.106	0.21	124	100%	0.000	0.098	107	97%	0.000	0.06
Animal and vegetable fats and oils	38	90%	0.004	0.11	38	90%	0.002	0.11	36	100%	0.000	0.12	53	97%	0.000	0.102
Alcoholic beverages	6	100%	0.000	0.002	6	84%	0.010	0.014	6	100%	0.000	0.005	6	84%	0.006	0.007
Drinking water**	451	88%	0.0001	0.003	452	78%	0.001	0.003	449	99%	0.000	0.002	449	85%	0.002	0.004
Food for infants and small children	11	100%	0.000	0.24	11	100%	0.000	0.15	10	90%	0.126	0.24	10	100%	0.000	0.24
Edible offal, farmed animals	495	80%	0.87	1.18	542	94%	0.092	0.36	285	84%	0.087	0.32	170	99%	0.014	0.52
Edible offal, game animals	903	4%	214	215	898	58%	5.48	8.18	105	10%	9.77	9.87	105	99%	0.010	2.52

1545 *Includes fungi; **without additives except carbon dioxide; includes water ice for consumption.

1546 N: number; %LC: Percentage left-censored; LB: Lower bound; UB: upper bound.

1548 Table 5: Mean levels (µg/kg) of PFOS, PFOA, PFNA and PFHxS in selected fish species and fish offal

1549

Fish species	PFOS				PFOA				PFNA				PFHxS			
	N	%LC	LB	UB	N	%LC	LB	UB	N	%LC	LB	UB	N	%LC	LB	UB
Herring (<i>Clupea</i>)	288	74%	0.32	0.62	290	96%	0.016	0.38	243	90%	0.023	0.38	237	99%	0.000	0.38
Sardine and pilchard (<i>Sardina</i>)	14	0%	4.73	4.73	28	64%	0.101	0.37	14	57%	0.084	0.53	14	64%	0.014	0.45
Anchovy (<i>Engraulis</i>)	5	0%	0.58	0.98	13	62%	0.044	0.12	-	-	-	-	-	-	-	-
Salmon and trout (<i>Salmo</i> spp.)	574	88%	0.31	0.83	521	95%	0.13	0.63	522	100%	0.003	0.70	365	100%	0.000	0.63
Mackerel (<i>Scomber</i>)	125	79%	0.36	0.93	136	81%	0.31	0.88	129	96%	0.004	0.74	122	99%	0.001	0.74
Tuna (<i>Thunnus</i>)	21	39%	0.16	0.26	34	100%	0.000	0.12	17	100%	0.000	0.13	17	100%	0.000	0.11
Cod and whiting (<i>Gadus</i> spp.)	174	67%	0.47	1.05	145	93%	0.012	0.74	130	92%	0.016	0.78	27	100%	0.000	0.53
Halibut (<i>Hippoglossus</i> spp.)	487	71%	0.26	0.81	106	99%	0.003	0.30	487	100%	0.000	0.77	487	100%	0.002	0.69
Carp (<i>Cyprinus</i>)	145	14%	14.12	14.21	149	32%	4.10	4.33	125	65%	0.84	1.47	126	97%	0.066	1.01
Eels (<i>Apodes</i>)	164	35%	9.23	9.44	177	96%	0.071	0.68	54	91%	0.98	1.66	58	98%	0.017	0.73
Fish offal	208	83%	3.38	4.99	208	100%	0.010	3.51	204	99%	0.011	2.41	202	100%	0.031	1.65

1550 N: number; %LC: Percentage left-censored; LB: Lower bound; UB: upper bound; -: no data provided to EFSA.

3.1.3. Comparison of previous and current occurrence

Occurrence of PFASs in foods were summarised in the EFSA Opinion on PFOS and PFOA in food – (EFSA CONTAM Panel, 2018). A direct comparison of reported data is difficult due to a variety of reasons. For example, different laboratories and methodologies lead to different sets of PFASs that are measured; data can be reported with very different LODs / LOQs; much of the literature data did not provide the mean concentration (LB and UB) required for an accurate comparison.

It was concluded that fish was the best studied of all food types in terms of this contaminant group. This is because several PFASs are present in fish at higher concentrations than in other food groups and because they are also useful as a marker of environmental quality resulting in their use as environmental indicators. PFOS is usually the PFAS that is present at the highest concentration in fish and shellfish, and lean predatory fish usually have the highest concentrations.

Many studies that had included a wide range of food types suffered from inadequate sensitivity resulting in left-censored data. The more recent studies that have been conducted with more sensitive methods (Haug et al., 2010; Lacina et al., 2011; Noorlander et al., 2011; Vestergren et al., 2012) reported concentrations for PFASs with a similar profile to those reported to EFSA and used for the current exposure assessment in this opinion. For these studies, fish had the highest concentrations of PFOS (0.013–5.4 µg/kg), followed by meat, meat products and chicken eggs which had concentrations of PFOS in the range 0.013–1.281 µg/kg – although there were some anomalies, such as a pooled Swedish egg sample that contained an unusually large amount of PFOS. PFOA has similar concentrations in food samples of both vegetable and animal origin (< 0.003–0.102 µg/kg), whereas PFOS is generally higher in foods of animal origin. These differences in homologue patterns may result from different pollution sources, differences in bioaccumulation, or could derive from different uptake and elimination pathways in terrestrial and aquatic food webs.

The concentration ranges reported in the literature for fish were in line with the mean concentrations reported to EFSA. PFOS levels for milk and eggs submitted to EFSA were also consistent to those reported in the literature, while for other foods of animal origin an accurate comparison was not possible due to limited information (e.g. information on meat type missing). For most foods of plant origin (e.g. olive oil, apple, potatoes, etc.), PFAS levels reported to EFSA were usually a little lower than those reported in the literature. Johansson et al. (2014) reported temporal trends in dietary exposure to PFASs. Archived samples of eggs, milk and farmed rainbow trout collected between 1999 and 2010, which covered a period when major production changes occurred, were assessed. The results showed significantly decreasing concentrations of PFOS in fish ($p < 0.002$) and eggs ($p < 0.001$). Concentrations of PFOS in fish and eggs decreased by a factor of 10 and 40, respectively. In eggs there was also a statistically significant decreasing trend for PFOA.

There have been several reports in the literature of PFASs in foods published since the EFSA opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018). Most of these have focused on PFOS and PFOA, although some do include other PFASs. In general, these data support the findings described in the current occurrence section above (Section 3.1.1).

In addition to the individual studies, a recent review collates data published in the period 2011-2016 for all PFASs (Domingo and Nadal, 2017). This review updated their previous report (Domingo et al., 2012) that covered the concentrations of PFASs in foodstuffs, human dietary exposure, and health risks. Although it is apparent that most of the data published is for PFOS and PFOA, there are also data for other PFASs included in the review. The limited amount of data for other PFASs supports the current occurrence data of PFASs as submitted to EFSA (Section 3.1.1).

A study by Scher et al. (2018) investigated PFASs in garden produce from homes that have elevated levels of PFASs in their municipal water supply. PFBA was the main PFAS present in the water supply, followed by PFPeA. Although PFBA, PFOA and PFOS were present in all soil samples analysed and were found at higher concentrations than the other PFASs, only PFBA was found to be translocated to plants. PFBA in the garden produce was found to be related to water that was applied to the garden via watering and the type of produce grown. The study confirmed that short-chain PFASs have the highest potential to translocate to and bioaccumulate in edible plants. PFBA was frequently detected across all plant types and parts examined, but differed widely in concentration, with median concentrations ranging from 0.11

1603 mg/kg in stems to 15 mg/kg in florets. PFHxA, PFHxS, and PFPeA also had the highest concentrations
1604 in florets.

1605 In a review on the accumulation of PFASs in agricultural plants, Ghisi et al. (2019) indicated a direct
1606 correlation between PFAS concentrations in soil and bioaccumulation in plants. It was established that
1607 plants could uptake PFASs from water, and that the amount of uptake was associated with chain length,
1608 functional group, plant species and organ. Low accumulation of PFOA and PFOS was found in peeled
1609 potatoes and cereal seeds, while short-chain compounds were found to be present in substantial
1610 amounts in leafy vegetables and fruits.

1611 Short-chain PFASs are increasing in terms of production volume as they are being used as substitutes
1612 for longer-chain compounds and are found in water due to their relatively high solubility and mobility
1613 (Blum et al., 2015).

1614 In summary, although it is not possible to make a direct comparison with data in the scientific literature
1615 due to differences in sampling and reporting, there is broad agreement with the data reported to EFSA
1616 that have been used for the exposure assessment. Most data reported in the literature are for fish.
1617 There is evidence that PFOS and PFOA concentrations in food are decreasing. Production and use of
1618 short-chain PFASs is increasing, and there is evidence that these compounds may be preferentially taken
1619 up by leafy vegetables and fruits.

1620 **3.1.4. Food processing**

1621 Most studies on food processing focus on PFOS and PFOA with only very limited information in the
1622 literature about other PFASs, partly due to the low levels found and insufficiently sensitive analytical
1623 methods. Where other PFASs have been measured these have been discussed in the EFSA opinion on
1624 PFOS and PFOA (EFSA CONTAM Panel, 2018).

1625 **3.1.4.1 Migration from food contact materials, including non-stick coatings used on** 1626 **cookware**

1627 As described in the EFSA opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018) there are several
1628 studies that found polytetrafluoroethylene (PTFE) cookware to contain residual PFOA in the low µg/kg
1629 range. These studies concluded that fluoropolymer food-contact materials were not likely to be a major
1630 source of PFASs. PFCAs, particularly PFOA, and fluorotelomer alcohols (FTOHs) have been shown to be
1631 released from coated cookware at normal cooking temperatures (179 to 233 °C surface temperature),
1632 and therefore have the potential to migrate into food during the cooking process, but studies are
1633 inconclusive and show that only small amounts are released into foods.

1634 Since the literature was searched for the previous opinion (EFSA CONTAM Panel, 2018), a
1635 comprehensive study of per- and polyfluoroalkyl substances in microwave popcorn bags has been
1636 published by Zabaleta et al. (2017). Different global regions were found to use different PFASs in their
1637 products. In bags from European and American countries, short-chain PFASs were detected, whereas
1638 long-chain PFASs were found to still be used in Asian countries. Whilst the study identified the PFAS
1639 compounds in the bags, the potential of PFASs to migrate into food was not fully established.

1640 A study by Schaidler et al. (2017) reported the presence of PFASs in grease-resistant packaging used
1641 for fast food. It was found that 46% of food contact papers and 20% of paperboard samples contained
1642 detectable fluorine (>16 nmol/cm²). A subset of 20 samples found PFOA (in 6 samples) and unidentified
1643 PFCAs, PFSAAs and other PFASs that were found using a non-targeted analysis approach with LC/MS.
1644 Concentrations in foods as a result of using these packaging materials were not measured.

1645 Jogsten et al. (2009) investigated sources of PFASs in a variety of foodstuffs as a result of food
1646 processing and packaging. PFHxS, PFOS, PFHxA, and PFOA were detected in at least one sample,
1647 whereas PFBS, PFHpA, PFNA, PFDA, PFUnDA, and PFDoDA were found to be <LOD in all samples. PFOS
1648 was most commonly found (8 out of the 20 composites), and PFHxA was detected in samples of raw
1649 veal, chicken nuggets, frankfurter sausages, and packaged lettuce. It was not sufficiently clear whether
1650 non-stick cookware, or packaging used for some foods, was a significant source of exposure.

Choi et al. (2018) investigated PFASs in food simulants after migration from fluorocarbon resin-coated frying pans, baking utensils, and non-stick baking papers on the Korean market. Estimated daily intakes as a result of migration for PFOA, PFNA, PFDoDA, PFTrDA, PFTeDA, PFHxDA, and PFODA were 0.35, 0.09, 0.35, 0.2, 0.1, 0.3, and 0.4 ng/kg bw per day, respectively. Hu et al. (2018) investigated whether profiles of PFASs in human serum were able to provide information on major exposure sources. Several associations of potential predictors with serum PFAS concentrations were established, including positive associations (p -value < 0.05) between serum PFHxS and increased use of Teflon cookware and preheated packaged foods. PFOS was also associated with increased use of packaged foods. Results were adjusted for PFAS exposure sources from other consumer goods, seafood consumption and demographic factors that may influence toxicokinetics, such as race, ethnicity, previous pregnancy and breastfeeding history.

Susmann et al. (2019) investigated associations between serum PFASs and consumption of restaurant food and popcorn in a representative sample of the US population. Associations between serum PFAS and popcorn consumption were found to be a potential consequence of PFAS migration from microwave popcorn bags. Inverse associations between serum PFAS and food eaten at home was consistent with less contact between home-prepared food and food contact materials, some of which contain PFASs.

In summary, PTFE cookware may contain residual PFOA in the low $\mu\text{g/kg}$ range, and food packaging may contain PFASs where they are used because of their grease-resistant properties. Studies conducted to date continue to support the conclusions reported in the previous opinion (EFSA CONTAM Panel, 2018) that the use of this type of material is likely to contribute to human exposure to PFASs.

3.1.4.2 Effect of cooking not related to coatings on cookware and food contact materials

The 2018 opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018) summarised the impact of cooking on concentrations of PFASs in foods. The limited number of studies gave an inconsistent view about whether or not losses or increases occur. Domingo (2011) found that it was the cooking process that had the greatest impact rather than the food type that was being cooked, but that different cooking methods could either reduce or increase the levels of chemical contaminants in food. Cooking procedures that released or removed fat from the product generally reduces the total concentrations of organic contaminants, but this was not necessarily the case for PFASs since they are not associated with the fat component of the food in the same way as for other organic contaminants.

Since the literature was searched for EFSA CONTAM Panel (2018), Alves et al. (2017) reported on changes in the bioaccessibility of several contaminants including PFASs, i.e. PFOS and PFUnDA, as a result of cooking of seafood. A range of contaminant classes was studied including brominated flame retardants (i.e. brominated diphenylether 47, brominated diphenylether 100, alpha-hexabromocyclododecane), pharmaceuticals and personal care products (i.e. venlafaxine, methylparaben and UV-filter octocrylene). Whilst there was some variation between contaminant class and seafood species, PFASs were found to be generally more available, i.e. to have higher bioaccessibility percentages as a result of cooking when compared to some of the other contaminant classes (between 71 and 95%).

Binninton et al. (2017) studied the effects of preparation on nutrient and environmental contaminant levels in traditional foods derived from the Arctic beluga whale (*Delphinapterus leucas*), and found that the process of roasting blubber increased concentrations of hydrophilic substances including certain PFASs (PFNA, PFDA, PFUnDA; based on measured concentrations of PFOS and the fact that all of these compounds were not present in oil from the blubber suggesting that concentrations in the remaining blubber after cooking must have increased).

Barbosa et al. (2018) examined the effect of steaming to cook seafood on a range of contaminants of emerging concern, including some PFASs. Out of all the PFASs measured, only PFUnDA, PFDoDA, PFTrDA, PFTeDA and PFOS were detected in raw and steamed samples of *Katsuwonus pelamis* and *Pleuronectes platessa*. Other PFASs, like PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFBS, PFHxS, PFHpS and PFDS were not detected (i.e. $< \text{LOD}$) in the analysed species (i.e. *P. platessa*, *Merluccius australis*, *Merluccius capensis*, *K. pelamis* and *Mytilus edulis*). In raw samples of *M. edulis*, only PFDA was detected but not after steaming ($< \text{LOD}$). However, in this species a large increase for PFBA ($> 100\%$) was detected after steaming. In *K. pelamis*, cooking by steaming resulted in a significant decrease of PFUnDA

1703 (68%), PFDoDA (10%) and PFOS (53%), but a significant increase of PFTTrDA (50%) and PFDA (>
1704 100%).

1705 In summary, the literature above covers the few papers describing the impact of cooking on PFASs in
1706 foods. This limited number of studies gives an inconsistent view about whether or not losses or increases
1707 occur as a result of cooking and processing.

1708 **3.2. Dietary exposure assessment**

1709 **3.2.1. Current exposure assessment**

1710 Among the 26 PFASs for which data were available after validation, quantified results were reported for
1711 17 PFASs (Section 3.1.1). The proportion of left-censored data across food categories was above 90%
1712 for all PFASs with the exception of PFOS for which it was 80%. The chronic dietary exposure assessment
1713 cannot be performed accurately if a large proportion of left-censored data is included (WHO/IPCS, 2009;
1714 EFSA, 2010b). The exposure is likely to be underestimated with the LB approach whereas it may be
1715 overestimated with the UB approach, even to a high extent. For compounds with very high proportions
1716 of results below LOD/LOQ and/or only limited availability of data, the exposure calculations should
1717 therefore be considered only as a rough indication of the range of chronic dietary exposure. Since this
1718 was the case for all the PFASs, results of the present assessment should be interpreted with caution.
1719 The CONTAM Panel concluded that the calculated LB exposure is likely to be more realistic than the UB
1720 exposure.

1721 The CONTAM Panel assessed the refined dietary chronic exposure (following the methodology described
1722 in Section 2.2.1) to the following 17 PFASs: PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA,
1723 PFDoDA, PFTTrDA, PFTeDA, PFBS, PFHxS, PFHpS, PFOS, PFDS and FOSA.

1724 Based on the available data with the limitations described, the chronic dietary exposure to the 17
1725 individual PFASs was estimated to be at the level of a few ng/kg bw per day considering all population
1726 groups. By analogy, for the PFASs for which no exposure assessment could be carried out, the chronic
1727 dietary exposure is likely to be lower.

1728 Tables 6 to 12 show summary statistics of the estimated chronic dietary exposure to 17 individual PFASs
1729 for each age group using the available occurrence data. Table 13 shows summary statistics of the
1730 estimated chronic dietary exposure to the sum of PFOA, PFNA, PFHxS, PFOS for each age group using
1731 the available occurrence data. Detailed mean and 95th percentile dietary exposure estimates calculated
1732 for all population groups and each of the 35 dietary surveys are presented in Annex A, Table A5.

1733

1734 Table 6: Summary statistics of the mean and 95th percentile LB and UB chronic dietary
 1735 exposure to 17 PFASs (ng/kg bw per day) for infants in 11 surveys across European
 1736 countries

PFAS	Range of mean dietary exposure in infants (LB-UB) (ng/kg bw per day)					
	Mean LB dietary exposure			Mean UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
PFBA	0.15	0.68	2.37	2.49	8.99	19.89
PFPeA	0.06	0.38	0.78	6.32	13.20	35.72
PFHxA	0.29	0.63	1.85	8.76	17.55	28.74
PFHpA	0.84	2.49	6.92	8.75	15.59	24.87
PFOA	0.11	0.19	0.39	8.88	17.33	27.76
PFNA	1.14	4.13	11.73	11.11	17.42	29.43
PFDA	0.99	3.73	10.48	10.55	16.49	27.85
PFUnDA	<0.01	0.01	0.22	10.34	18.83	29.37
PFDoDA	1.85	7.40	21.28	13.72	21.44	43.41
PFTTrDA	<0.01	0.01	0.13	3.85	11.50	22.24
PFTeDA	<0.01	<0.01	0.01	2.37	6.60	18.35
PFBS	0.03	0.10	0.12	11.37	18.73	30.47
PFHxS	0.09	0.19	0.36	11.00	16.74	29.03
PFHpS	<0.01	<0.01	<0.01	5.48	11.13	26.67
PFOS	0.23	0.36	1.26	11.78	20.10	31.44
PFDS	<0.01	<0.01	<0.01	5.05	10.81	26.47
FOSA	<0.01	0.01	0.14	15.78	30.52	47.63

PFAS	Range of 95th percentile dietary exposure in infants (LB-UB) (ng/kg bw per day)					
	95th percentile LB dietary exposure			95th percentile UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
PFBA	0.63	2.22	4.86	8.37	21.21	49.68
PFPeA	0.15	1.15	2.87	22.17	36.53	70.87
PFHxA	1.47	1.81	3.86	21.49	27.74	58.17
PFHpA	1.97	7.68	16.05	17.32	25.76	45.44
PFOA	0.37	0.58	1.02	21.83	26.88	54.70
PFNA	2.77	13.16	27.88	21.38	31.40	57.54
PFDA	2.63	11.70	24.93	20.76	29.94	54.52
PFUnDA	0.01	0.02	1.41	21.08	30.39	54.10
PFDoDA	4.92	23.74	50.70	23.47	45.99	84.67
PFTTrDA	<0.01	0.02	0.85	10.04	22.02	42.56
PFTeDA	0.01	0.01	0.06	5.09	20.05	41.99
PFBS	0.13	0.18	0.42	22.28	32.31	59.77
PFHxS	0.38	0.51	0.86	20.11	31.25	57.63
PFHpS	<0.01	<0.01	0.01	10.60	27.84	54.19
PFOS	0.74	1.59	4.15	26.36	32.56	61.95
PFDS	<0.01	<0.01	0.01	10.04	28.02	54.19
FOSA	0.02	0.03	0.65	37.83	48.84	90.22

1737

1738 Table 7: Summary statistics of the mean and 95th percentile LB and UB chronic dietary
 1739 exposure to 17 PFASs (ng/kg bw per day) for toddlers in 14 surveys across European
 1740 countries

PFAS	Range of mean dietary exposure in toddlers (LB-UB) (ng/kg bw per day)					
	Mean LB dietary exposure			Mean UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
PFBA	0.53	0.96	1.82	7.96	10.50	11.37
PFPeA	0.18	0.40	1.34	17.45	20.76	23.96
PFHxA	0.80	0.89	1.37	16.79	18.87	29.37
PFHpA	0.36	0.73	2.20	11.69	14.69	23.27
PFOA	0.25	0.41	0.59	16.58	18.87	29.03
PFNA	0.25	0.81	3.42	14.13	17.71	27.35
PFDA	0.21	0.80	3.08	13.38	17.02	26.51
PFUnDA	0.01	0.04	0.46	14.94	17.92	27.47
PFDoDA	0.28	1.29	6.05	14.29	17.81	25.37
PFTTrDA	0.01	0.03	0.29	2.36	4.48	7.78
PFTeDA	<0.01	0.01	0.07	1.38	2.46	6.26
PFBS	0.07	0.09	0.14	15.62	18.92	28.96
PFHxS	0.19	0.23	0.32	13.03	16.86	26.33
PFHpS	<0.01	<0.01	<0.01	3.61	4.69	9.28
PFOS	0.70	1.34	2.58	17.41	20.43	29.38
PFDS	<0.01	<0.01	<0.01	3.01	4.28	9.25
FOSA	0.02	0.08	0.23	31.26	35.68	48.62

PFAS	Range of 95th percentile dietary exposure in toddlers (LB-UB) (ng/kg bw per day)					
	95th percentile LB dietary exposure			95th percentile UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
PFBA	1.54	2.57	4.03	13.82	17.40	21.60
PFPeA	0.46	1.04	5.18	30.73	34.02	44.63
PFHxA	1.46	1.98	2.67	26.61	33.06	60.85
PFHpA	0.81	2.39	6.63	21.46	28.62	53.73
PFOA	0.59	0.88	1.23	26.63	33.44	59.09
PFNA	0.64	3.58	11.32	24.18	32.89	56.46
PFDA	0.61	3.53	10.09	23.25	32.54	55.01
PFUnDA	0.06	0.14	1.79	25.17	32.63	60.16
PFDoDA	0.97	6.24	20.55	24.01	36.70	56.35
PFTTrDA	0.04	0.10	1.17	4.96	9.26	17.71
PFTeDA	0.01	0.04	0.31	2.68	6.90	17.70
PFBS	0.14	0.17	0.23	25.98	33.26	58.83
PFHxS	0.43	0.48	0.80	22.99	31.70	54.90
PFHpS	<0.01	<0.01	0.01	6.09	10.83	22.45
PFOS	2.05	3.77	6.89	27.59	36.48	58.85
PFDS	<0.01	<0.01	0.01	5.90	10.44	22.44
FOSA	0.06	0.31	3.32	47.33	55.86	92.17

1741

Table 8: Summary statistics of the mean and 95th percentile LB and UB chronic dietary exposure to 17 PFASs (ng/kg bw per day) for other children in 19 surveys across European countries

PFAS	Range of mean dietary exposure in other children (LB-UB) (ng/kg bw per day)					
	Mean LB dietary exposure			Mean UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
PFBA	0.40	0.77	1.50	5.48	7.32	10.07
PFPeA	0.09	0.28	0.50	9.47	14.49	19.32
PFHxA	0.42	0.65	1.17	10.25	13.88	21.51
PFHpA	0.10	0.26	0.33	6.80	10.15	17.13
PFOA	0.16	0.30	0.63	10.06	13.82	21.23
PFNA	0.05	0.12	0.24	9.29	12.51	19.86
PFDA	0.07	0.14	0.26	8.86	12.05	19.18
PFUnDA	0.01	0.02	0.24	9.13	13.04	19.84
PFDoDA	0.02	0.08	0.33	7.50	11.50	18.34
PFTTrDA	0.01	0.02	0.16	1.29	2.06	3.56
PFTeDA	<0.01	0.01	0.03	0.85	1.23	1.52
PFBS	0.03	0.07	0.10	10.33	13.50	21.18
PFHxS	0.07	0.16	0.25	8.78	11.73	19.02
PFHpS	<0.01	<0.01	<0.01	1.73	2.53	3.70
PFOS	0.52	1.02	2.28	10.37	14.95	21.66
PFDS	<0.01	<0.01	<0.01	1.42	2.11	3.38
FOSA	0.02	0.06	0.54	20.21	26.36	36.69

PFAS	Range of 95th percentile dietary exposure in other children (LB-UB) (ng/kg bw per day)					
	95th percentile LB dietary exposure			95th percentile UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
PFBA	1.23	2.02	3.27	8.72	12.39	15.19
PFPeA	0.26	0.75	2.48	15.89	25.05	35.27
PFHxA	0.90	1.36	2.32	20.24	26.93	41.79
PFHpA	0.24	0.50	0.74	16.13	21.48	36.34
PFOA	0.40	0.65	1.03	19.97	27.67	41.74
PFNA	0.14	0.28	0.95	19.18	25.90	40.70
PFDA	0.18	0.31	1.49	18.80	25.31	40.42
PFUnDA	0.05	0.09	1.11	18.64	25.31	39.13
PFDoDA	0.05	0.14	1.50	17.22	23.14	37.11
PFTTrDA	0.04	0.07	0.66	2.68	4.14	5.64
PFTeDA	0.01	0.03	0.15	1.40	2.05	3.09
PFBS	0.06	0.12	0.19	20.05	26.10	41.69
PFHxS	0.21	0.35	0.58	18.79	24.54	40.54
PFHpS	<0.01	<0.01	<0.01	2.97	4.58	5.66
PFOS	1.92	3.12	8.51	20.27	29.48	42.05
PFDS	<0.01	<0.01	0.01	2.79	4.15	5.61
FOSA	0.09	0.23	2.93	34.22	45.12	67.20

1747 Table 9: Summary statistics of the mean and 95th percentile LB and UB chronic dietary
 1748 exposure to 17 PFASs (ng/kg bw per day) for adolescents in 18 surveys across European
 1749 countries

PFAS	Range of mean dietary exposure in adolescents (LB-UB) (ng/kg bw per day)					
	Mean LB dietary exposure			Mean UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
PFBA	0.22	0.43	0.93	2.43	3.95	5.26
PFPeA	0.05	0.11	0.21	4.34	6.96	11.83
PFHxA	0.23	0.36	0.65	5.36	6.99	10.67
PFHpA	0.05	0.15	0.21	4.09	4.96	8.68
PFOA	0.09	0.17	0.31	5.36	7.00	10.78
PFNA	0.02	0.04	0.08	4.93	6.25	9.92
PFDA	0.02	0.06	0.10	4.78	6.01	9.66
PFUnDA	<0.01	0.01	0.12	5.28	6.49	10.27
PFDoDA	0.01	0.02	0.09	4.37	5.57	9.35
PFTTrDA	<0.01	0.01	0.08	0.58	1.07	1.53
PFTeDA	<0.01	<0.01	0.02	0.45	0.68	0.87
PFBS	0.02	0.04	0.06	5.56	7.00	10.78
PFHxS	0.04	0.07	0.16	4.72	5.90	9.54
PFHpS	<0.01	<0.01	<0.01	0.74	1.23	1.69
PFOS	0.23	0.53	1.10	5.57	7.30	11.20
PFDS	<0.01	<0.01	<0.01	0.53	1.00	1.42
FOSA	0.01	0.04	0.52	11.07	13.17	18.42

PFAS	Range of 95th percentile dietary exposure in adolescents (LB-UB) (ng/kg bw per day)					
	95th percentile LB dietary exposure			95th percentile UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
PFBA	0.76	1.03	2.04	4.56	6.90	9.40
PFPeA	0.12	0.26	1.18	9.44	13.62	19.78
PFHxA	0.51	0.80	1.29	11.33	14.62	22.57
PFHpA	0.11	0.29	0.38	9.54	11.56	20.65
PFOA	0.23	0.40	0.59	11.33	14.59	22.64
PFNA	0.06	0.10	0.29	10.85	13.56	22.03
PFDA	0.08	0.16	0.36	10.59	13.28	21.83
PFUnDA	0.03	0.06	0.53	11.06	13.32	22.15
PFDoDA	0.03	0.05	0.47	10.38	12.55	21.44
PFTTrDA	0.02	0.05	0.32	1.28	2.12	3.36
PFTeDA	0.00	0.01	0.08	0.85	1.19	1.54
PFBS	0.03	0.07	0.12	11.63	14.69	22.72
PFHxS	0.13	0.19	0.29	10.55	13.12	21.66
PFHpS	0.00	0.00	0.00	1.45	2.27	3.50
PFOS	0.85	1.59	4.95	11.84	15.78	23.43
PFDS	0.00	0.00	0.00	1.17	2.06	3.16
FOSA	0.04	0.15	2.86	20.35	24.69	31.70

Table 10: Summary statistics of the mean and 95th percentile LB and UB chronic dietary exposure to 17 PFASs (ng/kg bw per day) for adults in 19 surveys across European countries

PFAS	Range of mean dietary exposure in adults (LB-UB) (ng/kg bw per day)					
	Mean LB dietary exposure			Mean UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
PFBA	0.12	0.32	0.51	2.19	2.68	3.11
PFPeA	0.04	0.07	0.15	3.97	4.80	5.80
PFHxA	0.22	0.29	0.37	3.47	4.05	5.58
PFHpA	0.06	0.12	0.17	2.45	2.84	4.59
PFOA	0.13	0.18	0.28	3.60	4.18	5.71
PFNA	0.02	0.04	0.07	3.08	3.68	5.25
PFDA	0.02	0.05	0.11	2.98	3.52	5.12
PFUnDA	0.01	0.02	0.06	3.32	3.76	5.46
PFDoDA	0.01	0.02	0.04	2.72	3.25	4.97
PFTTrDA	<0.01	0.01	0.04	0.48	0.66	0.85
PFTeDA	<0.01	<0.01	0.01	0.44	0.53	0.70
PFBS	0.03	0.03	0.04	3.46	4.04	5.68
PFHxS	0.06	0.08	0.11	2.86	3.45	5.06
PFHpS	<0.01	<0.01	<0.01	0.62	0.76	0.94
PFOS	0.29	0.58	0.93	3.82	4.47	5.94
PFDS	<0.01	<0.01	<0.01	0.51	0.67	0.91
FOSA	0.01	0.04	0.33	7.86	9.26	11.64

PFAS	Range of 95th percentile dietary exposure in adults (LB-UB) (ng/kg bw per day)					
	95th percentile LB dietary exposure			95th percentile UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
PFBA	0.48	0.86	1.29	3.52	4.46	5.30
PFPeA	0.10	0.21	0.88	7.51	9.32	12.09
PFHxA	0.43	0.61	0.77	6.31	8.02	15.78
PFHpA	0.14	0.25	0.30	4.16	6.12	14.70
PFOA	0.32	0.40	0.59	6.76	8.37	15.92
PFNA	0.06	0.10	0.18	5.80	7.46	15.42
PFDA	0.06	0.13	0.32	5.64	7.19	15.27
PFUnDA	0.04	0.08	0.30	5.62	7.26	15.76
PFDoDA	0.03	0.05	0.17	4.95	6.72	15.10
PFTTrDA	0.02	0.05	0.18	0.93	1.25	1.81
PFTeDA	<0.01	0.01	0.06	0.75	0.93	1.50
PFBS	0.05	0.07	0.09	6.19	8.10	15.88
PFHxS	0.15	0.18	0.24	5.36	7.13	15.22
PFHpS	<0.01	<0.01	<0.01	1.11	1.33	1.78
PFOS	0.84	1.71	4.79	7.53	9.31	16.31
PFDS	<0.01	<0.01	<0.01	1.09	1.37	2.00
FOSA	0.02	0.13	2.30	13.12	16.03	22.98

1757 Table 11: Summary statistics of the mean and 95th percentile LB and UB chronic dietary
 1758 exposure to 17 PFASs (ng/kg bw per day) for the elderly in 18 surveys across European
 1759 countries

PFAS	Range of mean dietary exposure in the elderly (LB-UB) (ng/kg bw per day)					
	Mean LB dietary exposure			Mean UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
PFBA	0.12	0.36	0.47	2.07	2.60	3.29
PFPeA	0.05	0.08	0.23	3.81	5.45	6.31
PFHxA	0.22	0.33	0.40	2.96	3.78	4.68
PFHpA	0.06	0.10	0.14	2.06	2.58	3.53
PFOA	0.15	0.17	0.29	3.04	4.01	4.88
PFNA	0.03	0.04	0.09	2.66	3.46	4.36
PFDA	0.03	0.06	0.09	2.51	3.35	4.25
PFUnDA	0.01	0.02	0.08	2.75	3.49	4.50
PFDoDA	0.01	0.02	0.05	2.38	3.01	4.07
PFTTrDA	<0.01	0.01	0.05	0.45	0.66	0.82
PFTeDA	<0.01	<0.01	0.01	0.42	0.48	0.70
PFBS	0.03	0.03	0.04	3.07	3.84	4.76
PFHxS	0.06	0.08	0.11	2.49	3.26	4.17
PFHpS	<0.01	<0.01	<0.01	0.56	0.76	0.91
PFOS	0.45	0.59	1.66	3.33	4.41	5.96
PFDS	<0.01	<0.01	<0.01	0.51	0.65	0.99
FOSA	0.01	0.03	0.30	7.20	9.18	11.44

PFAS	Range of 95th percentile dietary exposure in the elderly (LB-UB) (ng/kg bw per day)					
	95th percentile LB dietary exposure			95th percentile UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
PFBA	0.49	0.82	1.21	3.32	4.20	5.23
PFPeA	0.12	0.31	1.13	6.96	10.21	11.09
PFHxA	0.46	0.64	0.81	5.52	7.14	11.65
PFHpA	0.13	0.22	0.26	3.49	5.44	10.21
PFOA	0.27	0.36	1.13	6.10	7.33	12.03
PFNA	0.08	0.10	0.19	4.88	6.67	11.39
PFDA	0.09	0.13	0.29	5.07	6.49	11.25
PFUnDA	0.04	0.07	0.32	4.71	6.69	11.15
PFDoDA	0.03	0.05	0.21	4.37	6.12	10.88
PFTTrDA	0.01	0.05	0.19	0.88	1.17	1.67
PFTeDA	<0.01	0.01	0.07	0.75	0.88	1.32
PFBS	0.05	0.06	0.09	5.32	7.25	11.64
PFHxS	0.14	0.18	0.22	4.58	6.42	11.02
PFHpS	<0.01	<0.01	<0.01	1.07	1.29	1.63
PFOS	1.32	1.87	4.30	6.54	8.27	13.49
PFDS	<0.01	<0.01	<0.01	1.03	1.35	1.87
FOSA	0.02	0.10	1.98	12.35	15.43	20.39

Table 12: Summary statistics of the mean and 95th percentile LB and UB chronic dietary exposure to 17 PFASs (ng/kg bw per day) for the very elderly in 14 surveys across European countries

PFAS	Range of mean dietary exposure in the very elderly (LB-UB) (ng/kg bw per day)					
	Mean LB dietary exposure			Mean UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
PFBA	0.12	0.38	0.50	2.29	2.75	3.54
PFPeA	0.02	0.09	0.25	4.23	5.56	6.75
PFHxA	0.23	0.33	0.42	3.20	3.97	4.73
PFHpA	0.06	0.11	0.15	2.16	2.69	3.52
PFOA	0.08	0.15	0.37	3.29	4.08	4.82
PFNA	0.02	0.04	0.15	2.85	3.63	4.25
PFDA	0.04	0.05	0.14	2.72	3.50	4.11
PFUnDA	0.01	0.02	0.05	2.83	3.68	4.38
PFDoDA	0.01	0.02	0.07	2.56	3.07	3.94
PFTTrDA	<0.01	0.01	0.03	0.48	0.68	0.88
PFTeDA	<0.01	<0.01	0.01	0.42	0.51	0.73
PFBS	0.02	0.03	0.04	3.17	4.07	4.72
PFHxS	0.04	0.08	0.11	2.69	3.41	4.04
PFHpS	<0.01	<0.01	<0.01	0.65	0.80	0.93
PFOS	0.28	0.57	2.62	3.61	4.42	7.03
PFDS	<0.01	<0.01	<0.01	0.51	0.66	1.06
FOSA	0.01	0.03	0.21	7.21	9.54	12.10

PFAS	Range of 95th percentile dietary exposure in the very elderly (LB-UB) (ng/kg bw per day)					
	95th percentile LB dietary exposure			95th percentile UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
PFBA	0.39	0.87	1.47	3.22	4.47	5.19
PFPeA	0.05	0.41	1.56	7.96	9.22	12.72
PFHxA	0.47	0.65	0.95	5.41	7.27	10.51
PFHpA	0.12	0.21	0.30	3.93	5.39	8.99
PFOA	0.21	0.32	2.06	5.56	7.23	10.81
PFNA	0.05	0.11	0.39	5.10	6.82	10.27
PFDA	0.08	0.13	0.88	4.97	6.69	10.17
PFUnDA	0.04	0.06	0.26	5.18	6.61	10.06
PFDoDA	0.03	0.05	0.39	4.65	5.72	9.80
PFTTrDA	0.01	0.04	0.14	0.91	1.23	1.74
PFTeDA	<0.01	0.01	0.08	0.75	0.87	1.26
PFBS	0.05	0.06	0.21	5.53	7.40	10.58
PFHxS	0.09	0.15	0.23	4.86	6.55	9.88
PFHpS	<0.01	<0.01	<0.01	1.06	1.35	1.62
PFOS	1.03	1.83	7.40	6.25	8.37	12.27
PFDS	<0.01	<0.01	<0.01	1.10	1.27	1.79
FOSA	0.01	0.13	1.28	11.67	15.85	20.45

1767 Table 13: Summary statistics of the mean and 95th percentile for the combined LB and
 1768 UB chronic dietary exposure to PFOA, PFNA, PFHxS and PFOS (ng/kg bw per day) for
 1769 each age group across surveys

Age group	Range of mean dietary exposure (LB-UB) (ng/kg bw per day)					
	Mean LB dietary exposure			Mean UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
Infants	2.39	4.87	12.19	42.77	71.44	114.62
Toddlers	1.47	2.94	6.51	61.20	74.17	112.09
Other children	0.84	1.54	3.07	38.50	53.23	81.78
Adolescents	0.42	0.84	1.52	20.59	26.48	41.45
Adults	0.55	0.92	1.34	13.54	15.94	21.97
Elderly	0.71	0.89	2.08	11.51	15.07	18.77
Very elderly	0.42	0.86	3.10	12.56	15.41	19.85
Age group	Range of 95th percentile dietary exposure (LB-UB) (ng/kg bw per day)					
	95th percentile LB dietary exposure			95th percentile UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
Infants	4.50	13.65	27.88	92.77	122.25	224.84
Toddlers	3.35	7.55	13.69	100.65	134.01	229.04
Children	2.66	4.21	9.69	78.97	109.03	165.31
Adolescents	1.27	2.13	5.22	44.17	57.04	89.40
Adults	1.30	2.29	5.04	26.29	32.78	62.70
Elderly	1.76	2.37	5.58	22.96	28.77	46.73
Very elderly	1.32	2.23	9.93	21.90	28.32	42.03

1770

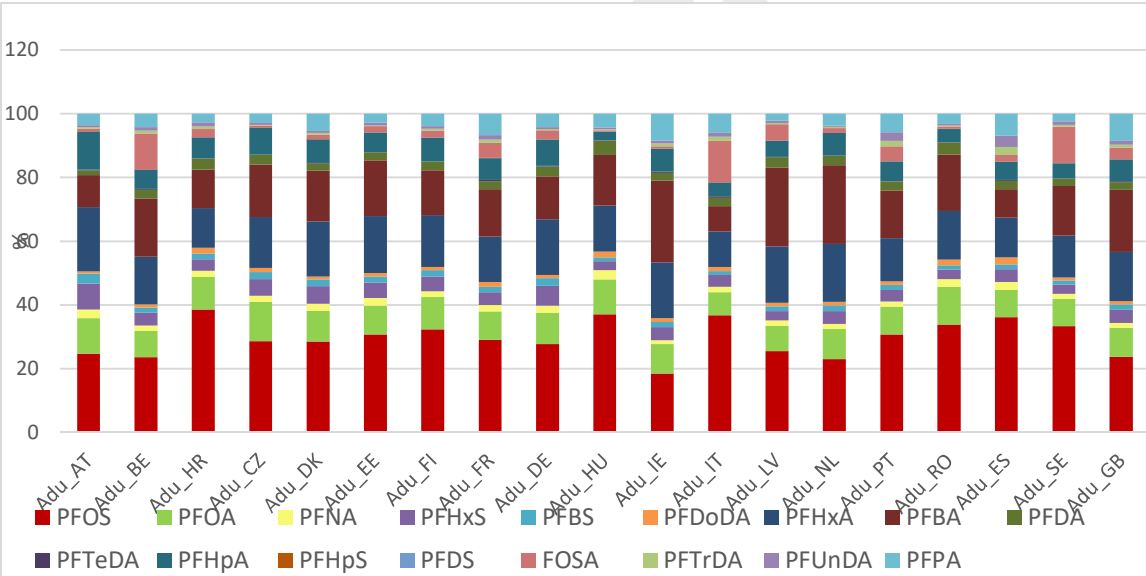
1771 Based on the medians across food surveys for the mean exposure, highest exposure for adults,
 1772 adolescents, elderly and other children was for PFOS, followed by PFBA, PFHxA, PFOA, and either PFHpA
 1773 or PFPeA. For infants, the highest median mean LB exposure was to PFDoDA, followed by PFNA, PFDA,
 1774 PFHpA, PFBA and PFHxA, so only a minor contribution for PFOS and PFOA. For toddlers, PFOS was most
 1775 important, as for older age groups, but this was followed by PFDoDA and then PFBA, PFHxA, PFNA and
 1776 PFDA. This implies that the median mean LB exposure to PFNA and PFHxS was relatively small for most
 1777 age groups

1778 In adults, the median of mean LB contribution to the sum of the 4 PFASs was for PFOA 21%, PFNA 4%,
 1779 PFHxS 10% and PFOS 66%. The contributions were similar in Other children, Adolescents, Elderly and
 1780 Very elderly. Overall, across surveys in adults the median contribution of these four was 46% (range
 1781 33-56%) to the sum of all PFASs for which the exposure was calculated, with contributions of 9%, 2%,
 1782 4% and 30% for, respectively, PFOA, PFNA, PFHxS and PFOS. Other PFASs that contributed more than
 1783 5% to this sum were PFBA (16%) and PFHxA (15%). This is illustrated in Figure 5.

1784 For infants and toddlers, the median of mean contribution of PFNA to the sum of the 4 PFASs was
 1785 respectively 79% and 26% (Table 14). This was highly influenced by one detected sample out of 10
 1786 samples in the food category "Food for infants and small children", and this sample had relatively high
 1787 PFNA concentration. Since the proportions of contributions across the other age groups are similar as
 1788 in adults, this high contribution from PFNA is not expected to be representative for the usual exposure
 1789 in infants and toddlers. This is further elaborated in Section 3.2.1.1.

1790 Table 14: Mean contribution (%) of PFOA, PFNA, PFHxS and PFOS to the dietary
1791 exposure to the sum of the four PFASs (minimum, medium, maximum across surveys)

	PFOA			PFNA			PFHxS			PFOS		
	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max
Infants	1	4	12	48	79	96	1	4	13	2	9	28
Toddlers	7	14	22	10	26	63	4	8	15	21	47	66
Other children	14	18	24	4	6	14	5	10	14	57	64	74
Adolescents	14	21	24	3	5	7	6	10	16	55	64	72
Adults	14	21	28	3	4	6	5	10	17	53	66	72
Elderly	12	20	23	4	5	6	4	9	12	63	66	80
Very elderly	8	19	23	3	5	7	3	9	12	60	68	84



1793
1794 **Figure 5:** Percent contribution of each PFASs to total exposure to 17 PFASs in adults.

1795 **3.2.1.1 Relative contribution from different food groups**

1796 The contribution (%) of each food category to the overall mean LB exposure for each PFAS, included in
1797 the exposure assessment, was calculated for each age group and dietary survey. It was decided to do
1798 this only for the mean LB exposure. The relative contribution of the different food categories is detailed
1799 in Annex A, Table A6, and shown for adults and toddlers for PFOA, PFNA, PFOS and PFHxS in Figures 6
1800 to 9, respectively. Similar FoodEx 1 food groups are shown for these PFASs, and no other food groups
1801 contributed to the LB exposure. Results should be interpreted with caution as neither all, nor the same
1802 food categories were analysed for the various PFASs and the proportion of left-censored data across
1803 food categories was above 89% for all PFASs. In principle, the relative contribution is determined by
1804 both occurrence and the amounts consumed, implying that food categories with high levels but rarely
1805 consumed may not be very relevant for mean exposure (like offal of wild game). It should also be
1806 pointed out that in some cases the mean level for a certain food category was only based on very few
1807 quantified results.

As shown in Figure 8, for PFOS "Fish and other seafood" was the most important contributor to the mean LB exposure, followed by "Eggs and egg products", "Meat and meat products", and especially for children also "Fruit and fruit products". Although to a lesser extent, "Fish and other seafood" was also an important contributor for PFOA (see Figure 6), again followed by "Eggs and egg products", "Meat and meat products", but also "Fruit and fruit products", "Vegetables and vegetable products" and "Drinking water".

"Fish and other seafood" was also among the main contributing food categories for most age groups for FOSA, PFPeA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, and PFTeDA. "Fruit and fruit products" was also among the main contributing food categories for most age groups for PFHxA, PFNA, PFTeDA, PFBS, and PFHxS. "Vegetables and vegetable products" was also among the main contributing food categories for most age groups for PFDoDA and PFDS. In addition to PFOA, "Drinking water" was among the main contributing food categories for most age groups for PFHxA, PFHpA, PFBS and PFHxS.

"Grains and grain-based products" was among the main contributing food categories for most age groups for PFDS. "Starchy roots and tubers" was among the main contributing food categories for most age groups for PFBA, PFHxA, PFHpA, PFDA and PFBS. For PFBA this is based on just one quantified result out of three samples, with a relatively high level. "Liquid milk" was a main contributing food category for all age groups for PFPeA, but based on 1 quantified result out of 13 samples. For children, infants and toddlers, "Food for infants and small children" was a main contributing category for PFDA, PFHpA, PFNA and PFDoDA. It should be stressed that this was based on 10 samples with only 1 quantified level for each of these PFASs. For PFNA, this sample had a quantified level above the mean in liver from farmed animals, and PFOS or PFOA were not detected in the same sample. Similarly, "Alcoholic beverages" was a main contributing category for PFHxS and PFOA in adults, elderly and very elderly, but again based on only one quantified result out of six samples.

For the combined exposure to PFOA, PFOS, PFHxS and PFNA, the main contributing categories were "Fish meat" and "Fruit and fruit products" for all population groups and "Food for infants and small children" was a main contributor for children, toddlers and infants, but only based on 1 quantified level for PFNA. The category "Eggs and egg products" was also a main contributor for all population groups except infants, based on detected levels for PFOS and PFOA in 15 and 14 samples out of 174 and 177, respectively.

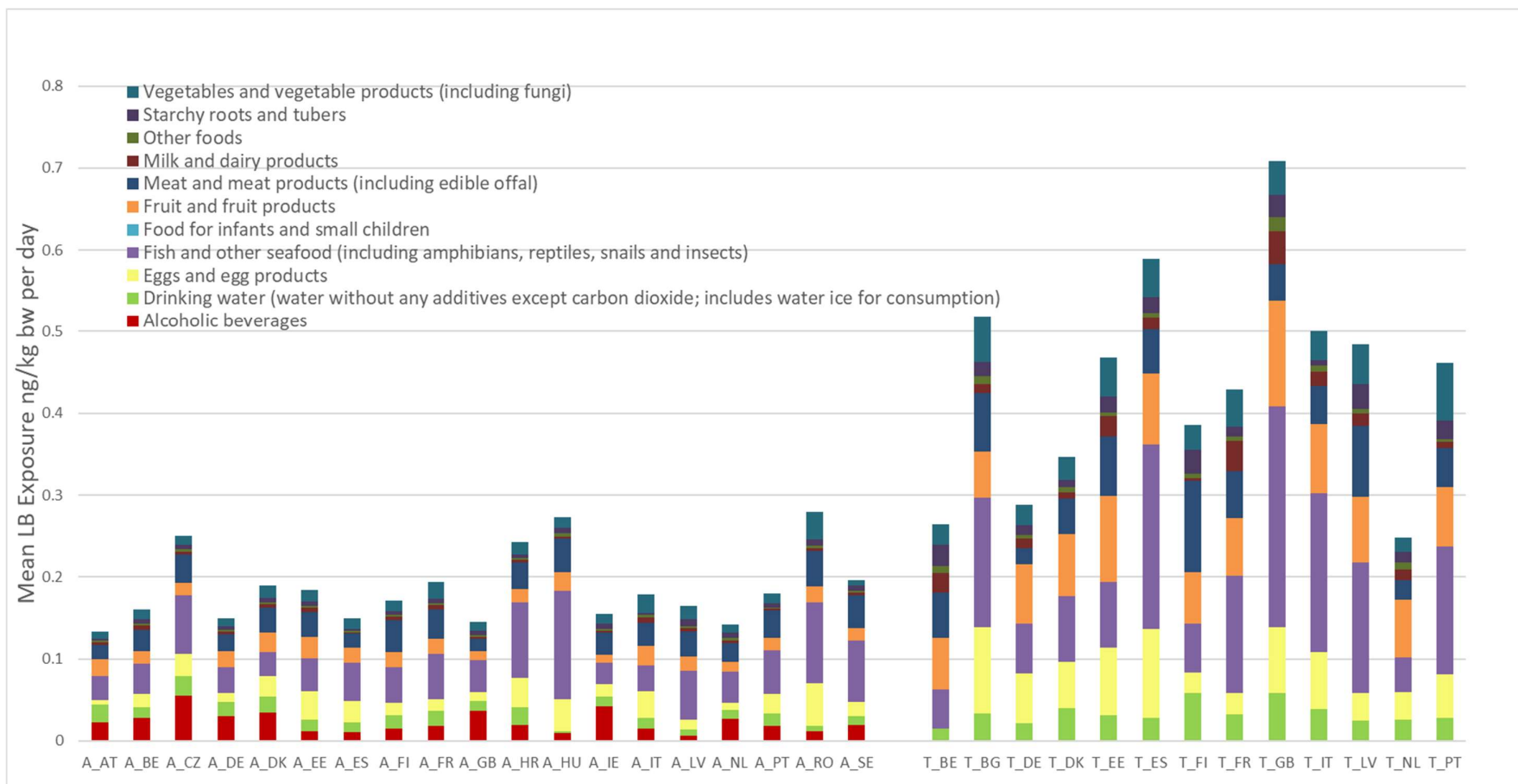


Figure 6: Mean LB exposure from different food categories of adults (A, left) and toddlers (T, right) to PFOA, for various surveys.

Perfluoroalkyl substances in food

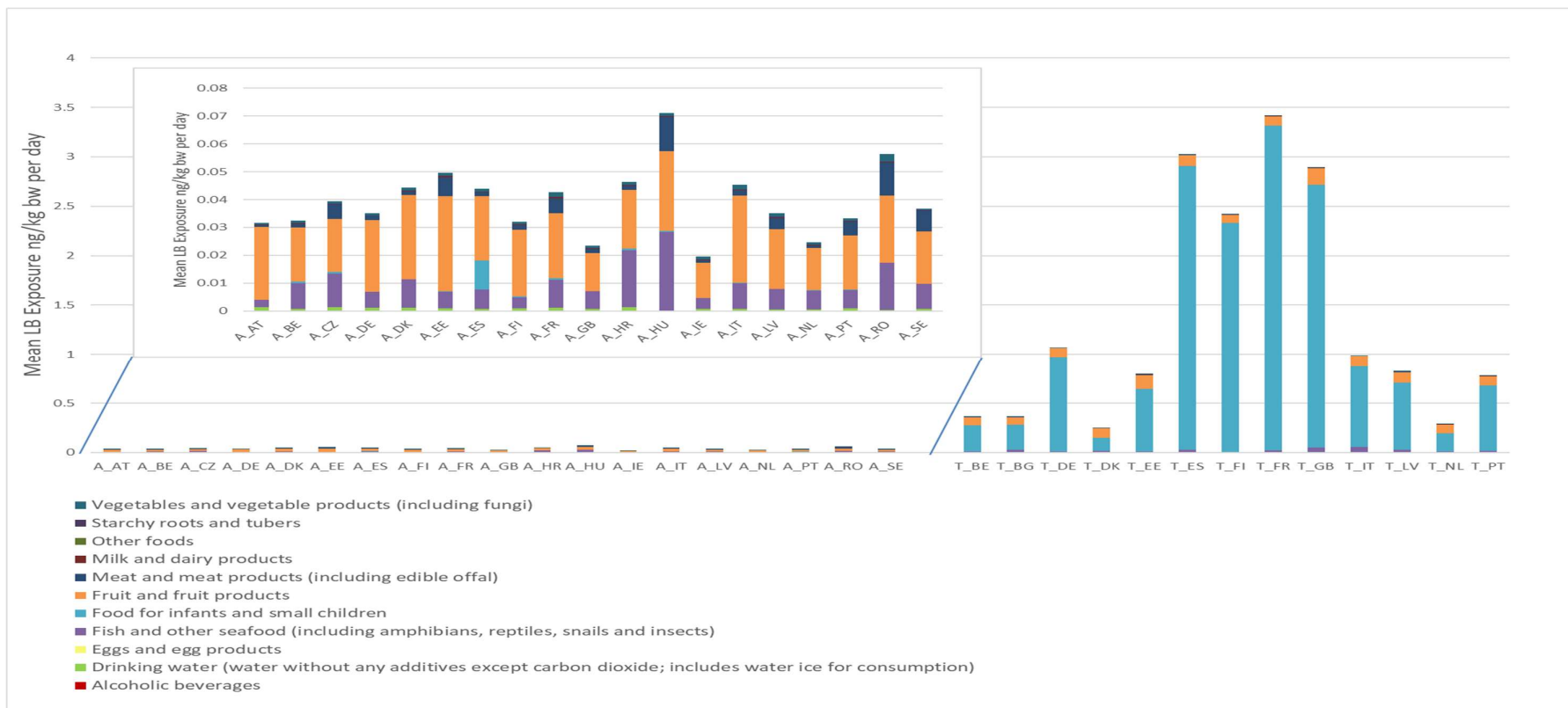


Figure 7: Mean LB exposure from different food categories of adults (A, left, inserted with different scale as toddlers) and toddlers (T, right) to PFNA, for various surveys. It should be stressed that the exposure from food for infants and small children was based on only one quantified level in 10 samples. This sample had a quantified PFNA level above the mean in liver from farmed animals, and PFOS or PFOA were not detected in the same sample, which is unexpected

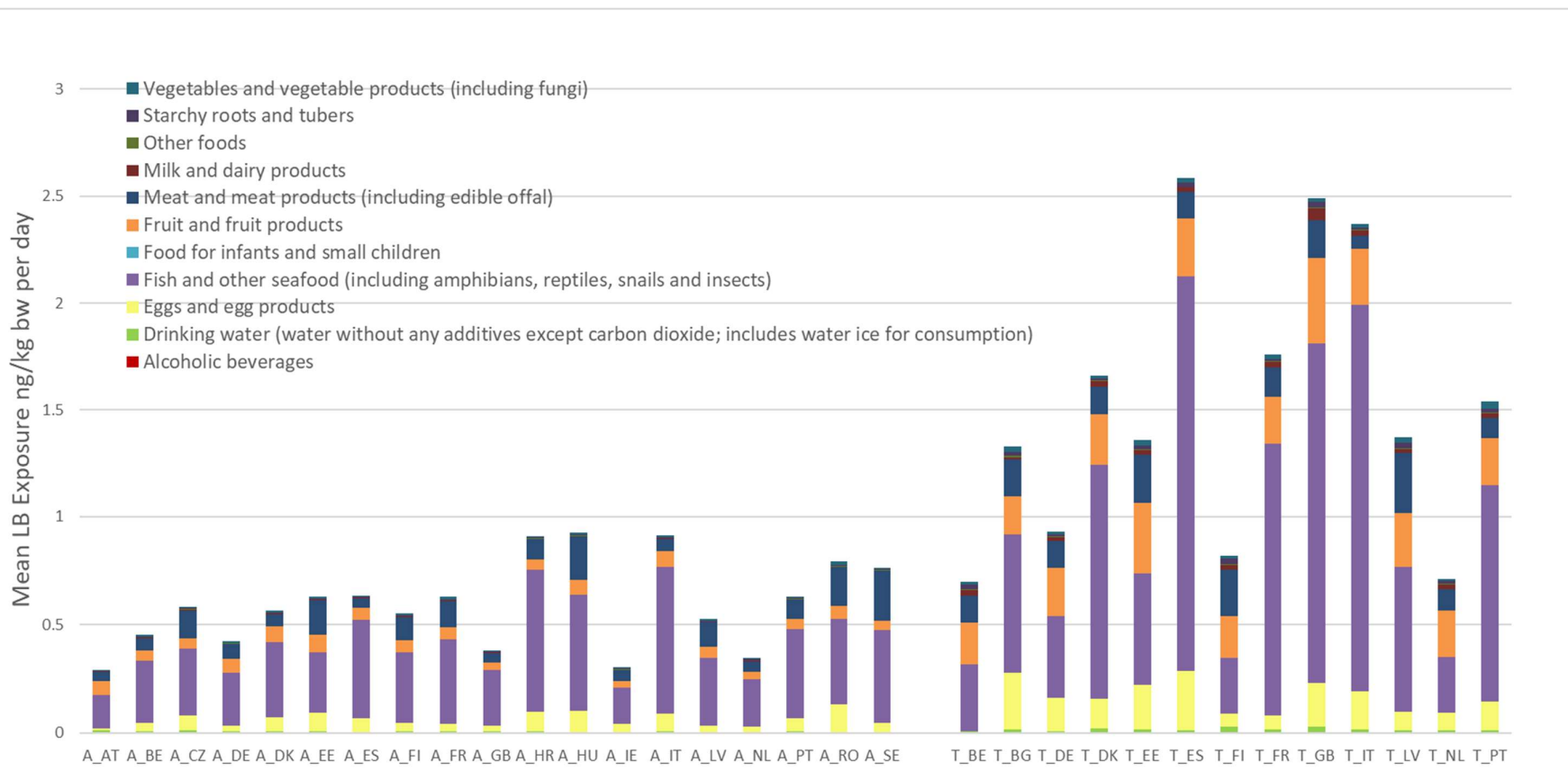


Figure 8: Mean LB exposure from different food categories of adults (A, left) and toddlers (T, right) to PFOS, for various surveys

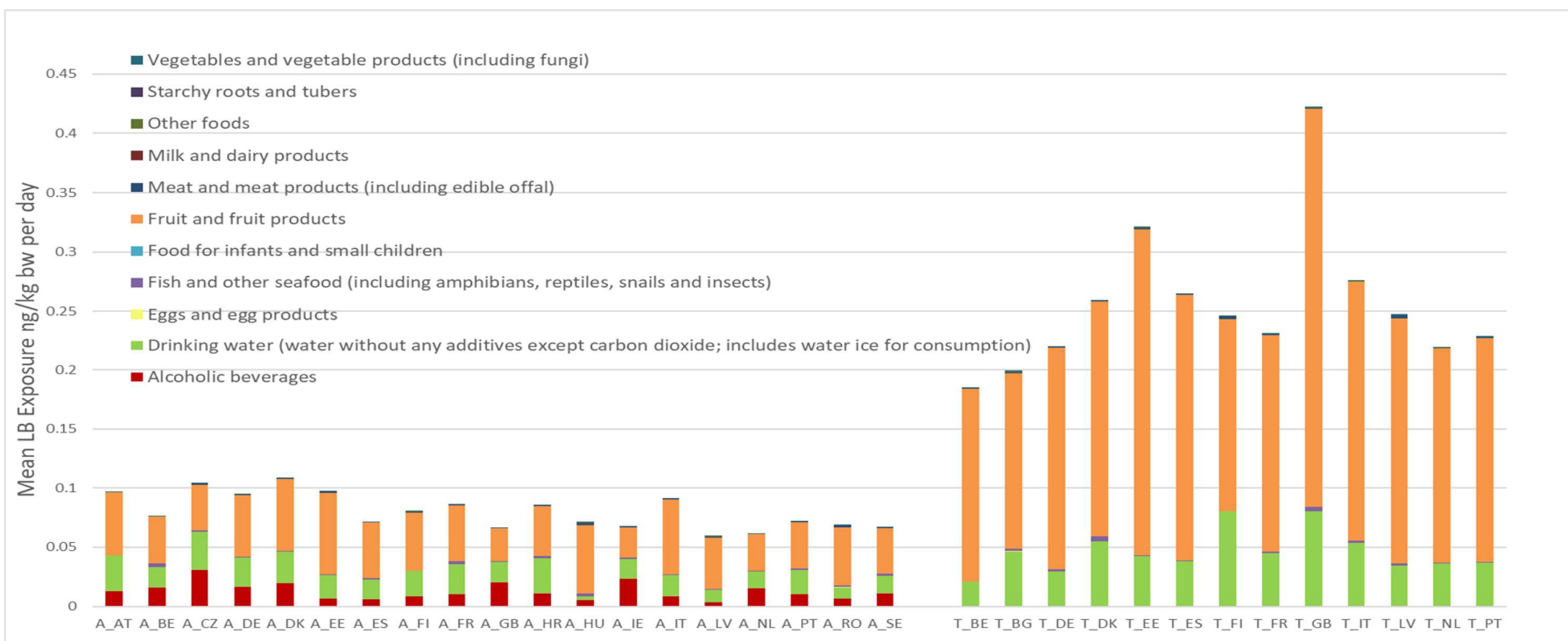


Figure 9: Mean LB exposure from different food categories of adults (A, left) and toddlers (T, right) to PFHxS, for various surveys

3.2.1.2 Exposure of breastfed infants

To assess the exposure of breastfed infants below 6 months of age, a median age of three months was selected, equivalent to a body weight of about 6.1 kg, with an estimated average daily milk consumption of about 800 mL and a high consumption of 1,200 mL. Levels in milk have been investigated but were often below the LOQs of the method. However, some studies determined the ratio between milk levels and maternal serum levels, being on average 0.015 for PFOS and 0.03 for PFOA. Based on the mean serum levels in adults of 7.5 ng/mL for PFOS and 2.1 ng/mL for PFOA (see section 3.3.2), this would result in milk levels of 0.113 and 0.063 ng/mL, respectively.

For PFOS, a daily average and high consumption of 800 or 1,200 mL milk (EFSA NDA Panel, 2013) would result in an intake of 90 and 135 ng PFOS per day or, respectively, 15 and 22 ng/kg bw per day (103 and 155 ng/kg bw per week). For PFOA the daily intake for average and high consumers would be 50 and 76 ng, or, respectively, 8 and 12 ng/kg bw per day (58 and 87 ng/kg bw per week).

For compounds that accumulate in the body over time and transfer to milk during breastfeeding, high levels in mothers will result in a prolonged high exposure of the infant. Using only the mean serum levels results in underestimating the exposure of these children. However, the CONTAM Panel decided that the available data on P95 serum levels are insufficient to estimate the exposure for higher milk levels.

3.2.2. Comparison with the exposure in the CONTAM Panel 2018 opinion

In the previous opinion, the CONTAM Panel estimated the exposure to PFOS and PFOA for the European population across the same age groups. To a large extent similar consumption surveys and occurrence data were used as those used in the present opinion. There are, however, some differences in the surveys, occurrence data and especially the way the data were treated in the present opinion, as shown in detail in Annex C:

- Updated and additional food consumption surveys and occurrence data were used
- Changes in cut-offs applied for LOQs
- Replacing missing occurrence data with values in similar food categories
- Mean occurrence levels changed, in particular in drinking water, fish, and meat, because occurrence was weighted for consumption
- Mean PFOA levels in milk were reduced due to withdrawal of data by the data provider

These changes led to differences in the LB and UB exposure assessments (see Annex C).

For exposure of adults to PFOA, when comparing the newly estimated mean LB exposure, they are on average 45% lower than the ones from 2018, and at the higher end (P95) 40% lower. Similar lower exposures are observed for most other age classes. For infants, the difference is even larger, the exposure estimate being 73% and 68% lower at the mean and P95, respectively. A major explanation for this large decrease lies in the fact that the LB level for milk in the present assessment was reduced to 0, as compared to 0.067 ng/g in the previous assessment. This was due to the withdrawal of all the samples with detected levels by the data provider (see Section 3.1.1.). Milk was one of the major contributors in the previous Opinion. Also the mean LB level of another important contributor, drinking water, was much lower in the present opinion, being 0.001 instead of 0.009 ng/g, due to weighing of the levels reported for bottled, drinking and tap water according to their relative consumption reported in the consumption surveys.

A factor that should have resulted in an increase in the exposure, was the decision to include all food categories, also those without reported data but considered analogous to those for which occurrence data were available. This led to including 1491 different food categories in the exposure assessment of PFOA and PFOS against the 184 and 406, respectively, in the previous opinion. It is unclear to what extent this affected the intake. As a result of the various changes, the relative contribution of food products to the LB exposure to PFOA also changed, with fish meat, eggs and egg products, fruit and

1901 fruit products and livestock meat being the most important sources, and for adults also alcoholic
1902 beverages (but based on one measured level).

1903 With respect to UB exposure, in order to reduce the uncertainty due to large numbers of LC data, it was
1904 decided to apply more cut-offs for LOQs in different food products and drinking water, resulting in the
1905 loss of some data points. This should have resulted in lower UB exposure estimates, but in practice the
1906 UB exposure for adults increased by a factor of 2.6 and 2.4 for the medians of respectively the mean
1907 and P95 exposure. A major reason is the decision to include more food categories based on occurrence
1908 in similar food category (see above). Since e.g. fruit and vegetables are highly consumed food
1909 categories, this has a major impact on the UB exposure.

1910 In the case of PFOS, the medians for the mean and P95 LB exposure of adults estimated in the current
1911 Opinion were similar as in the previous opinion (EFSA CONTAM Panel, 2018), being 4% and 13% lower.
1912 This was also the case for most other age classes, whereas for toddlers it was 79% higher. For the
1913 median P95 intakes by infants and toddlers, they were both higher, respectively 34 and 81%. Overall,
1914 it is difficult to point out a specific reason for the observed changes. The UB exposure estimates for
1915 PFOS were at least 2-fold higher than in the previous opinion (EFSA CONTAM Panel, 2018), but in some
1916 cases even up to 9-fold (infants, median of the means). This may be explained by the assumption that
1917 in principle all vegetables and fruits could be contaminated with PFOS, as explained above. As in the
1918 previous opinion (EFSA CONTAM Panel, 2018), fish meat was the most important source for the LB
1919 exposure, followed by eggs and egg products, but fruit and fruit products now became more important
1920 than meat and meat products.

1921 **3.2.3. Comparison with previously reported exposure assessments**

1922 EFSA reported details of occurrence and dietary exposure to PFASs in food in 2012 (EFSA, 2012). The
1923 report summarised occurrence data for PFASs collected in 13 European countries during the period 2006
1924 to 2012. The report considered 54,195 analytical results covering the same list of 27 substances included
1925 in this opinion. The overall proportion of quantified results was very low due to the analytical LODs of
1926 the methods used resulting in a large amount of left-censored data. PFASs were found more frequently
1927 in fish and other seafood and in meat and meat products (liver in particular) than in other food groups.
1928 Based on the available data with a low proportion of quantified results, the chronic dietary exposure to
1929 PFASs other than PFOS and PFOA was not quantified, but was expected to be in the low ng/kg bw per
1930 day range or even lower. Since this period, new data has been provided, some of which has been
1931 generated using methods with improved LODs, and hence an exposure estimate has been possible (see
1932 Section 3.2.1).

1933 The 2018 opinion on PFOS and PFOA in food (EFSA, CONTAM Panel, 2018), included details reported in
1934 the scientific literature for dietary exposure to PFASs. It was still difficult to make reliable dietary
1935 exposure estimates to compare with those reported in Section 3.2.1 due to uncertainties arising from
1936 the use of left-censored data, the fact that many reports were not clear whether UB or LB data had
1937 been used, and sampling strategies were not always reported. There are still insufficient data reported
1938 in the literature to make reliable dietary exposure estimates for most PFASs. There have been many
1939 studies that link data from blood monitoring studies to overall exposure and these show that blood can
1940 be a useful biomarker. For many individuals and locations, drinking water was shown to be a significant
1941 contributor to overall exposure to PFASs, either as a direct result of consumption or because of the use
1942 of water during cooking (EFSA, CONTAM Panel, 2018).

1943 Exposure to PFASs other than PFOS and PFOA have been reported by Haug et al. (2010). Associations
1944 were found between estimated individual total dietary intakes of PFASs and serum. PFAS concentrations
1945 in serum were associated with the consumption of lean fish, fish liver, shrimps and meat. The estimated
1946 dietary intakes of PFOA, PFUnDA and PFOS were 0.60, 0.34 and 1.5 ng/kg bw per day, respectively.
1947 Seafood (fish and shellfish) was the major dietary source contributing 38% of the estimated dietary
1948 intakes of PFOA, 93% of PFUnDA and 81% of PFOS. In a separate report, Haug et al. (2010) reported
1949 that in the general Norwegian adult population, the highest dietary intake was estimated for PFOA (31
1950 ng/day) followed by PFOS (18 ng/day), PFDA (13 ng/day) and PFNA (9.5 ng/day) using a value of 1/2
1951 LOD for left-censored data. For PFHxS, PFOS, PFHxA, PFHpA, PFOA, PFNA and PFDA more than 85%

- 1952 of the estimated intake was based on measurements above LOD. The estimated total dietary intake of
1953 PFASs for the general Norwegian adult population (all age groups and both genders) was 103 ng/day.
- 1954 Papadopoulou et al. (2017) estimated dietary exposure to PFASs in 61 Norwegian adults (74% women,
1955 average age: 42 years) using three methods: (i) a 1-day duplicate diet study; (ii) by estimating intake
1956 after combining food contamination with food consumption data, and iii) by a Food Frequency
1957 Questionnaire (FFQ). The PFASs found at highest concentrations in the duplicate diet samples were
1958 PFOA, PFOS and PFHxS where median total intakes were calculated to be 5.6 ng/day, 11 ng/day and
1959 0.78 ng/day, respectively.
- 1960 Johansson et al. (2014) investigated temporal trends of several different PFASs in commonly consumed
1961 food items over the period 1999–2010. Statistically significant decreasing trends were observed for
1962 concentrations of PFOS and PFHxS in fish and eggs. Concentrations of PFOS in fish and eggs decreased
1963 by a factor of 10 and 40, respectively. In eggs concentrations of PFOA were also shown to decrease.
1964 Whilst the concentrations of both linear and branch chained PFOS decreased in eggs, especially after
1965 2004, the concentration of the branched PFOS isomers dropped more quickly than the linear isomer,
1966 leading to an average increase of the ratio between the linear and sum of branched PFOS by around
1967 12% per year. A reduction in concentrations of PFHxS and PFOS was found in rainbow trout, whereas
1968 PFUnDA concentrations did not change significantly over the time period. The PFOS levels decreased by
1969 about 18% per year and PFHxS levels by about 4% per year between 1999 and 2010.
- 1970 Several papers have been published since the 2018 opinion (EFSA CONTAM Panel, 2018).
- 1971 Arrebola et al. (2018) reported on the differential contribution of animal and vegetable food items to
1972 persistent organic pollutant serum concentrations in Spanish adults, using data generated from the
1973 BIOAMBIENT.ES project. PFOS and PFDA serum concentrations were primarily influenced by fish
1974 consumption.
- 1975 Dietary exposure to PFASs for the general population and pregnant women in Taiwan was reported by
1976 Chen et al. (2018). Compared to Western populations, the Taiwanese were found to be exposed to
1977 higher levels of PFOA, PFDA, and PFUnDA (11.2, 85.1, 44.2, and 4.45 ng/kg bw per day, respectively),
1978 mainly due to the higher contaminations in food. The exposure of 8.0 mg PFOA / person per day in the
1979 95 percentile of pregnant women was largely due to frequent consumption of pork liver.
- 1980 Exposure to PFHxS and other PFASs in Baiyangdian Lake (China) associated with industrial
1981 contamination including from a photographic film production plant were reported by Cui et al. (2018).
1982 Estimated daily intake for the highest PFASs were for PFOS where exposure was 16.56 ng/kg bw per
1983 day for and PFHxS it was 16.11 ng/kg bw per day via aquatic food and drinking water. Fish consumption
1984 was the most important exposure pathway for residents to PFOA, PFHxS, PFOS, and 6:2 chlorinated
1985 polyfluorinated ether sulfonate (6:2 Cl-PFESA). The authors stated that this was the highest PFHxS
1986 levels ever recorded in surface water.
- 1987 Sznajder-Katarzyńska et al. (2018) examined exposure to PFASs as a result of contamination of fruits
1988 and vegetables. PFBA, PFOA and PFOS were the only 3 of 10 PFAS compounds that were quantitatively
1989 determined. The detection frequency for PFASs for the 55 samples analysed was < 10%. The major
1990 contributor to exposure was PFBA for which the concentration, reported only for banana, apple and
1991 orange samples, was 50.740 ng/g ww. PFOA was the most commonly detected PFAS. The origin and
1992 growing region were said to be possible factors that may influence the distribution profile of PFASs and
1993 their levels in food.
- 1994 Contaminated drinking water in New Hampshire was a source of exposure to PFOS, PFOA, and PFHxS
1995 (Daly et al., 2018), and PFOA and PFHxA were the dominant compounds in drinking water in central
1996 eastern China (Lu et al., 2018).
- 1997 As with the findings reported in the current exposure section above, fish and meat products such as
1998 offal are generally the highest reported sources of dietary exposure to most PFASs, except where
1999 contaminated drinking water has been used to prepare dishes with a high water content (Saito et al.,
2000 2004; Quinones and Snyder, 2009; Rayne and Forrest, 2009).
- 2001 Exposures in different parts of the world can vary according to usage of PFASs and sources of water
2002 contamination. Water is used extensively in the home for cooking and for food preparation, and

2003 therefore contaminated water can have a major influence in dietary exposure even if concentrations of
 2004 contaminants in foods are low.

2005 In summary, it is difficult to compare exposure estimates with literature sources due to several factors.
 2006 These include uncertainties arising from the use of left-censored data, the fact that many reports are
 2007 not clear whether UB or LB data had been used, and sampling strategies are not always reported. It is
 2008 nevertheless clear that drinking water can be an important factor, whether consumed directly or used
 2009 as part of the cooking process. PFASs are found more frequently in fish and other seafood, and in meat
 2010 and meat products (liver in particular) than in other food groups, although local sources can have an
 2011 impact in certain geographical regions. There is evidence that dietary exposure to some PFASs has
 2012 decreased over recent decades.

2013 3.2.4. Non-dietary exposure

2014 The large historical production volumes and widespread applications of PFASs in consumer products
 2015 represent a potential for contamination of the indoor environment. Recently, OECD (2018) has identified
 2016 4,730 PFAS-related CAS numbers. Non-dietary exposure to PFOS and PFOA has been described in the
 2017 previous opinion (EFSA CONTAM Panel, 2018). PFASs might be leaching from consumer products into
 2018 house dust as well as to both indoor and outdoor air. Thus, ingestion of house dust and inhalation of
 2019 air (in both gas and particulate phase) are potential exposure routes for PFASs. Exposure can also occur
 2020 through direct contact with consumer products such as all-weather clothing and other textiles. In
 2021 addition, PFAS manufacturing and downstream production may lead to occupational non-dietary
 2022 exposure to these compounds (Olsen, 2015).

2023 3.2.4.1 Exposure through air and dust

2024 Vapour pressures of PFCAs and PFASs in dissociated forms are expected to be low, thus exposure from
 2025 air primarily occurs through inhalation of neutral PFASs (Stock et al., 2010). Concentrations of PFASs in
 2026 indoor air usually exceed those in outdoor air considerably and thus exposure through inhalation of air
 2027 is mainly through indoor air (Harrad et al., 2010). Concentrations of PFASs in indoor air have been found
 2028 to vary a lot between homes (Fromme et al., 2015; Haug et al., 2011a; Ericson Jogsten et al., 2012).
 2029 FTOHs have in general been found in higher concentrations than perfluoroalkane sulfonamido
 2030 substances such as EtFASAs and EtFASEs. In a recent study from Germany with samples collected in
 2031 2014, the median concentration of 8:2 FTOH was 8679 pg/m³ (range 4,702-21,698 pg/m³), while
 2032 median concentrations of 69 pg/m³ (range 27-155 pg/m³) and < 54 pg/m³ (<54-119 pg/m³) were
 2033 reported for EtFOSE and EtFOSA, respectively (Fromme et al., 2015).

2034 Ingestion of house dust is also a potential exposure route for PFASs. As for indoor air, the concentrations
 2035 in house dust are quite variable. The distribution pattern is often following a lognormal distribution, with
 2036 some samples having concentrations far exceeding the mean and median values of the dataset (Harrad
 2037 et al., 2010). In a review by Eriksson and Kärrman (2015), concentrations of PFCAs, PFASs, PAPs as
 2038 well as perfluoroalkane sulfonamido substances in dust from various countries are described. The
 2039 highest median sum concentrations were observed for n:2 diPAPs, followed by PFCAs, PFASs and
 2040 perfluoroalkane sulfonamido substances. For example, the median sum concentrations of n:2 diPAPs,
 2041 PFCAs, PFASs and perfluoroalkane sulfonamido substances in Sweden were 62 ng/g, 44 ng/g, 4.6 ng/g
 2042 and < 0.1 ng/g (Eriksson and Kärrman 2015).

2043 3.2.4.2 Dermal exposure

2044 Dermal exposure to PFASs can occur through direct contact with consumer products, but so far, data
 2045 are very limited. Both ionic and neutral PFASs have been detected in a wide range of consumer products
 2046 (Kotthoff et al., 2015; Liu et al., 2014a) including carpets and textiles such as technical clothing
 2047 (Washburn et al., 2005; Gremmel et al., 2016), waxes and paints (Washburn et al., 2005), food contact
 2048 materials (Begley et al., 2005), non-stick cookware (Sinclair et al., 2007), and personal care products
 2049 including cosmetics (Fujii et al., 2013; Framtiden i våre hender, 2018) (see Section 1.3.3). So far very
 2050 little is known on the dermal absorption of PFASs. In a study by Fasano et al. (2015), the dermal
 2051 absorption of ammonium perfluorooctanoate was only 0.048% (Fasano et al., 2005), and for PFOS and
 2052 PFOA this pathway is thought to give a minor contribution to the intake both for infants, toddlers,

children and adults (Trudel et al., 2008). A more recent study indicates that the dermal absorption is dependent on the ionization state, and that there is a potential for a significant dermal absorption of PFOA through human skin. However, this is likely not the case when exposed through the environment, as PFOA then exists in its ionised form resulting in a low dermal absorption (Franko et al., 2012). Based on similarities in the kinetics of other PFCAs and PFSA's compared to PFOA and PFOS, it can be expected that the dermal exposure to these compounds is also low. No information on dermal exposure is available for the other PFASs covered in this opinion.

3.2.4.3 Relative contribution to overall exposure

In a very recent study (published after the literature deadline) from Norway, the relative exposure from ingestion of food and drinks, inhalation of air, ingestion of dust and dermal absorption was assessed in a study group from the general population (Poohong et al., 2020). For most of the participants, diet was the predominant exposure pathway, but for 7, 12, 14 and 18 of the 61 participants more than 20% of the exposure was attributed to other exposure pathways than diet for PFHxS, PFOS, PFOA and PFNA, respectively. For some individuals less than 50% of the exposure to one or more of the compounds studied came from ingestion of food and drinks. Also in a study from China, diet was identified as the major exposure pathway for ordinary residents while in occupational workers ingestion of dust contributed most to the total exposure (Fu et al., 2015). A study from Sweden demonstrated that, similar to PFOS and PFOA, contaminated drinking water may in particular cases be the major exposure pathway to PFHxS (Li et al., 2018a).

3.3. Hazard identification and characterisation

3.3.1. Toxicokinetics

This chapter reviews data on absorption, distribution, metabolism and elimination of PFASs in animals and humans. PFOS and PFOA toxicokinetics studies which have been published before 2017 are documented in previous EFSA opinions (EFSA, 2008; EFSA CONTAM Panel, 2018). Additional studies published since 2017 have been analysed and reported in the current opinion.

3.3.1.1 Experimental animals

Most of the information on the fate of PFSA's and PFCAs is based on PFOS and PFOA, respectively (EFSA CONTAM Panel, 2008, 2018). These compounds have been shown to be readily absorbed in the gastrointestinal tract in mammals, including humans, and to distribute predominantly to the plasma and liver. PFOS and PFOA are not metabolised and are excreted in both urine and faeces. They may be subject to extensive enterohepatic recirculation. For PFOS, the serum elimination half-lives in rats and mice were slightly higher than one month, whereas in rabbits and monkeys, they were 3–4 months. Significant sex differences in the elimination of PFOA are observed in some species such as rats, for which half-lives may vary from few hours (in females) to several days (in males). Differences in biological half-lives between species for both PFOS and PFOA and between sexes for PFOA are mainly due to differences in renal clearance. For both PFOS and PFOA, maternal transfer occurs prenatally to the foetus through placental transfer and postnatally through the consumption of maternal milk. A detailed description of the individual studies summarised here below is provided in Appendix C.

PFCAs

In the past decades, a limited set of data was published on the toxicokinetics of PFCAs other than PFOA, such as PFBA, PFHxA, PFHpA, PFNA, PFDA, PFUnDA, PFDODA, PFTrDA and PFTeDA. The most extensive studies have been carried out in rodents. Oral exposure, mainly by gavage, of experimental animals to PFCAs having a perfluorinated carbon chain length of 3 to 11 was shown to result in an estimated absorption fraction greater than 95 % of the administered dose (ATSDR, 2018). None of the experimental studies observed the formation of metabolites, suggesting, as previously reported for PFOA (EFSA CONTAM Panel, 2018), that the biotransformation of PFCAs is unlikely in mammals, irrespective of their chain length.

Although distribution of PFCAs shows species and sex differences, which are attributed, at least in part, to differences in elimination kinetics, blood, liver and kidney are the tissues exhibiting the highest concentrations of absorbed PFCAs. In blood, PFCAs were found to bind to serum albumin, the affinity generally increasing with PFCAs hydrophobicity, but decreasing for perfluorinated carbon chain length beyond 8 carbons.

The primary route of elimination of PFCAs having a carbon chain length below 10 is via urine, whereas for PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA, faecal excretion is predominant. In rodents, half-lives may vary from few hours (PFBA, PFHxA) to more than one month (PFNA, PFDA). Elimination of PFCAs exhibits pronounced sex differences in rats, with faster elimination in females than in males.

It was shown that transport proteins such as serum albumin, liver fatty acid binding proteins (L-FABP), and organic anion transporters play a key role in PFCA excretion and/or reabsorption (Appendix C).

PFSAs

In addition to PFOS, toxicokinetics data in experimental animals were identified for PFBS and PFHxS, showing that the gastrointestinal absorption of these compounds was almost complete. They distributed mainly to blood, liver, and at a lesser extent to kidney. Neither *in vitro* nor *in vivo* biotransformation was reported for PFSAs. As for PFCAs, a slower elimination rate in males, compared to females was also observed for PFSAs in rats.

PFSAs are primarily eliminated in urine, and to a lesser extent in faeces. They are excreted into the bile but undergo extensive intestinal reabsorption, explaining the limited faecal excretion. Half-lives of PFSAs are usually longer than for PFCAs with identical fluorocarbon chain length.

PFSAs are able to bind to serum albumin and with organic anion transporters, which are expressed in hepatocytes and enterocytes (Appendix C).

Other PFASs

Most studies on fluorotelomer-based polyfluoroalkyl substances have used FTOHs as the parent compound, showing the formation of PFOA and, to a smaller extent, PFNA and lower-chain-length PFCAs. In general, the yield is low, probably because of the multiple biotransformation pathways, including conjugation reactions in animals. The PAPs, were shown to be direct precursors of FTOHs and thus follow similar degradation pathways (Appendix C).

3.3.1.2 Humans

In the previous opinion dealing with PFOS and PFOA (EFSA CONTAM Panel, 2018), it was reported that both compounds were extensively absorbed in humans and mainly distributed in plasma (in which they are predominantly bound to albumin), liver and kidney. PFOS and PFOA does not undergo metabolism and are eliminated in urine and bile. Biliary excretion of PFOS and PFOA is significantly higher than elimination via the urine, but does not predominantly contribute to overall elimination, due to high biliary reabsorption. Humans have a high percentage of PFOA renal tubular reabsorption due to the high affinity of PFOA for human uptake transport proteins such as OAT1, OAT3 and URAT1. Although a limited number of papers have been published on PFOS transporting proteins, compared to PFOA, some authors showed that these proteins are involved in the transport of PFSAs, including PFOS (Zhao et al., 2015, 2017). These studies are described in detail in this opinion. Several studies estimated the half-lives of PFOS and PFOA in humans, most of them suggesting values between 2 and 6 years (Table 15). PFOS and PFOA have been detected in umbilical cord blood, breast milk and plasma samples of breastfed toddlers indicating that maternal transfer occurs pre- and postnatally.

Most of the human data published on the toxicokinetics of PFASs other than PFOS and PFOA are related to their distribution and elimination. Because humans are exposed to PFAS mixtures, these compounds will not be considered individually in this section, as it was the case for experimental animal data. Quantitative oral absorption data in humans were not identified, but reports of high levels of PFASs in blood of individuals exposed to contaminated water (see section 3.3.2.5) indicate that gastrointestinal absorption of these compounds had occurred. Although studies of metabolism of PFASs in humans were not identified, it is unlikely that these compounds could be biotransformed in human tissues. However, similarly to experimental animals, humans are able to transform precursors to PFCAs and PFSAs. The FTOH metabolites FTCAs and FTUCAs were detected in the blood from ski wax technicians exposed

through inhalation to high levels of 8:2 FTOH, suggesting metabolism of FTOH to PFOA and PFNA (Nilsson et al., 2013). Using human hepatocytes, Nabb et al. (2007) demonstrated the biotransformation of 8:2 FTOH to PFPeA, PFHxA, PFHpA, PFOA and PFNA (Appendix C, Table C.4). After having detected and quantified FTOH–sulfate conjugate in serum and/or urine of rats dosed with 8:2 FTOH and 8:2 diPAP (see Appendix C, Section C.4), Dagnino et al. (2016) found the presence of measurable levels of FTOH–sulfate in the serum of more than 5% of the general population and suggested that this metabolite can be considered as a biomarker of exposure to FTOHs and PAPs in humans.

Compared to PFOA and PFOS, a limited number of studies investigated the interaction of other PFASs with human serum albumin. However, evidence exists that PFBS, PFHxS, PFHpA, PFNA, PFDA, PFUnDA, PFDoDA can bind to human serum albumin (see Liu et al., 2019 for review). Among PFCA, the binding strength follows the order PFHpA < PFOA = PFNA < PFDA > PFUnDA; whereas for PFSA, the order is PFOS < PFHxS (Hebert and MacManus-Spencer, 2010).

The concentrations of 21 PFASs in 99 samples of autopsy tissues (liver, lung, kidney, brain and bone) from subjects who had been living in Tarragona (Catalonia, Spain) were measured by Perez et al. (2013). Unexpectedly, in the liver and brain, the PFAS found at the highest median concentration was PFHxA (68.3 and 141 ng/g, respectively), whereas in kidney and lung PFBA was predominant (263 and 807 ng/g, respectively). In bone, PFHpA was the main PFAS when the mean was considered (77.1 ng/g), but based on the median, PFOA was predominant.

Partitioning behaviours of PFASs between human plasma and blood cells were investigated by several authors (Kärman et al., 2006; Ehresman et al., 2007; Hanssen et al., 2013; Jin et al., 2016; Poothong et al., 2017). Median serum (or plasma) to whole blood ratio of PFOS, PFOA, PFHxS, PFNA, and PFUnDA was approximately 2. However, for other PFASs the ratio may be different, suggesting that blood can be a preferable matrix compared to serum or plasma for monitoring these compounds (see section 3.3.2.1). The toxicokinetics of PFCAs with six to fourteen carbon atoms (C6 to C14) was investigated in humans by Fujii et al. (2015). Urine from healthy volunteers, bile from patients who underwent biliary drainage, and cerebral spinal fluid (CSF) from brain drainage were collected for analysis. Urinary and biliary clearance of each PFCA was determined by dividing the cumulative urine or bile excretion in a 24-h period by the serum concentration of each PFAS. Urinary clearance decreased as a function of chain length, from 0.67 mL/kg per day for PFHpA to 0.005 mL/kg per day for PFUnDA. No differences were observed between PFUnDA, PFDoDA and PFTTrDA. For comparison, PFOA and PFOS renal clearance values were 0.03–0.04 and 0.015 mL/kg per day (Harada et al., 2005; Fuji et al., 2015), respectively. Biliary clearance varied from 1.2 mL/kg per day (PFNA) to 11.22 mL/kg per day (PFTTeDA). For PFOA and PFOS biliary excretion rates, reported mean values were 1.06–2.62 and 2.98 mL/kg per day, respectively (Harada et al., 2007; Fuji et al., 2015). These data suggest that shorter chain PFCAs, which are less bound to serum protein, are preferentially excreted in urine, whereas PFNA and longer chain PFASs are preferentially eliminated through the bile. This is in accordance with the conclusion reached by Zhang et al. (2013a) on the preferential excretion of short chain PFCAs in urine, whereas other excretion routes contributed to the elimination of longer PFASs. Levels of PFCAs in the human CSF ranged from 1.0 to 2.5% of the concentrations in serum, confirming that, irrespective of the chain length, crossing the blood–brain barrier occurred at a very limited extent (Fujii et al., 2015).

Available total clearance values (sum of urinary and biliary clearance) are presented in Table 15 and are generally 50–100 times smaller than in rodents (Appendix C, Table C.2 and C.3). Values for PFNA, PFDA, PFUnDA, PFDoDA and PFTTrDA estimated by Fujii et al. (2015) are approximately 0.07 mL/kg per day. Parts of these values are in accordance with the data reported by Zhang et al. (2013a). Higher values were determined for PFHpA and PFTTeDA (Fujii et al., 2015) and by Russell et al. (2013) for PFHxA.

Zhang et al. (2013b) investigated the binding affinity of twelve PFCAs with L-FABP. They found that binding affinity increased significantly with their perfluorinated carbon number from 4 to 11, and decreased slightly when the number was over 11, suggesting that PFNA, PFDA and PFUnDA may accumulate preferentially in the liver. PFSA have stronger L-FABP interactions than PFCAs with the same perfluorinated chain length (Zhang et al., 2013b, Ng and Hungerbühler, 2014).

Zhao et al. (2015) showed that human Na⁺/taurocholate co-transporting polypeptide (NTCP), which is highly expressed at the basolateral membrane of hepatocytes and mediates the uptake of bile acids into

hepatocytes, can transport PFBS, PFHxS and PFOS (Km values = 39.6, 112 and 130 μ M, respectively), whereas the human sodium-dependent bile salt transporter (ASBT), which is mainly localised to the brush border membrane in the terminal ileum and is involved in the enterohepatic circulation of bile acids, only transports PFOS (see Appendix C, Table C.1). Human organic solute transporter (OST) α/β , which is expressed at the basolateral membrane of enterocytes to mediate the efflux of bile acids, can also transport the 3 PFSA tested (Zhao et al., 2015).

The role of human renal organic anion transporters (OAT), organic anion-transporting polypeptide (OATP) and urate transporter (URAT) in transporting PFCAs was investigated by Yang et al. (2010). The inhibition of OATP1A2- or OAT4-mediated estrone-3-sulfate uptake or URAT1-mediated urate uptake has been compared for PFCAs with carbon chain lengths from 4 (PFBA) to 12 (PFDoDA). A chain length-dependent inhibition was observed, suggesting that PFCAs in general are substrates of OAT4 and URAT1, and to a lesser extent for OATP1A2, but with different levels of affinities to the transporters depending on their chain length. Regarding OATP1A2 and OAT4, the maximum inhibition was observed for PFNA and PFDA, whereas URAT1 was inhibited the most and roughly at the same levels by PFHpA, PFOA, PFNA, and PFDA. PFCAs with shorter or longer chain lengths showed relatively less inhibition. These data suggest that OAT4 and URAT1 are key transporters in renal reabsorption of PFCAs in humans and, as a result, may contribute to their long half-life.

Zhao et al. (2017) demonstrated that organic anion transporting polypeptides OATP1B1, OATP1B3, OATP2B1, which are expressed in human hepatocytes and/or enterocytes, can transport PFBS, PFHxS, PFOS, PFOA and PFNA, facilitating the enterohepatic recycling process. Furthermore, they showed that the organic solute transporter OST α/β was also able to transport PFBS, PFHxS and PFOS.

All these transporters can potentially play a role in the accumulation of PFASs in the liver and contribute to their long elimination half-life in humans.

The half-lives of various PFASs have been estimated in humans (Table 15). Short-chain PFASs were found to have half-lives ranging from few days (PFBA) to approximately one month (PFBS, PFHxA). For comparison, estimated half-lives for PFOA and PFOS vary from 2.3 to 8.5 years and from 3.1 to 7.4 years, respectively (EFSA, 2008, EFSA CONTAM Panel, 2018; ATSDR, 2018). Values for PFHxS were between approximately 4.7 and 8.5 years according to Olsen et al. (2007) (based on data from retired fluorochemical production workers) and Li et al., (2018a) (from the general population mainly exposed to PFASs through drinking water). Higher values were reported by Zhang et al. (2013a) (general population biomonitoring) and Worley et al. (2017) (drinking water exposure to PFASs). According to these authors, the half-life estimated by Zhang et al. (2013a) on the basis of renal clearance should be viewed as upper limits due to the possibility that there might be other elimination routes than via the urine. Other uncertainties in the results provided by Zhang et al. (2013a) include the estimation of half-lives from single time-point serum measurements and based on volume of distribution values reported by Thomson et al. (2010) for PFOA. The half-life calculation published by Worley et al. (2017) is based on serum concentrations measured at two time points and on intake rates estimated from PFHxS concentrations in drinking water supplying a residentially exposed population in Alabama (USA). The values, which are reported as means but without indications of range or inter-individual variability, may be overestimated, due to other sources of ongoing exposure. Bearing in mind these limitations in kinetic calculation or reporting, the CONTAM Panel considered that Olsen et al. (2007) and Li et al. (2018a), (see Table 15) provided the best estimate.

Gao et al. (2015) measured the levels of linear and branched PFOS, PFOA, and PFHxS isomers in serum and urine samples collected from 36 occupational workers in a fluorochemical manufacturing plant in China from 2008 to 2012 and they evaluated the daily clearance of these compounds. The median concentration of PFHxS isomers in the serum was 995 ng/mL (min - max = 12.8 – 10,546 ng/mL), with a mean linear isomer proportion of 93%. In urine, concentrations varied from LOQ (see supporting information) to a maximum of 40 ng/mL, with a mean proportion of linear isomers of 74%. The renal clearance was estimated on the basis of paired serum and urine concentrations and was found to be 0.04 mL/kg bw per day (0.03 for linear isomer and 0.19 for branched isomers).

In the study published by Zhang et al. (2013a), estimated geometric mean elimination half-lives for PFNA in young females and in a group of males and older females were 1.7 and 3.2 years, respectively

(Table 15). Corresponding values for PFHpA and PFDA were 1.0 years/0.82 years and 4.0 years/7.1 years. The values for PFUnDA (4.0 years/7.4 years) were similar to those of PFDA (Table 14). Some uncertainties regarding these estimated values were already mentioned in this section (see above).

For PFHxS, ATSDR reported fetal/maternal serum ratios ranging from 0.23 to 1.25. For PFNA, PFDA, PFUnDA, and PFDoDA, the ratios were 0.4-1.18, 0.03-0.41, 0.29-0.53 and 0.61-0.68, respectively (ATSDR, 2018). The median, calculated from the ratios reported by Inoue et al. (2004), Fromme et al. (2010), Kim et al. (2011a), Gützkow et al. (2012), Hanssen et al. (2013), Lee et al. (2013), Ode et al. (2013), Porpora et al. (2013), Zhang et al. (2013c), Cariou et al. (2015), Fisher et al. (2016), Eryasa et al. (2019) for PFHxS, PFOS, PFOA and PFNA were 0.56, 0.36, 0.74, and 0.48, respectively. These values have been used for the calculation of the serum level at birth (see Section 3.4 on PBPK/TWI calculation).

The maternal-infant transfer via breast milk was estimated using the ratio of the concentrations in milk versus plasma. The ratio is in the range of 0.01-0.07 for PFHxS, PFNA, PFDA PFUnDA and FOSA (ATSDR, 2018), indicating that the transfer occurs at similar levels as for PFOS (ratio of 0.01-0.02), but at a lesser extent than for PFOA (ratio of 0.03-0.12) (EFSA CONTAM Panel, 2018). Mondal et al. (2014), calculated that each month, breastfeeding was associated with 1 and 2% decrease in maternal serum concentration of PFHxS and PFNA, respectively compared to 3% for both PFOS and PFOA. Several authors estimated the decline in PFAS concentrations in breast milk, due to breastfeeding. The calculation of the decline was based (1) on repeated analyses of breast milk samples collected at different time points during lactation (Thomsen et al., 2010), (2) on measured concentrations in maternal serum at different time points during lactation (Fromme et al., 2010), (3) on measured concentrations in maternal serum of mothers that had breastfed their children during variable time (Bjermo et al., 2013; Branstaeter et al., 2013; Mondal et al., 2014; Lauritzen et al., 2016). In addition, calculations can be based on intakes, distribution volumes, breastfeeding amount and ratios between breast milk and serum concentrations. All these methods show that monthly decrease in breast milk concentrations ranged from 1.2 to 3.1% for PFOS and from 1.3 to the 7.7% for PFOA. Percentages reported for PFHxS and PFNA varied between 1 and 6.7% and between 1.1 and 2.8%, respectively.

Table 15: Toxicokinetics parameters for PFASs in humans

PFAS	Gender	Half-life	Clearance _{tot} (mL/kg/day)	Reference
PFBA	M (n=7), F (n=2)	2.5 d (1.8-6.3)	NR	Chang et al. (2008)
PFHxA	M (n=7)	32 d ^a (14-49)	4.33	Russell et al. (2013)
PFHpA	M (n=5), F (n=5) F ^b (n=12) M ^c + F ^d (n=31)	NR 1.0 y ^a (0.11-3.3) 0.82 y ^a (0.12-5.1)	0.674 0.61 ^k (95% CI 0.022-1.2) 0.61 ^k (95% CI 0.38-0.83)	Fujii et al. (2015) Zhang et al. (2013a) Zhang et al. (2013a)
PFOA	M (n=20) M+F (n=4) M (n=24), F (n=2) M+F (n=200) M+F (n=643) ⁱ M+F (n=1029) ^j M (n=5), F (n=5) F ^b (n=20) M ^c + F ^d (n=66) M+F (n=207) M (n=22), F (n=23)	NR NR 3.8 y (95% CI 3.1-4.4) 2.3 y (95% CI 2.1-2.4) 2.9 y (95% CI 2.3-3.8) 8.5 y (95% CI 7.1-10.1) NR 1.5 y ^a (0.19-5.2) 1.2 y ^a (0.04-14) 1.7 y ^a 3.9 y	0.132 0.150 0.150 NR NR NR 0.096 0.30 ^k (95% CI 0.11-0.49) 0.77 ^k (95% CI 0.47-1.1) NR NR	Harada et al. (2005) Harada et al. (2007) Olsen et al. (2007) Bartell et al. (2011) Seals et al. (2011) Seals et al. (2011) Fuji et al. (2015) Zhang et al. (2013a) Zhang et al. (2013a) Fu et al. (2016) Worley et al. (2017)
PFNA	M (n=5), F (n=5) F ^b (n=16) M ^c + F ^d (n=50)	NR 1.7 y ^a (0.38-7.7) 3.2 y ^a (0.34-20)	0.062 0.25 ^k (95% CI 0.13-0.37) 0.15 ^k (95% CI 0.099-0.20)	Fujii et al. (2015) Zhang et al. (2013a) Zhang et al. (2013a)
PFDA	M (n=5), F (n=5) F ^b (n=19) M ^c + F ^d (n=60)	NR 4.0 y ^a (1.2-7.7) 7.1 y ^a (0.58-60)	0.066 0.066 ^k (95% CI 0.035-0.097) 0.096 ^k (95% CI 0.050-0.13)	Fujii et al. (2015) Zhang et al. (2013a) Zhang et al. (2013a)
PFUnDA	M (n=5), F (n=5) F ^b (n=19) M ^c + F ^d (n=63)	NR 4.0 y ^a (1.1-7.8) 7.4 y ^a (1.1-104)	0.065 0.064 ^k (95% CI 0.033-0.095) 0.065 ^k (95% CI 0.050-0.080)	Fujii et al. (2015) Zhang et al. (2013a) Zhang et al. (2013a)
PFDoDA	M (n=5), F (n=5)	NR	0.070	Fujii et al. (2015)
PFTTrDA	M (n=5), F (n=5)	NR	0.077	Fujii et al. (2015)
PFTeDA	M (n=5), F (n=5)	NR	0.224	Fujii et al. (2015)
PFBS	M (n=5), F (n=1)	27.7 d (95% CI 16.1-39.3)	NR	Olsen et al. (2009)
PFHxS	M (n=24), F (n=2)	8.5 y (95% CI 6.4-10.6)	NR	Olsen et al. (2007)

PFAS	Gender	Half-life	Clearance _{tot} (mL/kg/day)	Reference
	F ^b (n=19) M ^c + F ^d (n=64) M (n=22), F (n=23) M (n=20) F (n=30)	7.1 y ^a (2.3-13) 25 y ^a (1.6-182) 15.5 y 7.4 y (95% CI 6.0-9.7) 4.7 y (95% CI 4.6-6.0)	0.039 ^k (95% CI 0.020-0.057) 0.027 ^k (95% CI 0.018-0.037) NR NR NR	Zhang et al. (2013a) Zhang et al. (2013a) Worley et al. (2017) Li et al. (2018a) Li et al. (2018a)
PFOS	M (n=20) M+F (n=4) M (n=24), F (n=2) F ^b (n=19) M ^c + F ^d (n=64) M (n = ca 1000) F (n = ca 1000) M+F (n=207) M (n=22), F (n=23) M (n=20) F (n=30)	NR NR 5.4 y (95% CI 3.9-6.9) 5.8 y ^a (3.2-10) 18 y ^a (1.6-121) 4.7 y (95% CI 4.2-5.3) 4.3 y (95% CI 4.1-4.5) 1.9 y ^a 3.3 y 4.6 y (95% CI 3.7-6.1) 3.1 y (95% CI 2.7-3.7)	0.066 0.106 NR 0.05 ^e (95% CI 0.037-0.064) 0.037 ^e (95% CI 0.026-0.049) NR NR NR NR NR NR	Harada et al. (2005) Harada et al. (2007) Olsen et al. (2007) Zhang et al. (2013a) Zhang et al. (2013a) Wong et al. (2014) Wong et al. (2015) Fu et al. (2016) Worley et al. (2017) Li et al. (2018a) Li et al. (2018a)

Values are means and 95% confidence interval (95 % CI) or means and range

y = years; d = days

NR = Not Reported; M = male; F = female

a = geometric mean

b = age ≤ 50 years

c = 20 y < age < 88 y

d = age > 50 years

e = age 22±0.9

f = age 68±5

g = age 23±3

h = age 69±5

i = <4 years elapsed in a water district with high exposure levels

j = <9 years elapsed in a water district with low exposure levels

k = renal clearance (mean)

To summarise, based on the high levels of PFASs observed in the blood of individuals exposed to contaminated water and by analogy to what is known for PFOS and PFOA, it can be assumed that the gastrointestinal absorption of most of the PFASs occurs to a significant extent in humans. PFASs are widely distributed in the body, with the highest concentrations generally found in blood, liver and kidney. In blood PFASs bind to albumin. Metabolism of PFASs and PFCAs has never been observed, however, precursor compounds such as FTOHs and PAPs can be biotransformed in the human body to PFCAs and other metabolites. PFASs are eliminated in urine and in faeces, but breast milk was also found to be a substantial route of excretion. Shorter chain PFCAs, are preferentially excreted in urine, whereas PFNA and longer chain PFASs are preferentially eliminated through the bile and subsequently the feces. Extensive uptake from enterohepatic circulation and reabsorption by OATs in the kidneys are believed to be more active processes in humans compared to rodents, slowing down the excretion of these substances. Short-chain PFASs were found to have half-lives ranging from few days (PFBA) to approximately one month (PFBS, PFHxA), whereas for PFHxS, PFOS, PFOA, PFNA, PFDA, and PFUnDA estimated half-lives can exceed 3 years.

Based on levels in cord blood, PFASs can be transferred to the foetus during pregnancy with varying transfer rates depending on the structure of the compound. Generally, longer fluoroalkyl chain length and a terminal sulfonate group are associated with lower fetal/maternal ratios.

Limited data were identified on the toxicokinetics of FTOHs and other precursors in humans.

3.3.1.3 PBPK modelling

Published PBPK models for PFOS and PFOA were already summarised in the previous opinion (EFSA CONTAM Panel, 2018). Since then, other models have been developed, such as the PFOS PBPK model published by Chou and Lin (2019) in which a Bayesian statistical analysis using Markov chain Monte Carlo simulation was performed to optimize the model and to characterize the uncertainty and interspecies (rat, monkey and humans) variability of chemical-specific parameters. Although this type of model can be applied to other PFASs, there are some limitations in this extrapolation. A limited

number of validated PBPK models specifically based on PFASs other than PFOS and PFOA has been published.

Kim et al. (2018) developed a model for simulating the kinetics of PFHxS in male and female rats. The model includes compartments representing plasma (including a bound and free fraction), kidney and renal glomerular filtrate, gastrointestinal tract as absorption site, liver (taking into consideration enterohepatic circulation), brain, heart, lung and the rest of the body. A storage compartment was included in the model, receiving PFHxS from the glomerular filtrate. Absorption from the gastrointestinal tract is simulated as the balance between first-order absorption and fecal excretion of unabsorbed chemical. A PBPK model for PFOA and PFOS in monkeys was referred to describe the renal tubular reabsorption (Loccisano et al., 2011). The tissue to plasma partition coefficients used in the model were calculated from data obtained in rats 14 days after iv injection of PFHxS at a dose of 0.5–10 mg/kg per bw. The transporter maximum, the transporter affinity constant, the rate constant to storage compartment and the free fraction of PFHxS in plasma were estimated by fitting the plasma and urine concentration data in rats.

To validate the PBPK model, male and female rats were both orally administrated 1 and 4 mg/kg bw doses and the model was evaluated according to the values observed for the PFHxS concentrations in plasma, tissues and urine, compared to the predicted concentrations at different time points. The authors concluded that the experimental results fitted well with the model by visual inspection.

The developed PBPK model was extrapolated to humans, considering the interspecies differences of physiological and chemical-specific parameters using the following allometric equation:

$$k_{\text{human}} = k_{\text{rat}} \cdot (BW_{\text{human}}/BW_{\text{rat}})^{-0.25}$$

where k_{human} and k_{rat} are the rate constants in human and rats, respectively and $BW_{\text{human}}/BW_{\text{rat}}$ refers to the body weight of humans and rats, respectively.

Kim et al. (2019) explored gender differences in humans for PFNA and PFDA using a PBPK model based on an *in vivo* study in male and female rats. The PBPK model was validated by comparing visually the data obtained from the analysis of the biological samples collected during the *in vivo* experiment with the predicted concentrations by model simulation. In addition, a normalized sensitivity analysis was carried out comparing the area under the curve (AUC) obtained from the original parameter and the changed parameter after increasing by 1%. The rat PBPK model was extrapolated to humans, considering the interspecies physiological parameters, on the basis of the same allometric equation described by Kim et al. (2018), as reported above.

3.3.1.4 Transfer from feed to food

Numata et al. (2014) estimated the bioaccumulation factors (ratio of the concentration in the tissue vs concentration in feed) of several PFASs and PFCAs in growing pigs fed for 21 days a diet containing PFOS, PFBS, PFHxS, PFHxA, PFOA, PFHpA, PFHpS at concentrations of 137, 132, 91.3, 47.8, 22.4, 10.2 and 4 µg/kg dry feed, respectively. Although the steady state was not reached at the end of the experiment for most of the investigated substances, bioaccumulation factors were reported for the whole body as well as for some tissues. For PFBS, the bioaccumulation factors calculated for the whole animals, meat and liver were 1.2, 0.8 and 6.0, respectively, whereas it was 17.9, 9.7 and 503, respectively for PFOS. The corresponding values for PFHxS were 20.1, 13.1 and 48, whereas for PFHpS, the values were 12.7, 8.3 and 81, respectively. The bioconcentration factors for PFHpA were 2.7, 1.8 and 7.0 for the whole animal, meat and liver whereas for PFOA corresponding values were 7.9, 5.3 and 32.8. For PFHxA the bioconcentration factor was <1 in each case.

Kowalczyk et al. (2013) investigated the transfer of PFBS, PFHxS, PFOS and PFOA into milk in dairy cows fed a PFAS-contaminated feed for 28 days. On the basis of the daily intake of PFAS-contaminated grass silage and hay, the total intake of PFBS and PFHxS was 3.4 ± 0.7 and 4.6 ± 1.0 µg/kg bw per day, respectively, compared to 7.6 ± 3.7 and 2.0 ± 1.2 µg/kg bw per day for PFOS and PFOA, respectively. The estimated transfers of PFOS, PFBS, PFHxS and PFOA into milk were found to be approximately 14, 0.01, 2.5 and 0.1%, respectively. The accumulated amount of PFASs in tissue samples

of dairy cows showed the highest accumulation potential for PFOS (approximately 18% and 43% of ingested dose in liver and muscle, respectively), less for PFHxS (0.6 and 9%, respectively) as well as negligible potential for PFBS and PFOA accumulation.

Martin et al. (2003) exposed juvenile rainbow trout (*Oncorhynchus mykiss*) to a mixture of PFASs for 34 days in the diet. The concentrations of PFCAs in feed were 0.50, 0.52, 0.46, 0.42, 0.39, 0.57, 1.1 and 1.2 mg/kg, for PFPeA, PFHxA, PFHpA, PFOA, PFDA, PFUnDA, PFDoDA and PFTeDA, respectively, whereas concentrations of PFSAs were 0.32, 0.51, 0.54 mg/kg, for PFBS, PFHxS and PFOS, respectively. PFBS, PFHxA and PFHpA were not detected or quantified in fish carcasses (LOQ not specified), suggesting that their bioaccumulation potential was negligible. For other PFASs, the bioaccumulation factors increased with increasing perfluoroalkyl chain length, varying from 0.04 for PFOA to 1 for PFTeDA. Overall, these values indicate that dietary exposure does not result in bioaccumulation of PFASs in juvenile trout.

This result was confirmed by Goeritz et al. (2013) in adult rainbow trout dietary exposed for 28 days to a mixture of PFASs (PFBS, PFHxS, PFOS, PFOA and PFNA incorporated into the feed at 0.50 mg/kg each). In these experimental conditions, bioaccumulation factors were calculated as 0.42 for PFOS, >0.23 for PFNA, >0.18 for PFHxS, >0.04 for PFOA, and >0.02 for PFBS.

Gaillard et al. (2017) investigated the fate of EtFOSE in juvenile perch (*Perca fluviatilis*) dietary exposed for 45 days to EtFOSE-based phosphate diester incorporated into feed at a concentration of 1.6 mg/kg feed. EtFOSE and metabolites, including PFOS, were monitored in fish tissues (muscle, liver, serum) to study the bioaccumulation. Although a bioaccumulation factor cannot be calculated from the data provided by the authors, the concentrations found in muscle, liver and serum for EtFOSE and the sum of corresponding metabolites (< 0.04 µg/g) suggest that bioaccumulation does not occur.

3.3.2. Biomonitoring

3.3.2.1 Selection of biomarker and appropriate matrix for assessing internal dose

Due to their chemical structure, perfluoroalkyl sulfonates (PFSAs) and perfluoroalkyl carboxylates (PFCAs) are highly persistent and are not metabolised in the human body. Thus, when biomonitoring studies are conducted, PFSAs and PFCAs can be measured unchanged in biological matrices. The most frequently assessed PFSAs and PFCAs are PFOS and PFOA, followed by PFHxS and PFNA (see Appendix B, Table B.1). Quite a few studies have also reported levels of PFDA and PFUnDA, while data on other compounds are scarce. Biomonitoring of PFOS and PFOA has been described in detail in the previous opinion (EFSA CONTAM Panel, 2018).

Blood, specifically plasma or serum, is generally the preferred matrix to assess internal doses of PFSAs and PFCAs in humans (Butenhoff et al., 2006). As described in detail in Section 3.3.2.3 below, PFHxS, PFOS, PFOA and PFNA are in general the most abundant PFSAs and PFCAs. Studies on whole blood have also been conducted, with the advantage that whole blood represents the entire circulating fluid. However, due to practical reasons, serum and plasma are more frequently used. When comparing levels in whole blood, serum and plasma, it has been common to multiply the whole blood concentration with a factor of 2 to be comparable with concentrations in serum and plasma (Ehresman et al., 2007). In a study on Norwegian adults (n = 61), a range of PFCAs and PFSAs were determined in plasma, serum and whole blood. For PFHxS, PFNA and PFUnDA, as well as for PFOS and PFOA, the ratio between serum/plasma and whole blood was close to 2, while for PFBS, PFHpS, PFDS, PFDA, PFDoDA and PFTeDA the results were more variable (Poothong et al., 2017). This could be due to differences in the distribution between blood compartments, but the very low concentrations (median <0.4 ng/mL) of these PFASs in all three matrices may also have had an influence on this, as the measurement uncertainty is considerably higher for such low concentrations. Furthermore, PFHxA was detected in all samples of whole blood but none of the serum/plasma samples from the same individuals (Poothong et al., 2017). Based on this study, whole blood is the preferred blood matrix for PFHxA.

For PFASs other than PFCAs and PFSAs, much less is known. Methods for determination of most of these compounds in blood have been established, but the data are very limited (see below). Similar to PFHxA, a study on Norwegian adults (n = 61) found considerably higher concentrations of FOSA in

whole blood compared to serum/plasma samples from the same individuals, and thus whole blood is also the preferred blood matrix for FOSA (Poonthong et al., 2017).

As described in Section 1.3.1, FTOHs and PAPs can be transformed in the human body to PFCAs, and EtFASEs and EtFASAs to FASAs and further to PFASs, and thus contribute to the internal dose of PFCAs and PFASs. As EtFOSA is transformed to EtFOSAA in the body, EtFOSAA would be the preferred biomarkers of exposure to EtFOSA.

Some studies on breast milk have been conducted, and a limited number of studies have assessed the levels of blood and breast milk from the same individuals. The levels in breast milk are substantially lower than in serum. In a study including 61 paired samples of breast milk and serum from France, only PFHxS in addition to PFOS and PFOA was observed above the LOQ in some of the milk samples (15% >LOQ), with a mean concentration of 0.026 ng/mL (Cariou et al., 2015). The ratio between the mean concentrations of PFHxS in breast milk and serum was 0.01. In a Swedish study on matched samples of breast milk and serum, mean ratios (breast milk:serum) of 0.02, 0.07 and 0.01 were reported for PFHxS, FOSA and PFNA (Kärman et al., 2007). Distribution of PFASs between serum and breast milk were also explored in a Korean study, and a ratio (breast milk:serum) of 0.008 was reported for PFHxS (Kim et al., 2011b). In a study on matched serum and breast milk samples from China, ratios (breast milk:serum) of 0.04, 0.03 and 0.04 were reported for PFNA, PFDA and PFUnDA, respectively (Liu et al., 2011).

Also, some few studies have reported concentrations of PFASs and PFCAs in urine, and the concentrations are in general low. In a study on matched urine and serum samples from children in South Korea, short-chain PFCAs were the compounds most frequently detected in urine, while none of the PFASs were observed above the LOD in any of the urine samples (Kim et al., 2014). In general, the detection frequency in urine was very low except for PFPeA which was found above the LOQ in 70% of the samples with a mean concentration of 2.34 ng/mL (range; not detected–11.6 ng/mL). In comparison, the mean PFPeA concentration in serum (detection frequency of 37%) was 0.497 ng/mL (range; not detected–0.942 ng/mL). In a study from China with samples collected in the Hebei province, 86 paired samples of urine and blood were collected (Zhang et al., 2013a). The median concentrations in urine were 0.0011, 0.025, 0.00082, 0.019, 0.0017, 0.00022, 0.00030 and 0.00044 ng/mL for PFHxS, PFOS, PFHpA, PFOA, PFNA, PFDA, PFUnDA and FOSA, respectively. The ratios between the mean serum and urine concentrations were 30, 33 (whole blood:urine), 163, 218, 600, 682, 760 and 1,320 for PFHpA, FOSA, PFOA, PFNA, PFUnDA, PFDA, PFOS and PFHxS. In another study from China, 64 paired samples of urine and serum were analysed (Li et al., 2013). The median concentrations of PFOA and PFOS in urine were approximately 125 and 175 times lower than the corresponding median serum concentrations, respectively. In a study of 55 highly exposed fishery employees from Wuhan, China, with paired samples of urine and serum, high correlations (Spearman Rank Correlation >0.65) between concentrations in urine and serum were observed for PFBA, PFOA, PFBS, PFHxS, and PFOS, and the corresponding median ratios were 0.139, 0.003, 0.0001, 0.0003 and 0.0006, respectively (Wang et al., 2018a).

Other non-invasive matrices, which have been assessed, are hair and nails, but it is still not clear how to interpret these results (Li et al., 2013; Alves et al., 2014, 2015).

3.3.2.2 Time trends

The production and use of PFASs and PFCAs have changed dramatically during the last decades (see Section 1.3.3). To understand how concentrations of various PFCAs and PFASs have changed over time, several time-trend studies have been conducted (see detailed information in Appendix B, Table B.1). The time frames of the studies vary, and so do the observed trends.

To illustrate this, results from a time-trend study conducted in Germany based on samples from the German Environmental Specimen bank (Yeung et al., 2013a,b) are described in detail below. This study covers many sampling years and many different PFASs, including both males and females, and the LOQs are satisfactory low to be able to determine a wide range of PFASs in the samples.

In this particular study, 420 blood samples were collected in Münster (n = 270) and Halle (n = 150) from students in the age group 20–29. The samples from Münster (mean age 24 years) were collected

between 1982 and 2009, and PFASs concentrations were determined in ten samples per year except in 2006 (n = 9) and 2004 (n = 0). Samples were collected in Halle during the period 1995 to 2009, and ten samples per year were analysed to determine PFAS concentrations except in 2006 (n = 11). The mean age of the individuals that donated samples in Halle was 23 years. The gender distribution was approximately even in the samples from both Münster and Halle.

Information on the trends of PFOS and PFOA in this study is given in the EFSA CONTAM Panel (2018) Opinion.

PFHxS: no particular trend was observed for either the Münster or Halle samples. In the samples from Münster, the mean concentration in males varied between 1.28 ng/mL in 1985 and 2.49 ng/mL in 2002. The lowest observed mean concentration in males from Halle was 1.21 ng/mL in 1995, while it was 1.91 ng/mL in 2003.

PFNA: in the Münster samples a steep increase was observed from 1982, with a mean concentration in males of 0.13 ng/mL, to 1989 where the mean concentration in males was 1.58 ng/mL. Following this, a decreasing trend was observed up to the year 2000. The mean concentration in males was then 0.34 ng/mL. From 2000 to 2006 the concentration increased again, with a mean concentration in males of 0.95 ng/mL in 2006. In the Halle samples the concentration of PFNA was quite stable from 1995 to the end of the century, with a mean concentration in males of 0.30 ng/mL in 1995. From around the year 2000 an increasing trend was observed with a mean concentration in males of 0.86 ng/mL in 2008.

PFDA: an increasing concentration was observed in the Münster samples from 1982 up to 2006 after which it seemed to stabilise. The mean concentration in males was 0.061 ng/mL in 1982 while it was 0.42 ng/mL in 2006. A comparable trend and similar concentrations were observed for the Halle samples.

PFUnDA: the trend in the Münster samples was similar to the trend for PFOS and PFOA with an increasing trend from 1982 to around 1990, followed by decreasing concentrations between 1990 and 1995 and a new increasing trend from 1995 to 2009. The mean concentration in males in 1982 was 0.034 ng/mL while it was 0.218 ng/mL in 1989 and 0.127 ng/mL in 2009.

No particular trends were observed for the remaining detected substances (PFHxA, PFHpA, PFDoDA, PFTrDA, PFTeDA, 8:2 diPAP, PFDS, EtFOSAA).

The general trends observed in the time-trend studies (see Appendix B, Table B.1) can be summarised as follows: (i) the concentrations of most PFSAs and PFCAs increased from the early 1970s up to around the year 2000, (ii) the concentrations of PFOS and PFOA have in most studies been observed to decrease from approximately the year 2000, while in many studies PFNA, PFDA and PFUnDA have increased or at least remained stable during the same time period, (iii) variable trends have been reported for PFHxS, while no particular change has been reported for some of the PFASs that are present in low concentrations in humans. This is in line with the conclusions in a recent review on time trends of PFASs (Land et al., 2018).

3.3.2.3 Levels in general European populations with serum or plasma samples collected from 2007–2008 and onwards

In Appendix B (Tables B.2 and B.4) concentrations of all PFASs included in this Opinion in plasma and serum from adults and children are reported, respectively. Furthermore, in Appendix B (Table B.3) concentrations of PFHxA and FOSA in whole blood are presented. European studies with samples collected from general populations in 2007–2008 and onwards are included. For the time-trend studies, only results from the most recent years have been included. Descriptive statistics summarising the studies in Appendix B (Tables B.2 to B.4) are presented in Tables 16 and 17 for adults and Table 18 and 19 for children, respectively. The summary statistics on PFOS and PFOA are given in the EFSA CONTAM Panel (2018) Opinion but are also included in Tables 16 to 19 for comparison.

Despite variations in design, populations (e.g. sex, age, race), analytical methods, and geographic location the median concentrations in the different European studies are similar, with ratios between the highest and lowest median concentration usually being less than 10. The European levels are also

2525 comparable to levels observed in general populations world-wide (Haines and Murray, 2012; Wan et al.,
2526 2013; Zhang et al., 2014; Khalil et al., 2016).
2527 No European studies reporting concentrations of PFHxDA, PFODA and EtFOSE were identified. Two
2528 studies from Australia and China have reported that PFHxDA and PFODA were determined, but not
2529 observed above LOD (<0.2 ng/mL) in any of the samples (Eriksson et al., 2016b; Gao et al., 2016).

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Table 16: Summarising statistics describing serum concentrations of PFCAs in the European adult population based on studies included in Tables B.2. and B.3. in Appendix B (from 2007–2008 and onwards)

		Concentration (ng/mL)											
		PFBA	PFPeA	PFHxA	PFHxA whole blood	PFHpA	PFOA ^(a)	PFNA	PFDA	PFUnDA	PFDoDA	PFTTrDA	PFTeDA
Statistics based on medians reported in studies included in Tables B.2. and B.3. in Appendix B ^(b)	Median	0.01	0.05	0.03	0.620	0.05	1.9	0.61	0.30	0.28	0.05	0.05	-
	Mean	0.01	0.05	0.03	0.620	0.06	2.1	0.74	0.35	0.46	0.14	0.08	-
	Minimum	0.01	0.01	0.01	0.620	0.03	0.76	0.30	0.07	0.06	0.03	0.01	-
	Maximum	0.013	0.09	0.045	0.62	0.14	4.9	2.64	1.34	2.49	0.54	0.21	-
	Number of studies^(c)	1	2	2	1	6	32	37	33	22	7	4	0
Information based on individual samples reported in studies included in Tables B.2. and B.3. in Appendix B	Minimum individual samples	<LOQ (0.013)	<LOQ (0.013)	<LOQ (0.0003)	0.14	<LOQ (0.01)	0.03	<LOQ (0.013)	0.013	<LOQ (0.013)	<LOQ (0.0036)	<LOQ (0.009)	<LOQ (0.002)
	Maximum individual samples	3.59	2.02	1.58	1.65	0.70	80.8	8.6	19.2	18.2	3.9	0.9	0.029
	Country reporting maximum individual samples	Italy	UK	Germany	Norway	Sweden	France	Germany	Germany	Greenland	Germany	Greenland	Czech Republic
	Reference for maximum	Ingelido et al. (2018)	Heffernan et al. (2018)	Yeung et al. (2013a)	Poothong et al. (2017)	Stubleski et al. (2016)	Denys et al. (2014)	Fromme et al. (2017)	Fromme et al. (2017)	Long et al. (2015)	Fromme et al. (2017)	Long et al. (2015)	Sochorov á et al. (2017)

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NR: not reported. LOQ: limit of quantification.
 For studies with median concentration lower than LOQ, the LOQ was used for calculations. For studies reporting the concentrations in ng/g it was assumed that 1 g = 1 mL of serum/plasma.
 (a): Data from EFSA CONTAM Panel (2018) Opinion.
 (b): Based only on studies where median levels were reported.
 (c): Only studies where median is >LOQ are included in this number.

Table 17: Summarising statistics describing serum concentrations of PFSA and other selected PFASs in the European adult population based on studies included in Table B.2. and B.3. in Appendix B (from 2007–2008 and onwards)

		Concentration (ng/mL)												
		PFBS	PFHxS	PFHpS	PFOS ^(a)	PFDS	FOSA	FOSA whole blood	8:2 monoPA P	8:2 diPAP	EtFOSA	EtFOSAA	FC-807	PFOSI
Statistics based on medians reported in studies included in Tables B.2. and B.3. in Appendix B ^(b)	Median	0.03	0.67	0.20	7.7	0.03	0.025	0.140	0.045	0.009	0.025	-	-	-
	Mean	0.03	4.94	0.25	7.5	0.03	0.023	0.140	0.045	0.009	0.025	-	-	-
	Minimum	0.03	0.20	0.08	1.7	0.01	0.007	0.140	0.045	0.009	0.004	-	-	-
	Maximum	0.04	152	0.6	27.4	0.06	0.035	0.14	0.045	0.009	0.045	-	-	-
	Number of studies ^(c)	2	37	8	32	2	4	1	1	1	2	0	0	0
Information based on individual samples reported in studies included in Tables B.2. and B.3. in Appendix B	Minimum individual samples	0.006	0.008	0.05	0.06	0.005	0.0018	0.05	0.045	0.0005	0.004	0.27	0.0004	0.15
	Maximum individual samples	4.6	1790	13.3	392.3	0.245	0.52	2.35	0.045	0.11	0.05	0.27	0.0007	NR
	Country reporting maximum individual samples	Italy	Sweden	France	France	Czech Republic	Sweden	Norway	Norway	Norway	Norway	Spain	Germany	Denmark
	Reference for maximum	Ingelido et al. (2018)	Li et al. (2018a)	Denys et al. (2014)	Denys et al. (2014)	Sochorová et al. (2017)	Stableski et al. (2016)	Poonthong et al. (2017)	Poonthong et al. (2017)	Poonthong et al. (2017)	Haug et al. (2011)	Bartolome et al. (2017)	Yeung et al. (2013b)	Joensen et al. (2013)

NR: not reported.

For studies with median concentration lower than LOQ, the LOQ was used for calculations. For studies reporting the concentrations in ng/g it was assumed that 1 g = 1 mL of serum/plasma.

(a): Data from EFSA CONTAM Panel (2018) Opinion.(b): Based only on studies where median levels were reported. (c): Only studies where median is >LOQ are included in this number.

Table 18: Summarising statistics describing serum concentrations of PFCAs in the European children population based on studies included in Table B.4. in Appendix B (from 2007–2008 and onwards)

		Concentration (ng/mL)									
		PFBA	PFPeA	PFHxA	PFHpA	PFOA ^(a)	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA
Statistics based on medians reported in studies included in Table B.4. in Appendix B ^(b)	Median			0.08	0.09	3.3	0.79	0.30	0.19	0.05	0.10
	Mean			0.08	0.09	3.3	0.92	0.29	0.20	0.06	0.10
	Minimum			0.08	0.03	0.49	0.5	0.13	0.15	0.04	0.1
	Maximum			0.08	0.15	6.9	2.13	0.4	0.29	0.08	0.1
	Number of studies ^(c)	0	0	1	4	8	9	7	4	4	1
Information based on individual samples reported in studies included in Table B.4. in Appendix B	Minimum individual samples	<0.1 (IQR)	<0.05 (IQR)	0.03	<0.03 (IQR)	0.45	0.12	< 0.05	0.03	0.01	0.05
	Maximum individual samples	<0.1 (IQR)	<0.05 (IQR)	1.34	1.47	19.5 (P95)	23.96	1.89	1.08	0.24	0.17
	Country reporting maximum individual samples	Faroe Islands	Faroe Islands	Norway	Norway	Germany	Norway	Norway	Norway	Norway	Norway
	Reference for maximum	Dassuncao et al. (2018)	Dassuncao et al. (2018)	Averina et al. (2018)	Averina et al. (2018)	Fromme et al. (2010)	Papadopoulou et al. (2016)	Averina et al. (2018)	Papadopoulou et al. (2016)	Averina et al. (2018)	Papadopoulou et al. (2016)

P95: 95th percentile. IQR: interquartile range.

For studies with median concentration lower than LOQ, the LOQ was used for calculations. For studies reporting the concentrations in ng/g it was assumed that 1 g = 1 mL of serum/plasma.

(a): Data from EFSA CONTAM Panel (2018) Opinion.

(b): Based only on studies where median levels were reported.

(c): Only studies where median is >LOQ are included in this number.

Table 19: Summarising statistics describing serum concentrations of PFSA in the European children population based on studies included in Table B.4. in Appendix B (from 2007–2008 and onwards)

		Concentration (ng/mL)							
		PFBS	PFHxS	PFHpS	PFOS ^(a)	PFDS	FOSA	MeFOSAA	EtFOSAA
Statistics based on medians reported in studies included in Table B.4. in Appendix B ^(b)	Median	0.10	0.60	0.14	3.2	0.03	0.03	0.04	0.03
	Mean	0.10	0.56	0.13	3.3	0.03	0.06	0.04	0.03
	Minimum	0.1	0.3	0.07	0.49	0.03	0.03	0.03	0.03
	Maximum	0.1	0.81	0.19	8.6	0.03	0.11	0.05	0.03
	Number of studies^(c)	2	9	4	8	2	3	2	2
Information based on individual samples reported in studies included in Table B.4. in Appendix B	Minimum individual samples	<0.1 (IQR)	<0.03	0.03	0.47	<0.03 (IQR)	<0.03	<0.03 (IQR)	<0.03 (IQR)
	Maximum individual samples	<0.1 (IQR)	84.7	7.62	23.0	0.04 (IQR)	0.16	0.08 (IQR)	<0.03 (IQR)
	Country reporting maximum individual samples	Faroe Islands	Norway	Norway	Denmark	Faroe Islands	Norway	Faroe Islands	Faroe Islands
	Reference for maximum	Dassuncao et al 2018	Averina et al 2018	Averina et al 2018	Mørck et al. 2015	Dassuncao et al 2018	Papadopoulou et al. 2016	Dassuncao et al 2018	Dassuncao et al. 2018

IQR: interquartile range.

For studies with median concentration lower than LOQ, the LOQ was used for calculations. For studies reporting the concentrations in ng/g it was assumed that 1 g = 1 mL of serum/plasma.

(a): Data from EFSA CONTAM Panel (2018) Opinion.

(b): Based only on studies where median levels were reported.

(c): Only studies where median is >LOQ are included in this number.

3.3.2.4 Levels in blood from occupationally exposed adults

Fluorochemical production workers have in several studies been reported to have elevated exposure to PFASs. Most frequently, levels of PFOS and PFOA are presented, with mean concentrations typically in the range 500–7,000 ng/mL (Fromme et al., 2009), but also studies on PFHxS have been described with geometric mean concentrations up to 700 ng/mL (Olsen, 2015). In Olsen (2015), levels in fluorochemical production workers have been discussed in further detail.

Elevated concentrations of a range of PFCAs in serum from professional ski waxers have been reported in two Nordic studies (Nilsson et al., 2010; Freberg et al., 2010). As an example, in a Swedish study, the whole blood concentrations of PFNA were in the range 0.8–163 ng/mL, while the range of concentrations for PFDA and PFUnDA were 0.9–24 ng/mL and 0.1–2.8 ng/mL, respectively.

Several studies have found increased concentrations of different PFASs and PFCAs in blood from firefighters compared to the general population. In a study from Australia, elevated serum levels of PFOS and PFHxS were observed (Rotander et al., 2015). Median concentrations of 66 and 25 ng/mL were reported for PFOS and PFHxS, respectively. The PFOS and PFHxS concentrations were around 6–15 times higher than the corresponding concentrations in the general population. Elevated concentrations of PFOA, PFNA and PFDA compared to the general US population were observed in two studies on Californian firefighters (Shaw et al., 2013; Dobraca et al., 2015). Further, Tao et al. (2008), concluded that New York State employees and National Guard personnel were exposed to PFASs and PFCAs, especially PFOA, PFNA, and PFHxS, through inhalation of dust and smoke released during and after the collapse of the World Trade Center. In a study on Finnish firefighters, PFNA and PFHxS concentrations in serum increased during three consecutive training sessions, although this was not possible to test statistically due to few included individuals (Laitinen et al., 2014).

3.3.2.5 Levels in blood from populations with elevated drinking water exposure

Drinking water contaminated with PFASs has been identified in many countries, and several biomonitoring studies have observed elevated concentrations of PFASs in blood from general populations drinking the contaminated water (Hölzer et al., 2008; Frisbee et al., 2009; MDH, 2012; ATSDR, 2013; Jakobsson et al., 2014; Gyllenhammar et al., 2015). Most of the studies have focused on PFOA and PFOS, but also increased concentrations of other PFASs such as PFHxS and PFNA are described. For example, in a study from Sweden a maximum concentration of PFHxS of 1,790 ng/mL was reported (Li et al., 2018a). These findings emphasise that, even though most general populations world-wide are similarly exposed to PFASs and PFCAs, elevated exposure may be experienced by large populations (Post et al., 2012).

3.3.2.6 Factors that may have an impact on the internal dose of PFASs

Exposure to PFASs can occur throughout our entire lives. Trans-placental transfer has been confirmed for many PFASs (Apelberg et al., 2007a; Kim et al., 2011a; Gützkow et al., 2012; Porpora et al., 2013; Ode et al., 2013; Zhang et al., 2013c; Zhang and Qin, 2014; Fisher et al., 2016), and the exposure continues after birth among others through breastfeeding (Fromme et al., 2010; Haug et al., 2011; Papadopoulou et al., 2016).

In contrast to some other environmental contaminants, no clear regional differences in PFAS concentrations have been reported for general populations, but there are indications that low income countries may have somewhat lower prevalence of these compounds (Kannan et al., 2004; Fromme et al., 2009).

The body burden of PFASs may be influenced by several biological factors. In general, lower concentrations have been observed in women compared to men (Fromme et al., 2009; Ingelido et al., 2010; Haines and Murray, 2012; Bjermo et al., 2013). Factors that may explain this are differences in exposure (Kato et al., 2015a), physiological differences including urinary elimination (Han et al., 2012), menses (Wong et al., 2014), use of oral contraceptives (Rush et al., 2018), pregnancy and lactation (Brantsæter et al., 2013). The relation between PFAS concentrations and age is more questionable (Fromme et al., 2009), and due to temporal changes in exposure it is hard to distinguish between the effect of age and the effect of changes in exposure (Nøst et al., 2014). However, due to long elimination

half-lives for several PFASs (see Section 3.3.1), bioaccumulation and thereby increasing concentrations with increasing age, is anticipated.

With respect to ethnicity, lower PFSA and PFCA concentrations have been reported in Mexican Americans compared to non-hispanic whites (Calafat et al., 2007; Khalil et al., 2016). The effect of body weight / body mass index (BMI) on PFAS concentrations in blood has been evaluated in many studies, and both inverse associations (Kato et al., 2014; Lauritzen et al., 2016; Eriksen et al., 2011), positive associations (Halldorsson et al., 2008) and no associations (Rylander et al., 2009) have been reported. Also, the impact of socio-economical status on the concentrations of PFASs in blood has been assessed in a range of studies. Increasing PFAS concentrations with increasing income has frequently been reported (Calafat et al., 2007; Nelson et al., 2012; Brantsæter et al., 2013; Kato et al., 2014; Jain, 2014; Sagiv et al., 2015), while the impact of education is not as clear (Apelberg et al., 2007b; Calafat et al., 2007; Halldorsson et al., 2008; Nelson et al., 2012; Brantsæter et al., 2013; Kato et al., 2014; Jain, 2014; Sagiv et al., 2015).

3.3.2.7 Relative contribution and correlations between PFASs

The relative contribution of different PFASs in blood varies between individuals. In Figure 10, the relative contribution based on medians of medians presented in Table 16 and 17 (adults) and Table 18 and 19 (children) are shown. The seven most prominent PFASs (PFOS, PFOA, PFHxS, PFNA, PFDA, PFUnDA, PFHpS) contributed with 96.6% and 93.4% of the total for adults and children, respectively. As can be seen from Figure 6, for adults the most prominent PFAS was PFOS (64%) followed by PFOA (16%), PFHxS (5.6%) and PFNA (5.1%). For children, PFOS and PFOA contributed almost the same with 35.0% and 36.6% of the total, followed by PFNA (8.8%) and PFHxS (6.7%). Also in most of the individual studies contributing to calculations of medians of medians, both for adults and children, these four PFASs were predominant (for example Fromme et al., 2010; Haug et al., 2011; Bjeremo et al., 2013; Bao et al., 2014; Denys et al., 2014; Mørck et al., 2015; Stubleski et al., 2016; Hansen et al., 2016; Bach et al., 2016; Timmermann et al., 2016; Papadopoulou et al., 2016; Bartolome et al., 2017; Sochorová et al., 2017; Grandjean et al., 2017a; Ingelido et al., 2018; Heffernan et al., 2018; Averina et al., 2018). As can be noted from Figure 4, the relative contribution of the different PFASs differs between children and adults. Several factors may partly explain this, such as differences in the transplacental transfer of various PFASs, differences in the relative transfer of PFASs during breastfeeding, and differences in the relative contribution of various exposure pathways for children and adults.

Pair-wise correlations between PFAS concentrations have been assessed in several studies. Pair-wise correlations for the four most prominent PFASs in general European populations (PFOA, PFNA, PFHxS and PFOS) were summarised based on various studies (without known pronounced contamination of drinking water) from Table B.2 in Appendix B (Bao et al., 2014; Cariou et al., 2015; Hansen et al., 2016; Bach et al., 2016; Sochorová et al., 2017; Ingelido et al., 2018 (non-exposed subjects)). High correlations indicate similar exposure pathways among PFASs, while low correlations indicate distinct exposure pathways for specific compounds. The correlations varied to a large extent for all compounds (PFOS vs PFOA: 0.40–0.71; PFOS vs PFHxS: 0.38–0.83; PFOS vs PFNA: 0.46–0.89; PFOA vs PFHxS: 0.23–0.87; PFOA vs PFNA: 0.58–0.82; PFNA vs PFHxS: non-significant–0.74). This shows that it is difficult to predict concentrations of one PFAS based on another PFAS.

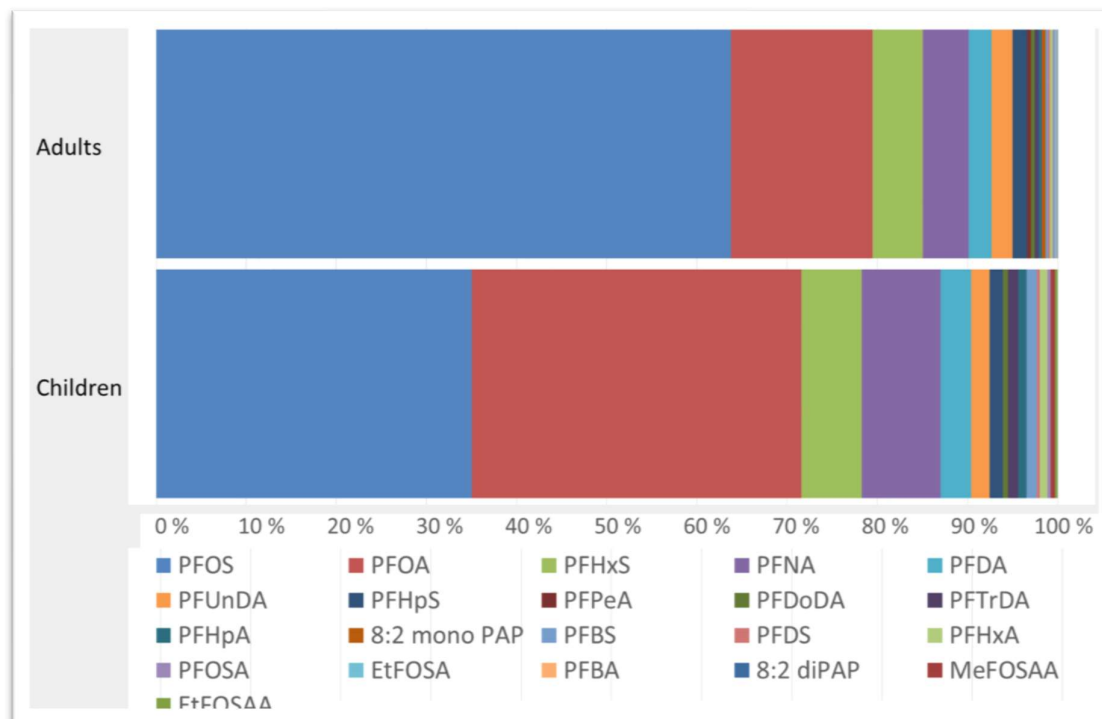


Figure 10: The relative contribution of various PFASs based on medians of medians for adults presented in Tables 16 and 17 and for children presented in Tables 18 and 19

3.3.2.8 Linear and branched isomers

PFCAs and PFSAAs may exist as both linear and branched isomers (see Section 1.3.1). So far most studies have been focussing on isomers of PFOS and PFOA (see Section 1.3.1 of EFSA CONTAM Panel (2018)), but isomer patterns may be of particular interest for exploring sources of exposure also for other PFASs (Martin et al., 2010; Miralles-Marco and Harrad, 2015).

3.3.2.9 Summary

Taken together, blood serum and plasma have been the most commonly used matrices for biomonitoring of PFASs, even though for some specific compounds whole blood may be more appropriate. Different time trends have been observed for the various PFASs the last fifty years. Generally, decreasing concentrations have been observed for PFOS, PFOA and in some studies PFHxS after the year 2000, while the concentrations of PFNA, PFDA and PFUnDA have increased in the same time span. In samples collected from general populations in 2007/2008 and onwards, the median of the median concentrations of all PFASs (except for PFOA and PFOS) in all included studies were below 1 ng/mL for both adults and children. However, considerably higher concentrations have been observed for some individuals, and for occupationally exposed adults with for example mean PFHxS concentrations up to 700 ng/mL. Further, higher levels have also been reported following elevated exposure from for instance contaminated drinking water. Age, gender and socio-economic status may also have an impact on the determined PFAS concentrations.

3.3.3. Toxicity in experimental animals

With a few exceptions, information is missing as to whether or not linear or branched forms of PFASs were applied. The NOEL/LOEL and NOAEL/LOAEL values indicated, have been derived by the authors and agreed by the CONTAM Panel, unless otherwise stated.

3.3.3.1 Effects following acute exposure

Considering the limited number of published data on acute exposure effects, studies on oral as well as non-oral exposure were considered. Reports on effects following acute exposure to PFOS and PFOA, which have been published between 2008 and 2016, are documented in the previous opinion (EFSA CONTAM Panel, 2018).

For the group of PFCAs, studies on PFHxA and PFDA were identified. The LD₅₀ for PFHxA ranged between 1750 and 5000 mg/kg bw in female rats and for PFDA between 120 and 129 mg/kg bw in female mice. In male mice PFDA lowered major transporters for bile acids into the liver; as a result, 80 mg/kg bw increased serum bile acid concentrations. The same dose elevated the hepatic expression of Mrp3 and Mrp4 interfering with the efflux of bilirubin and bile acids to serum. Hepatocellular injury and inflammation at 50-80 mg of PFDA/kg bw were reported as well.

With regard to other PFASs, EtFOSE did not alter peroxisomal β -oxidation or relative liver weights, when applied i.p. to male rats at 100 mg/kg bw. Cynomolgus monkeys, treated with a single dose of 9 mg PFOS/kg bw by gavage, showed no significant alterations. A single gavage of 8:2 FTOH at 500 and 2000 mg/kg bw exerted no effects in male and female rats. For details see Appendix D.

3.3.3.2 Effects following repeated exposure

Studies on effects following repeated exposure to PFOS and PFOA, which have been published between 2008 and (deadline of 2017), are documented in the previous opinion (EFSA CONTAM Panel, 2018).

3.3.3.2.1 PFCAs

The available repeated dose studies for PFBA, PFHxA, PFHpA, PFNA, PFDA, PFUnDA, PFDODA, PFTeDA, PFHxDA and PFODA are listed in Appendix E, Table E1. Reports on effects following repeated exposure to PFOA, which have been published between 2008 and 2017, are documented in the EFSA opinion of 2018. For PFTrDa no studies have been identified.

In two independent studies, **PFBA** increased the liver weight in male mice (28 days) and male rats (28 or 90 days) at oral doses of 35 (Foreman et al., 2009) and 30 mg/kg bw per day (Butenhoff et al., 2012), respectively. Butenhoff et al. (2012) treated male and female SD rats (10/sex/group) by gavage with ammonium PFBA at doses of 0, 6, 30 and 150 mg/kg bw per day for 28 days and at doses of 0, 1.2, 6 and 30 mg/kg bw per day for 90 days. At the lowest dose there were no treatment-related alterations in females. In males, PFBA induced dose-dependently an increased liver weight, accompanied by hepatocellular hypertrophy and coagulative necrosis. In females, the relative liver weight remained unchanged. In males, total serum cholesterol was lowered. Thyroid-follicular hypertrophy and hyperplasia were also noted. In the 28-day study a reduced total and free serum thyroxin level was observed in males already at 6 mg/kg bw per day but no changes occurred in female animals in any group. In the 90-day study the serum thyroxin levels were decreased again in males only at 30 mg/kg bw per day. The considerably higher NOAEL in females was attributed to significantly lower levels of PFBA in serum and the liver, e.g., after 28 days of 150 mg PFBA/kg bw per day levels in males reached 82.2 μ g/mL serum and 37.4 μ g/g in males, while females showed levels of 10.2 μ g/mL serum and 2.7 μ g/g in liver tissue only. The same trend could be seen also after 90 days of 30 mg PFBA/kg bw per day. Females exhibited 5.2 μ g/mL serum and 0.9 μ g/g liver tissue and males 52.2 μ g/mL serum and 16.1 μ g/g liver.

PFHxA, increased absolute and relative liver weights after oral application in male and female rats (WIL Lab 2005; Chengelis et al., 2009; Loveless et al., 2009; NTP 2019a) and after *i.p.* application in female mice (Kudo et al., 2006) occurring at doses of 100 mg/kg bw per day and above. In male rats liver enzymes in serum were increased at 20 mg/kg bw per day (Loveless et al., 2009). The most sensitive parameter was increased relative kidney weight in male animals, treated with the lowest dose of 10 mg/kg bw per day for 90 days (Chengelis et al., 2009). This finding could not be reproduced by another study, applying the same rat strain and treating the animals for an almost identical period of time (Loveless et al., 2009). Here, an increased relative kidney weight occurred in both sexes only at the highest dose of 500 mg/kg bw per day. Further sensitive endpoints were degenerations and atrophy in the nasal cavity occurring in male and female rats at 100 mg/kg bw per day (Loveless, et al., 2009). Loveless et al. (2009) performed also a one-generation reproduction study in rats (see Section

3.3.3.3.3). In this part of the study body weight of parental males was reduced at 500 mg/kg bw per day after exposure for approximately 105 days.

In the NTP (2019a) study male SD rats exhibited 1.6- to 2.9-fold higher plasma concentration compared to females, which was consistently seen among all treatment groups. Increased relative liver weight occurred at doses of 250 (males) and 500 mg/kg bw per day (females) and above. Serum cholesterol concentrations were significantly decreased in all male dose groups. Lowered haematocrit, haemoglobin and RBC were evident in males from 62.6 and in females from 250 mg/kg bw per day upwards. ALT, AST were elevated in both sexes at 500 mg/kg bw per day. Total thyroxine (T4), free T4, and triiodothyronine (T3) concentrations were significantly decreased in all male dose groups, while females exhibited no alterations. In the histopathological evaluations the most prominent findings were degeneration and hyperplasia of the olfactory epithelium occurring in both sexes at doses of 250 mg/kg bw per day and above.

A few studies on the subacute effects of *i.p.* administration of **PFHpA** indicate that PFHpA induces peroxisomal β oxidation enzymes in rats at a dose of 160mg/kg bw/day (sex not specified) and in male mice at 20 mg/kg bw per day. PFHpA elevates the relative and absolute liver weight in male mice (Kudo et al., 2000; Kudo et al., 2006; Abe et al., 2017). Female mice required higher doses to show liver enlargement and induction of peroxisomal β -oxidation (Kudo et al., 2006).

Reports on **PFOA**, being published between 2008 and 2017 are documented in the EFSA opinion of 2018. In the NTP (2019a) study on PFOA male SD rats exhibited increased absolute and relative liver weights as well as increased relative kidney weights at 0.625 mg/kg bw per day, when given for 28 days. Relative and absolute liver weights were elevated also in other studies applying doses above 1 mg/kg bw per day to mice or rats (Zheng et al., 2017; Hui et al., 2017; Wang et al., 2017c; Wu et al., 2018). Li et al. (2019) showed that the PFOA-induced liver enlargement was due to elevated hepatocellular DNA replication, as demonstrated in mice receiving 1 mg/kg bw per day for up to 8 weeks.

With regard to hepatotoxic effects of PFOA, male rats exhibited increased ALT and ALP serum levels at already 0.625 mg/kg bw per day (NTP 2019a). PFOA elevated transaminases and induced hepatocellular necrosis at 1 mg/kg bw per day and above also in mice (Hui et al; 2017; Wu et al., 2018) and increased the malondialdehyde concentration in rat liver at 5 mg/kg bw per day.

Most of the recent studies on PFOA focused on metabolic effects of this compound. PFOA-exposed mice showed elevated hepatic peroxisomal β -oxidation, reduced triglyceride and free fatty acid contents in serum, as well as elevated levels of hepatic triglycerides (Hui et al., 2017). Liver sections displayed visible vacuolation of hepatocytes. This was associated with up-regulated mRNA as well as protein of hepatic lipoprotein lipase (LPL) and fatty acid translocase (CD36) and down-regulated mRNA and protein of apolipoprotein-B100 (APOB). These findings suggest dysregulations of fatty acid trafficking. Also, Wang et al. (2017) observed that the frequency of CD36-positive cells was elevated and of ApoB-positive cells reduced. Li et al (2019) reported that in mice, kept on a high-fat diet, 1mg PFOA/kg bw per day decreased the severity of hepatic steatosis within a treatment period of 2-16 weeks.

Zheng et al. (2017) studied the impact of PFOA on liver glucose homeostasis. In male mice 4 weeks of 1.25 mg/kg of PFOA per day increased fasting blood glucose levels and decreased glycogen and glucose content in the liver. In blood insulin levels remained unchanged while those of glucagon were elevated, possibly contributing to the lowered protein levels of glycogen synthase and elevated levels of glucokinase in the liver and to the hyperglycemia observed in the treated animals. PFOA exposure did not affect muscle glucose or glycogen levels. Indirect calorimetry revealed elevated energy consumption in PFOA-treated animals. The findings give indication that PFOA may induce glycogenolysis and gluconeogenesis in murine livers. In contrast to observations of Zheng et al. (2017), Wu et al. (2018) found dose-dependent but insignificant increases in insulin and decreases in glucose in blood of fasted mice, starting at 1 mg PFOA/kg bw per day. This was associated with increased insulin-positive cells in the pancreas of the animals. Furthermore, PFOA downregulated the protein amount of hepatocellular fibroblast growth factor 21 (FGF21) dose-dependently. In the liver FGF21 expression is regulated by PPAR α and the effect of this hepatokine is additive to the activity of insulin with regard to glucose uptake in the peripheral tissues. The observations of Zheng et al. (2017) and Wu et al. (2018) indicate complex alterations in the regulation of carbohydrate metabolism.

In the NTP study (2019a) many hemotological and clinical chemistry parameters were altered in male rats at already 0.625 mg/kg bw per day, such as decreased serum cholesterol and triglycerides, increased albumin/globulin ratios and lowered levels in T3, free and total T4. In females, some of these alterations occurred at much higher doses, such as elevated albumin/globulin ratios at 12.5 mg/kg bw per day or lowered total and free T4 at 100 mg/kg/bw per day. At the pathohistological examination cytoplasmic changes of hepatocytes and degeneration and inflammation in the olfactory epithelium were observed at 0.625 mg/kg bw per day in the males. Similar changes were seen in females at 12.5 or 25 mg/kg bw per day and above. This indicates a marked sex difference in the sensitivity towards PFOA, related to the much lower serum levels in female rats.

For **PFNA** most repeated-dose toxicity studies focused on hepatic effects. Sensitive endpoints were increased relative liver weight and increased intrahepatic levels of total cholesterol and triglycerides in male mice at 0.2 mg/kg bw per day (Wang et al., 2015), and decreased serum HDL and cholesterol at 0.2 mg/kg bw per day in male rats (Fang et al., 2012a,b). Hepatotoxic effects, as indicated by elevated serum transaminases, became evident at 1 mg/kg bw per day and more (Fang et al., 2012b and 2015; Wang et al., 2015). Subsequent studies (Rosen et al., 2017; Das et al., 2017; Abe et al., 2017) focused on the MoA of PFNA and applied doses of 1 mg/kg bw per day and above (see also Section 3.3.5). Again, increased relative and absolute liver weights as well as intrahepatic lipid storage were reported. Das et al. (2017) showed a decrease in DNA content per mg liver but an overall increase in the amount of total DNA per liver, suggesting both hypertrophy and hyperplasia, as also shown for PFOA in this study.

In the NTP study (2019a) on PFNA, most male and female animals died at the higher dose groups (5 and 10 mg/kg bw per day for males, 12.5 and 25 mg/kg bw per day for females). Body weights decreased at lower dose levels. The absolute and relative liver weights and the relative kidney weights were increased, starting at 0.625 mg/kg bw per day in male and 1.56 mg/kg bw per day in female SD rats. Blood cholesterol was consistently lowered in males exposed to 0.625 and 1.25 mg/kg bw per day but not in those exposed to 2.5 mg/kg bw per day, while in females no changes were found. Urea concentrations and the albumin/globulin ratio were elevated in almost all dose groups in males and in females at 3.12 and 1.56 mg/kg bw per day, respectively. In male rats increased bile acid concentrations were evident at 0.625 mg/kg bw per day, followed by elevated bilirubin, AST, ALT and ALP at 1.25 mg/kg bw per day. In females, similar alterations were observed at higher PFNA doses, possibly related to the lower serum levels in females at similar doses. In almost all PFNA treated groups of males free and total T4 concentrations were decreased. In the histopathological evaluations alteration in the cytoplasm and hypertrophy of hepatocytes was the most sensitive endpoint occurring already at the lowest doses in both sexes.

Kawashima et al. (1995) treated male rats with a diet containing **PFDA** at concentrations ranging from 1.5 - 12 mg/kg bw per day for one week. PFDA treatment induced absolute and relative liver weights, peroxisomal beta-oxidation, microsomal 1-acylglycerophosphocholine acyltransferase and cytosolic long-chain acyl-CoA hydrolase in a dose-dependent manner. In male and female rats, PFDA was a potent inducer of peroxisomal β -oxidation in the liver; hepatic concentrations of PFDA were similar in both sexes (Kudo et al., 2000). Abe et al. (2017) observed an increased relative liver weight in mice at an i.p. dose of 20 mg/kg bw per day.

Frawley et al. (2018) reported on the effects of PFDA on the immune system, when administered by gavage to female rats and mice for a period of 28 days (for further details see section 3.3.3.5). The authors provide some additional information on the changes in the organ weights. In female SD rats the relative liver weight was increased from 0.125 mg/kg bw per day onwards, the absolute liver weight and relative kidney weight at 0.25 mg/kg bw per day and above and the absolute kidney weight at the highest dose of 0.5 mg/kg bw per day. At this dose single necrotic hepatocytes occurred in the centrilobular region in 3/8 rats. Mice were less sensitive than rats and showed an increased relative and absolute liver weight from 0.625 mg/kg bw per day and elevated relative spleen weight from 1.125 mg/kg bw per day onwards.

In the NTP study (2019a) on PFDA absolute and relative liver weight were increased in both, male and female SD rats, from the lowest dose onwards. The relative and absolute weight of the thyroid gland was elevated in females only at 0.312 mg/kg bw per day and above, whereas males showed decreased absolute weights of adrenal glands in all dose groups. The albumin/globulin ratio significantly increased

with 0.156 mg/kg bw per day or greater in both sexes. A decrease in blood cholesterol levels was evident in males at the 3 lower dose groups only and in females only at the high dose. ALT, AST, and ALP were significantly elevated at lower doses in both, male and female rats. Free and total T4 concentrations were lowered in males at the higher PFDA dose groups, while females showed only a decreased FT4 level at the highest dose level. In the histopathological evaluations, alteration in the hepatocellular cytoplasm and hypertrophy was the most sensitive endpoint occurring already at the lowest doses in both sexes.

For **PFUnDA**, decreases in body weight gain in male and female rats were noted, which were most pronounced at the highest dose (Takahashi et al., 2014). Further effects were increases in relative liver weight, blood urea nitrogen, centrilobular hypertrophy of hepatocytes and focal hepatocellular necrosis. A decrease in serum cholesterol observed at 0.3 mg/kg bw per day was not evident at the highest dose of 1 mg/kg bw per day. The most sensitive parameter was an increase in the relative liver weight in the males at the dose of 0.3 mg/kg bw per day.

When **PFDoDA** was applied to male and female rats, the most consistent treatment-related effects were described for the liver. The majority of alterations occurred at gavage doses of 0.5 mg/kg bw per day and above, and comprised increases in relative liver weight and serum ALP (Kato et al., 2015b; Liu et al., 2016). Several studies provided direct or indirect evidence for intrahepatic lipid peroxidation (Zhang et al., 2008; Liu et al. 2016). Lowered serum cholesterol, altered blood levels of albumin and glucose, as well as renal alterations occurred in an inconsistent way.

PFTeDA was administered to male rats by daily gavage at 1, 3 or 10 mg/kg bw per day for 42 days. One of the most sensitive endpoints was the lowered absolute weight of the seminal vesicles occurring at 1 mg/kg bw per day. The middle and the high dose caused increases in relative and absolute liver weight, hepatocyte hypertrophy and fatty changes in the center of liver lobules, reduced absolute and relative weight of the pituitary gland, reduced hindlimb strength, as well as follicular cell hypertrophy in the thyroid gland (Hirata-Koizumi et al., 2015).

PFHxDA was applied at 4, 20 or 100 mg/kg bw by daily gavage to male rats for 6 weeks. Liver enlargement, hepatocellular hypertrophy and steatosis and an elevated relative weight of the thyroid gland were observed mainly at the dose of 100 mg/kg bw per day (Hirata-Koizumi et al., 2015).

Hirata-Koizumi et al. (2012) treated male rats with **PFODA** at 0, 40, 200 or 1,000 mg/kg bw per day by gavage for 42 days. Rats were mated after about 2 weeks of treatment. Alterations of the liver (increased absolute and relative liver weight, centrilobular hepatocyte hypertrophy, necrosis) and of haematological parameters (decreased red blood cells count, lowered hemoglobin/hematocrit) appeared to be the most sensitive endpoints and occurred at a dose of 200 mg/kg bw per day.

In summary, the most consistent and most sensitive endpoint was the increased relative liver weight, especially in male rodents, seen for all PFCAs studied. The disturbances in lipid metabolism, hepatotoxic effects and signs of cholestasis were mostly evident at higher dose levels. For some PFCAs increased relative kidney weight, alterations of the mucosa in the nasal cavity and olfactory epithelium and disturbed thyroid hormone levels were among the most sensitive endpoints. For details see Appendix E.

3.3.3.2.2 PFSA's

The available repeated dose studies for PFBS, PFHxS and PFOS (appearing after literature deadline of the previous opinion (EFSA CONTAM Panel, 2018)) are listed in Appendix E, Table E2. For PFHpS and PFDS no studies have been identified.

In a 4-week study **PFBS** lowered phosphorus and potassium in the serum and increased relative and absolute liver weights in male rats at 300 and 900 mg/kg bw per day, (NICNAS, 2005). No alterations were noted for female rats. Lieder et al (2009a) treated male and female rats with PFBS at 0, 60, 200, and 600 mg/kg bw per day by gavage for 90 days. The most sensitive endpoints were decreased absolute and relative spleen weights. Further treatment related effects comprised haematological

parameters, such as decreased haemoglobin concentration and haematocrit. Histological alterations in the kidneys were found at the two highest doses (modest hyperplasia of the epithelial cells of the medullary and papillary tubules and the ducts in the inner medullary region) but were not considered to be adverse due to an absence of any changes in clinical chemistry or urinary parameters. In the liver signs of inflammation became evident at the highest dose in 3 of 10 males and in 4 of 10 female rats. In a further study Lieder et al (2009b) noted increased absolute and relative liver weight in male rats at a dose of 300 mg/kg bw per day, applied over a period of 10 weeks.

In the NTP study (2019b) on the effects of PFBS in SD rats, increases in the relative right kidney weights (females) and in relative liver weights (males and females) occurred in most dose groups. Decreases in T3 and free and total T4 occurred in both sexes at all doses applied. Haematocrit, red blood cell counts and blood cholesterol were decreased at most doses in males, but in females only a decrease in cholesterol was observed and only at the highest dose. Also for this PFAS, serum levels were lower in females than males. An increased albumin/globulin ratio was evident in males at females at 125mg/kg bw per day and above. In the histopathological evaluation hepatocellular cytoplasmic alteration and hypertrophy became evident at 125 mg/kg bw per day and above. Animals exhibited degeneration, necrosis, inflammation and/or hyperplasia of the olfactory epithelium, being significant at 125 mg/kg bw per day in females and at 250 mg/kg bw per day in males.

Butenhoff et al. (2009) treated male SD rats with PFHxS at 0, 0.3, 1, 3 or 10 mg/kg bw per day by gavage, during cohabitation (see Section 3.3.3.3). At day 42 low doses of PFHxS reduced serum cholesterol and prothrombin time (0.3 mg/kg bw per day corresponding to 44.2 µg/mL of PFHxS in serum). The prolonged prothrombin time at 0.3 mg/kg bw per day was not evident at the next higher dose. Increased liver-to-body weight ratio, centrilobular hepatocellular hypertrophy, hyperplasia of thyroid follicular cells, and decreased hematocrit occurred at 3 mg/kg bw per day (corresponding to 128.7 µg/mL serum or 338.7 µg/g liver tissue). Decreased body weight gain and elevations in serum albumin, BUN, ALP, Ca²⁺ and albumin/globulin ratio were observed at the highest dose only. Similar findings were reported in an industry study, reviewed by the Swedish Environmental Protection Agency (2012).

Das et al. (2017) studied the impact of PFHxS on hepatic gene expression patterns and lipid metabolism in male mice (see section on mode of action). An elevated relative liver weight was observed at a dose of 3 mg/kg bw per day and above; the increases in organ weight appeared to be due to hepatocellular hypertrophy, as reflected by decreases in DNA content per mg of liver (Das et al., 2017). Oil Red O stained sections revealed lipid accumulation in PFHxS groups, accompanied by elevated hepatic triglyceride levels and indicating the development of liver steatosis.

Chang et al. (2018) studied PFHxS for reproductive/developmental toxicity in CD-1 mice. In F0 males, 0.3-3 mg/kg bw per day was administered before mating, which induced centrilobular hepatocellular hypertrophy at 0.3 mg/kg bw per day and increased relative liver weight at 1 mg/kg bw per day. Decreased serum cholesterol and increased alkaline phosphatase were observed as well. Hepatotoxicity became evident at 3 mg/kg bw per day, as indicated by hepatocellular lipid vesicles and necrotic hepatocytes (see section 3.3.3.4 for further study details in relation to reproductive/developmental toxicity).

In the NTP study (2019b) on the potassium salt of PFHxS, absolute and relative liver weights were elevated at 1.25 mg/kg bw per day in males and 3.12 mg/kg bw per day (lowest dose) in females, and above. In males, serum levels of T3, total and free T4 were reduced already at the lowest dose (0.625 mg/kg bw per day). Blood cholesterol levels were lowered from 1.25 mg/kg bw per day upwards. Females showed reductions in total T4 at 6.25 mg/kg bw per day and free T4 at 12.5 mg/kg bw per day, but no significant decrease for T3, and also not for cholesterol. Serum PFHxS levels in females were lower than in males. In the histopathological evaluation, hepatocellular hypertrophy became evident at 2.5 mg/kg bw per day in males only. Female rats exhibited degeneration, necrosis, inflammation and/or hyperplasia of the olfactory epithelium, but in the highest dose group only.

PFOS elevated the relative liver weight in mice at already 0.2 mg/kg bw per day, when applied over a period of 4 weeks (Huck et al., 2018). Elevated levels of ALT were reported for rats after treatment with 1 mg/kg bw per day for 4 weeks (Han et al., 2018a, b). This was associated with increased serum levels of the pro-inflammatory cytokines TNFα and IL6, elevated content of malondialdehyde (a lipid

peroxidation product), a decrease in glutathione and increased occurrence of macrophages in the liver. This may indicate hepatocellular lipid peroxidation and damage, followed by a pro-inflammatory response in the affected organ. Similar alterations were observed by Lv et al. (2018) and Su et al. (2019), after application of 10 mg/kg bw per day for 3 weeks to mice. At doses of approximately 0.6 mg/kg bw, applied every second day over a period of a month, DNA fragmentation became obvious, which may be a primary effect of PFOS or secondary due to induction of apoptosis or necrosis of hepatocytes (Eke et al., 2017). This is substantiated by the observation that PFOS induces caspase-3 cleavage in the liver (Lv et al., 2018). Huck et al. (2018) reported on hepatic steatosis and elevated levels of blood glucose and serum cholesterol and triglycerides in mice, exposed to 1 mg PFOS/kg bw per day for 6 weeks. Similar alterations were seen when animals were kept on a high-fat diet. Mechanistic studies revealed that PFOS affected the expression of lipid trafficking genes, such as CD36, the major lipid transporter of cells. The combination of high-fat diet with PFOS, however, reduced steatosis and hepatomegaly probably by leaving the expression of genes of the lipid-transport machinery more or less unchanged.

In the NTP study (2019b), increased absolute and relative liver weights were observed in both male and female SD rats at 0.312 mg/kg bw per day. At 0.312 mg PFOS/kg bw per day, serum levels of free and bound T4 were reduced in both sexes, and T3 levels at 0.625 mg/kg bw per day and higher. In males, cholesterol blood levels were lowered from the lowest dose upwards (0.312 mg/kg bw per day), in females only significantly at the highest dose of 5 mg/kg bw per day. Serum levels of PFOS were similar in males and females. In the histopathological evaluation in both sexes, decreased extramedullary hematopoiesis and hypocellularity became evident at 1.25 mg/kg bw per day and hepatocellular alteration (hypertrophy and/or cytoplasmic alterations) at 2.5 mg/kg bw per day.

Lai et al. (2018) studied the impact of PFOS on the energy metabolism and the gut microbiome by feeding PFOS at 0.3 or 3 mg/kg bw per day to female CD mice over a period of 7 weeks. The authors noted that PFOS treatment elicited disturbances in the lipid and glucose metabolism and changes in the abundance of metabolism-associated bacteria belonging to the phyla Firmicutes, Bacteroidetes, Proteobacteria, and Cyanobacteria in the gut. This was associated with alterations in the gut metabolism of amino acids, methane, and butanoate.

In summary, an elevated absolute and relative liver weight was the most sensitive endpoint for PFBS, PFHxS and PFOS, no repeated dose toxicity studies were available for PFHpS and PFDS. Disturbed lipid metabolism, necrosis and inflammation in the liver was mostly seen at higher dose levels. Also alterations in the kidney and disturbed thyroid hormones were documented repeatedly. For details see Appendix E.

3.3.3.2.3 Other PFASs

The available repeated dose studies for 8:2 FTOH and EtFOSE are listed in Appendix E, Table E3. For FOSA and EtFOSA, no studies have been identified.

Iwase et al. (2006) applied **8:2 FTOH** at 240, 480 or 960 mg/kg bw per day via diet to male rats for a period of 2 weeks. The treatment elevated dose-dependently the relative liver weight and the proportion and content of 18:1 (n-9) fatty acid due to the induction of palmitoyl-CoA chain elongase and stearoyl-CoA desaturase. As a result, there was a relative decrease in 16:0 fatty acid in the liver starting at a dose of 240 mg/kg bw per day. The body weight remained unaffected. Ladics et al. (2008) treated rats with 0, 1, 5, 25 or 125 mg/kg bw per day for 90 days. There were no treatment-related alterations in body weight, food consumption or neurobehavioral parameters. The liver was the main target organ, as indicated by increased organ weight and focal necrosis, peroxisome proliferation and induction of hepatic β -oxidation at 25mg/kg bw per day and above. Chronic progressive nephrotoxicity was observed in females at 125 mg/kg bw per day. The authors derived a NOAEL of 5 mg/kg bw per day. Wang et al. (2018b) applied 8:2 FTOH to adult male C57BL/6 mice at 10, 30, and 100 mg/kg bw per day by gavage for 28 days. Liver toxicity became evident by increased absolute and relative liver weights and histological changes comprising vacuolation, cell swelling, immune cell infiltration, karyopyknosis and nuclear swelling. Thymus and spleen were hardly affected. 8:2 FTOH reduced the concentration of glutathione and the activity of superoxide dismutase (SOD) in the serum.

Xie et al. (2009) treated female rats with **EtFOSE** at 5.0 mg/kg bw per day for a period of 21 days (once daily by gavage for 5 days per week, no administration on days 6 and 7). The EtFOSE metabolite levels in liver and serum decreased in the order PFOS > PFOSA and N-ethyl perfluorooctanesulfonamidoacetate > perfluorooctanesulfonamidoethanol and EtFOSE. EtFOSE treatment lowered the body growth rate, increased relative liver weight and activity of catalase (uterus), superoxide dismutases (CuZnSOD and MnSOD; liver and uterus) and lowered the activity of glutathione peroxidase (GPX, uterus). Peroxisomal β -oxidation activity was elevated on average by 16-fold but did not reach significance. Absolute weights of liver and absolute and relative weights of uterus, kidney and spleen remained unaffected.

In summary, repeated dose studies were available for 8:2 FTOH and EtFOSE, while for FOSA and EtFOSA no studies have been identified. 8:2 FTOH treatment increased dose-dependently the relative liver weight and hepatic β -oxidation. Liver toxicity became evident by histological changes, comprising vacuolation, cell swelling, immune cell infiltration, karyopyknosis and nuclear swelling. Several EtFOSE metabolites occurred in liver and serum, with PFOS and PFOSA being predominant. EtFOSE treatment lowered the body growth rate and increased the relative liver weight. Peroxisomal β -oxidation activity was elevated insignificantly. For details see Appendix E.

3.3.3.3 Developmental and reproductive toxicity

The previous opinion (EFSA CONTAM Panel, 2018) documented reproductive and developmental toxicity studies for PFOS and PFOA, which have been published between 2008 and 2016. These studies are included in Appendix F (Tables F.6 - F.8), in which also some key studies evaluated by EFSA in the PFOS and PFOA opinion in 2008 (EFSA, 2008) are included. Additional studies (Keil et al., 2008; Abbott et al., 2009; Suh et al., 2011), not reviewed in EFSA CONTAM Panel (2018), were identified based on the literature list in the previously reviewed studies and these are also included in Appendix F.6.

3.3.3.3.1 PFOS

Developmental studies on PFOS show effects in offspring at doses similar to, or below, those showing maternal toxicity (EFSA 2008, EFSA CONTAM Panel, 2018). Among effects observed in rats and/or mice are high mortality early after birth, reduced fetal weight, reduced postnatal growth, increased liver weight, anasarca, impaired immune effects, cardiac abnormalities, cleft palate, delayed ossification of bones, and a decrease in placental weight and capacity. Increase in liver weight (NOAEL 0.3 mg/kg bw per day) effects on placental physiology (LOAEL 0.5 mg/kg bw per day, NOAEL not determined) and aspects of glucose homeostasis (LOAEL 0.3 mg/kg bw per day, NOAEL not determined) were the most sensitive endpoints (EFSA CONTAM Panel, 2018). One study (Liang et al., 2019), that did not change this conclusion, was identified by the updated literature search and included in Appendix F Table F.6.

3.3.3.3.2 PFOA

Regarding reproductive effects, in adult male mice reproductive organs and male sex hormone levels were affected by PFOA (NOAEL 0.3 mg/kg bw per day). Among developmental effects in rodents were increased resorption of litters, dead foetuses, reduced postnatal survival (NOAEL 0.3 mg/kg bw per day), impaired development of mammary gland (LOAEL 0.00045 mg/kg bw per day, NOAEL not determined), reduced postnatal weight, pathological alterations in liver and increased liver weight in pups (LOAEL 0.1 mg/kg bw per day, NOAEL not determined) (EFSA CONTAM Panel, 2018). Relevant studies identified by the updated literature search are included in the Appendix F Table F.7 (Song et al., 2018; Li et al., 2018b; Li et al., 2019; NTP 2019a), but they did not change these previous conclusions.

The most sensitive developmental effect observed is delayed and impaired development of the mammary gland upon prenatal and early postnatal exposure. The effect is also observed after higher exposure from puberty onwards. Delay in mammary gland development can be documented by decreased scores in whole mounts of mammary glands in early neonatal life or in puberty and later ages, including pregnancy and the first day after birth, or by increased scores in dams at the time of weaning, when the mammary gland is reducing and stopping the milk production in a process called involution (see Section 3.3.5.5). Delays were reported in all these stages. The eight studies on the developmental effects on mammary gland were performed in four different mouse strains. They are described in more detail below and summarised in Appendix F Table F.1. The table also includes a

selection of the PFOA concentrations measured in serum or tissues at different time points, and the NOAEL/LOAEL in each study is indicated. First the studies with exposure in utero and/or via lactation are listed chronologically, followed by studies with pubertal exposure.

Prenatal and early postnatal exposure via milk

White et al. (2007) studied the consequences of gestational exposure on mammary gland development, since prenatal PFOA exposure in previous studies led to postnatal death and a dose-related decrease in neonatal body weight, which could be indicative of lower milk production by the dam. Pregnant CD-1 mice were exposed to 0 and 5 mg/kg bw per day from either GD 1-17, GD 8-17 or GD 12-17. The body weight of offspring at PND 1-20 was reduced in all treated groups. Mammary gland differentiation was significantly reduced in the dams at PND 10 (time of maximal milk production), as assessed by the amount of epithelial tissue filling of the gland and the presence of well-formed, productive alveoli (presence of milk), after treatment during GD 1-17 and 8-17 (not significant for GD 12-17). At PND 20 the normal presence of apoptotic bodies in the mammary gland during involution at weaning was observed in controls but to a lesser extent in the treated dams. Development of the mammary gland in dams and female pups was scored on a 1 (poor) to 4 (normal) age-adjusted developmental scale by two independent scorers, blinded to the treatment. The scoring included assessment of the number of primary ducts, number of large secondary ducts, lateral side-branching, appearance of budding from the ductal tree and longitudinal outgrowth of the epithelia. In the pups, the mammary gland development was significantly impaired at both PND 10 and 20 in all three treatment groups, showing stunted epithelial branching and longitudinal growth. Differences in blood PFOA levels between the groups were small at PND 10, but larger at PND 20, dams and pups treated during GD 1-17 showing the highest levels. Liver PFOA levels in pups also increased with treatment duration at all three time points (PNDs 1, 10 and 20) but were significantly different only at PND 20. The study showed that the susceptible period for mammary gland development in offspring is in late gestation or postnatal.

In a follow-up study, White et al. (2009) confirmed that the effects in mammary gland development in pups (examined on PND 29 and 32) were similar when the dams were only treated during GD 15 to 17, as compared to much longer treatments starting at GD 7, 10 or 13 (daily dose 5 mg/kg bw per day). Furthermore, the effects of in utero versus lactational exposure were investigated by cross-fostering pups of untreated and treated dams (3 and 5 mg/kg bw per day, GD 1-17 and 5 mg/kg bw per day, GD 8-17). Pups from untreated dams receiving milk from treated dams (5 mg/kg bw per day) had mammary glands with impaired development at all investigated time points (PND 1, 3, 5, 10, 22, 42 and 63). Similar effects were seen in pups exposed in utero and cross-fostered by untreated dams. The PFOA concentration in serum from treated pups was highest on PND 1 in both the groups nursed by treated and untreated dams. In pups from untreated dams, but nursed by treated dams, levels increased up to PND 10 and to a level similar to that in pups only exposed in utero. At PND 63 the serum levels in all groups of pups were close to that in controls. The study showed that both late gestational or only lactational exposure to PFOA can result in impaired development of mammary glands, and that this can still be observed in adult animals.

Macon et al. (2011) studied the effect of lower doses during gestation in CD1 mice treated during GD 1-17 (0, 0.3, 1.0, 3 mg/kg bw per day) or during GD 10-17 (0, 0.01, 0.1, 1.0 mg/kg bw per day). Exposure during GD 1-17 led to a decrease in mammary gland development scores in pups at all doses at PND 14, 21, 42 and 84. After exposure during GD 10-17, the overall mammary gland developmental score in pups at PND 21 was dose-dependently and significantly decreased already at 0.01 mg/kg bw per day, and no NOAEL could be derived. The overall developmental score was composed of, but not restricted to, longitudinal and lateral growth, change in longitudinal and lateral growth, the number of terminal end buds (TEBs) and the number of terminal ends. The number of TEBs was the most sensitive individual parameter and showed a dose-related reduction at all doses, which was significant at 0.1 and 1.0 mg/kg bw per day. This study confirmed that the impairment of mammary gland development was permanent and that the sensitive period is late gestation or the first weeks postnatally. The LOAEL for exposure during GD-10-17 was 0.01 mg/kg bw per day. This treatment resulted in serum PFOA concentrations in pups of 285, 184, 151, 80 and 17 ng/mL at PNDs 1, 4, 7, 14 and 21 respectively.

White et al. (2011) performed a three-generation study, exposing CD-1 dams (P0) to 0, 1 or 5 mg/kg bw per day from GD 1 to GD 17. In addition, groups of dams dosed with 0 or 1 mg/kg bw per day

during GD 1-17 received a continued lifelong exposure to 5 µg/L of PFOA through the drinking water. This started on GD 8 in the dams and continued also in pups throughout F1 and F2 (estimated to correspond to a dose of 0.00045 mg/kg bw per day³⁰). At weaning (PND 22), maternal mammary gland indices were increased in all dosed P0 dams, indicating a delay in involution. The female F1 generation was used for breeding (F1 dams) or assessment of developmental scores (F1 females). Mammary gland development scores were significantly decreased in all treated F1 pup groups on PND 22, 42 and 63, indicating that at the time when F1 females became pregnant, the mammary glands were not fully developed. This effect was still observed in the F1 dams on PND 10, but such changes were not consistently seen on PND 22. Effects of treatment on mammary gland development were also less evident in F2 pups with significant decreases only observed at PND 42 and only in the groups receiving contaminated drinking water. A lactational challenge in F1 dams on PND 10 did not indicate lower milk production or change in time to initiate milk production in any of the dosed groups. Furthermore, growth of the F2 offspring was not altered. Nursing behaviour, such as number of nursing events or duration of nursing per event was not investigated. No other impact such as on the number of live fetuses, prenatal loss, postnatal survival, body and liver weight were reported following chronic exposure to 5 µg/L of PFOA through the drinking water. The serum PFOA concentration increased with age at different developmental time points after chronic exposure to 5 µg/L PFOA in the drinking water and corresponded to a lifetime average serum concentration in F1 females of 60 ng/mL and in F2 females of 51 ng/mL. At PND 22 the serum concentrations were 21 and 27 ng/mL in F1 and F2 pups, respectively.

In Sv/129 mice a PFOA dose of 3 mg/kg bw per day during GD 1-17 induced postnatal lethality in offspring but no changes in mammary gland parameters on PND 20 in offspring (Albrecht et al., 2013). The CONTAM Panel noted limitations in the study in that only one dose and one time point was investigated, the number of animals was low and that the mean number of TEBs per gland was reported to be only 2.1.

The effects of PFOA in CD-1 mice were confirmed in a comparative study with CD-1 and C57Bl/6 mice (Tucker et al., 2015). In this study, timed pregnant dams received oral daily doses of 0, 0.01, 0.1, 0.3 or 1.0 mg/kg bw per day of PFOA from GD 1 to 17. No treatment group showed an effect on absolute and relative body or liver weight, or on puberty onset measured by vaginal opening. Mammary gland development scores in CD-1 mice were significantly decreased in all dose groups at PND 35 and PND 56 and from 0.1 mg/kg bw per day on PND 21. C57Bl/6 mice appeared to be less sensitive since the decrease in the scores at PND 21 and 61 were only significant for the treatment with 0.3 and 1.0 mg/kg bw per day. However, the CONTAM Panel noted high uncertainty regarding differences in strain sensitivity since the treatment groups for C57Bl/6 were smaller than for CD-1. The concentration of PFOA in serum in general also appeared to be lower in C57Bl/6 than in CD-1 mice.

Pubertal exposure

Balb/c and C57BL/6 mice were orally treated with PFOA at doses of 0, 1, 5 and 10 mg/kg bw per day during 5 days a week for 4 weeks starting at PND 21 (Yang et al., 2009). An increase in absolute (at 5 and 10 mg/kg bw per day) and relative (at 1, 5 and 10 mg/kg bw per day) liver weight was observed in both strains. In Balb/c mice, a significant delay in vaginal opening was observed for 1 mg/kg bw per day and no vaginal opening at all for 5 and 10 mg/kg bw per day. In C57BL/6 mice a significant delay of vaginal opening was seen at 5 mg/kg bw per day and no vaginal opening at 10 mg/kg bw per day. Uteri of Balb/c mice showed a dose-dependent decrease of absolute and relative weight, whereas in C57BL/6 mice a dose of 1 mg/kg bw per day resulted in increased absolute and relative uterine weight and only 10 mg/kg bw per day led to a decrease in uterine weight. The ductal length, the number of mammary gland TEBs and the number of terminal ducts of the developing mammary gland were also investigated. In Balb/c mice, a dose-related decrease in all three parameters was observed and reached statistical significance at 5 mg/kg bw. In C57BL/6 mice, a decrease of all three parameters was only observed at 10 mg/kg bw per day, whereas at 1 and 5 mg/kg bw per day the number of terminal end buds and stimulated terminal ducts was increased, being significant at 5 mg/kg bw per day. Since the two mouse strains responded with different sensitivity, which might be indicative for strain specificity,

³⁰ Based on standard conversion factors in EFSA Scientific Committee (2012), Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data.

Balb/c mice were also treated with 0.1 mg/kg bw per day with no findings in comparison to controls (data not shown).

The decrease in mammary gland development in Balb/c and C57BL/6 mice was confirmed by Zhao et al. (2012). The effect was seen at a dose level of 2.5 mg/kg per day for Balb/c and a dose level of 7.5 mg/kg bw per day in C57BL/6 mice treated from PND 28 for four weeks with dosing at five days a week. No other dose levels were applied.

The mechanistic results from the studies on pubertal exposure are described further in section 3.3.5.5.

In summary, PFOA exposure was shown to impair normal development of the mammary gland in CD-1 and C57BL/6 mice exposed late in gestation or via lactation. This impairment in development of the gland was still observed at PND 84 (12 weeks) in CD-1 mice and is thus present beyond sexual maturity. Normal involution in CD-1 dams at weaning was also disturbed (as indicated by increased scores). Exposure starting at puberty also caused delay or impairment of mammary gland development in Balb/c and C57BL/6 mice. However, this was observed at substantially higher doses and serum levels than after exposure during gestation and lactation. In C57BL/6 mice an inhibitory effect on mammary gland development was seen at 7.1 mg/kg bw, but a stimulatory effect at dose levels 0.7 and 3.6 mg/kg bw, all three levels being inhibitory in Balb/c mice.

At present, the impaired development of mammary glands, as shown in two mouse strains (CD-1 and C57BL/6), is the most sensitive developmental outcome. No NOAEL has been identified in CD-1 mice based on the three studies using low doses during gestation (Macon et al., 2011; White et al., 2011; Tucker et al., 2015). The LOAEL of 0.01 mg/kg bw per day in CD-1 mice dosed during GD 10-17 resulted in a serum concentration in pups on PND 1 of 285 ng/mL, which decreased to 16.5 ng/mL on PND 21 (Macon et al., 2011). A same LOAEL of 0.01 mg/kg bw per day was observed in another study with dosing during GD 1-17, leading to a serum concentration of 74.8 ng/mL in CD-1 pups on PND 21 (Tucker et al., 2015). In the 3-generation study by White et al. (2011), a chronic dose of 5 µg/L in drinking water (0.00045 mg/kg bw per day) from GD 7 in P0 dams and continued for the F1 pups led to a LOAEC serum concentration of 21.3 ng/mL in the pups on PND 22. These LOAEC serum concentrations in pups on PND 21/22 are quite similar in the three studies. In the mice in the 3-generation study by White et al. (2011) under constant exposure via drinking water, the LOAEC of 21.3 ng/mL increased to 66.2 ng/mL on PND 63, at an age the mice get pregnant. Thus, the maternal LOAEC in the experiment was around 66 ng/mL and the average lifetime serum concentrations in F1 and F2 females were 60 and 51 ng/mL, respectively.

3.3.3.3.3 PFCA's

Reproductive and developmental toxicity studies were identified for PFBA, PFHxA, PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, PFHxDA and PFODA. No studies were identified for PFPeA, PFHpA, PFTTrDA, PFPeDA, and PFHpDA. Appendix F Table F.1 contains reproductive and developmental studies with exposure during pregnancy and Appendix F Table F.2 studies with exposure in puberty or adulthood. In the text below, all developmental and reproductive studies are described together for each PFAS.

PFBA was tested at doses of 0, 35, 175 and 350 mg/kg bw per day following oral gavage from GD 1-17, to 10 mice per dose group (Das et al., 2008). Serum levels in dams at the end of dosing were <0.01, 3.8, 4.4 and 2.5 µg/mL and liver levels were <0.01, 1.41, 1.6 and 0.96 µg/g. On the maternal side, a modest (30%) increase in absolute and relative liver weight became apparent at 175 and 350 mg/kg bw per day, and there was an increase in full litter resorption at the highest dose. There were no changes in maternal weight gain, and survival in newborns and postnatal weight gain were not affected. Liver weight at 175 and 350 mg/kg bw per day was also increased in the offspring at PND 1, but not on PND 10. Eye opening of pups was delayed by between 1 and 1.5 days at all doses. A dose-related 2-3 days delay in vaginal opening became significant at 175 mg/kg bw per day and a delay in preputial separation was seen at 350 mg/kg bw per day. Expression of genes regulated by PPARα and PXR was unaltered in liver from pups at PND1. Overall, gestational exposure to PFBA induced a delay in developmental markers with a LOAEL of 35 mg/kg bw per day for delayed eye opening, and a modest increase in liver weight in dams and in pups on PND 1 with a NOAEL of 35 mg/kg bw per day. The early neonatal mortality and decrease in postnatal growth that were seen with PFOS and PFOA were not observed for PFBA.

A study with **PFHxA** (NH_4^+ -PFHxA) on developmental and perinatal/postnatal reproduction toxicity was performed in CD-1 mice (Iwai and Hoberman, 2014). In the first phase of the experiment, mice (20 per dose group) were orally treated with 0, 100, 350 and 500 mg/kg bw per day on GD 6 to 18. In the second phase of the experiment the dosage was modified to 0, 7, 35 and 175 mg/kg bw per day. Increased neonatal mortality, reduced viability index, reduced weight gain during lactation and an increased liver to body weight ratio in the offspring were detected at doses of 350 mg/kg bw per day and higher in the first phase experiment. In the second phase there was an increased number of stillborn pups and pups dying on PND 1 and decreased pup weight on PND 1 at 175 mg/kg bw per day. No developmental changes were reported in F1. The overall NOAEL for maternal and developmental effects in the two studies was 100 mg/kg bw per day. Iwai et al. (2019) undertook a reassessment of the two studies, combining the two experimental phases in statistical analyses, and concluded that there was no significant decrease in viability at 175 mg/kg bw per day.

Loveless et al. (2009) performed a comprehensive toxicological evaluation of Na^+ -PFHxA, which included a 90-day subchronic, a one-generation reproduction and a developmental toxicity study in rats (see also Section 3.3.3.2). In the one-generation reproduction studies, parental animals were exposed from 70 days prior to cohabitation until weaning with 0, 20, 100 and 500 mg/kg bw per day by gavage. There were no detectable effects related to reproduction. In the developmental study, females were exposed from GD 6 - 20 to 0, 20, 100 and 500 mg/kg bw per day by gavage, according to OECD guideline 414. No effects linked to developmental processes could be identified. A NOAEL of 100 mg/kg bw day was delineated by the authors based on reduced maternal and fetal body weight in the 500 mg/kg bw per day exposure group.

In a 28-days study in male rats orally treated with PFHxA at doses from 62.6 to 1000 mg/kg bw per day did not show significant effects on sperm count per g cauda epididymis or serum testosterone, but a slight (5%) decrease in epididymal weight was reported for the highest dose. Oestrus cyclicity was normal in dosed females (highest dose tested 500 mg/kg bw per day) (NTP 2019a).

The effects of the free acid of **PFNA** (97% pure) were assessed in a study design that was delineated from PFOS and PFOA studies. PFNA was tested in CD-1 mice ($n = 11 - 27$ dams per dose group) with exposure during GD 1 to 17 at doses of 1, 3, 5 or 10 mg/kg bw per day (Das et al., 2015). Dams in the dose group of 10 mg/kg bw per day could not carry their pregnancies and were not subjected to follow-up studies. Serum concentrations in pregnant dams at term at 1, 3 and 5 mg/kg bw were approximately 20, 25 and 75 $\mu\text{g/mL}$ and the corresponding liver levels were 100, 270 and 320 $\mu\text{g/g}$. Liver weight was dose-dependently increased at 1, 3 and 5 mg/kg bw per day in both non-pregnant animals and pregnant animals at term. Neonatal survival was around 90% for the 0, 1 and 3 mg/kg per day dose groups, but decreased during the first 10 days in the 5 mg/kg bw per day group and was at weaning only 20%. Serum levels in pups in the 1, 3 and 5 mg/kg per day dose groups were around 25, 50 and 75 $\mu\text{g/mL}$ at PND 1 and decreased to control levels at weaning for the two lower dose groups. In pup livers the PFNA levels were similar at PND 1 and PND 10 (60, 150 and 200 $\mu\text{g/g}$ in the three dose groups) and thereafter decreased, but were still elevated at PND 40 and 70. The liver to serum ratio in pups was approximately 3:1 the first 10 weeks after birth. In surviving pups a dose-dependent reduction of postnatal body weight gain reached statistical significance at 3 and 5 mg/kg bw per day. The lower body weight persisted up to PND 49 in females and up to PND 297 in males. Relative liver weight was dose-relatedly increased in pups in all dose groups at PND 1, 10 and 24, but was not anymore significantly increased in the lower dose at PND 42, and no significant effects were seen at PND 60 in any of the groups. Together with decreased postnatal growth, the surviving pups also showed a dose-dependent delay in developmental landmarks (eye opening, preputial separation, and vaginal opening). The authors also provided benchmark dose estimates. The most sensitive ones were $\text{BMD}_5/\text{BMDL}_5$ values of 0.43/0.27 mg/kg bw per day for increased relative liver weights in mothers and $\text{BMD}_5/\text{BMDL}_5$ of 0.24/0.19 mg/kg bw per day for increased relative liver weight of pups at PND1.

Rogers et al. (2014) comparatively assessed the impact of several compounds on parameters related to cardiovascular functions in Sprague-Dawley rats. Animals were exposed from GD 1 to 20 to PFNA at 5 mg/kg bw per day, and 10 to 12 offspring animals per group were followed-up for testing cardiovascular functions. On the maternal side a delayed weight gain was observed. For the offspring a lower birth weight was recorded in females. Blood pressure was found to be increased but only at the time point

of 10 weeks of age of the offspring. A reduced nephron endowment (nephrons per kidney) was found in males at 22 weeks of age.

PFNA (males dosed at 0, 0.625, 1.25 and 2.5 mg/kg bw per day, females at 0, 1.56, 3.12, 6.25 mg/kg bw per day) showed effects on reproductive parameters in the 28 days study in rats conducted by NTP (2019a). Epididymal weight decreased dose relatedly (33% reduction at the highest dose) and was significantly reduced from the lowest dose. Cauda epididymal sperm count was significantly decreased, but was similar to the control per g tissue. There were also histopathological findings in epididymis at the highest dose (hypospermia, exfoliated germ cells, epithelial apoptosis, granuloma sperm). Testis weight was reduced in the two highest dose groups, and was accompanied by histopathological changes (degeneration, spermatid retention, interstitial cell atrophy). Testosterone was also significantly decreased in the two higher dose groups (28% and 81% respectively). In females, testosterone was dose-relatedly and significantly increased (66% increase at highest dose) but vaginal cytological data indicated cyclicity similar to controls.

Singh and Singh (2019a) exposed Parkes mice orally to PFNA (0, 2 and 5 mg/kg bw per day, 10 dams per group) from GD 12 to parturition, and testes of two male pups per dam were investigated on PND 3. In dams the body weight was not affected, and there were no changes in birth rate, number of pups per dam, and weight of male pups. Testicular testosterone was decreased in the highest dose group, together with reduced levels of proteins involved in testosterone synthesis (StAR, CYP11A1, 3 β and 17 β -HSD), gonad development (WT1 and SF1) and cell proliferation (PCNA). However, testis weight and testis histology were not affected.

Singh and Singh (2019b) investigated the consequences of PFNA exposure on parameters of male fertility (Appendix F Table F.3). After a dose range finding study, 14 male Parkes mice were orally treated at 0, 0.2 and 0.5 mg/kg bw per day for 90 days using a feeding needle, starting at prepubertal age (PND 25) and ending on PND 114. At 24 h after the last dosing, the authors observed a dose-related reduction of sperm number in cauda epididymis (significant at the highest dose, 74% of control), motility and viability. This was associated with a decreased proliferation (number of PCNA labelled cells) and an increase in apoptosis (caspase 3 staining) in the testicular tissue. Testis weight was not affected. In a reproduction assay, exposure of male mice led to a dose-related decrease in litter size (significant at the highest dose, 50% of control). The authors also showed a dose-dependent decrease of total serum cholesterol (67% of control) and of testosterone (69% of control), also being significant at 0.5 mg/kg bw per day. On a molecular level, decreased levels of proteins and mRNA involved in the steroidogenic process (StAR, Cyp11A1, 3 β -HSD and 17 β -HSD [isoform not specified]) were observed at the highest dose. Finally, the dose of 0.5 mg/kg bw per day of PFNA increased lipid peroxidation. This effect was accompanied by a decrease of SOD and catalase activity which was significant at 0.2 mg/kg bw per day.

The same authors also investigated parameters related to male reproduction and steroidogenesis in Parkes mice (n = 10 per group) orally exposed to PFNA (97% purity) at 0, 2 and 5 mg/kg bw for 14 days, from PND 25 to PND 38 (Singh and Singh, 2019c,d). Mice were sacrificed 24 h after the last treatment. A dose-related decrease in body weight gain became statistically significant at 5 mg/kg bw per day. Serum and intratesticular testosterone levels (5 mice per group) were decreased in both dose groups, and the percent of seminiferous tubules showing degenerative changes were increased. A dose-related decrease of levels of markers involved in steroidogenesis (SF-1, StAR, CYP11A1 and 3 β and 17 β -HSD) in testis was reported. Lipid peroxidation was increased, whereas SOD, catalase and glutathione S-transferase were decreased in testis, indicating oxidative stress. Proliferation index measured by % PCNA positive cells per tubule was decreased, whereas the apoptotic index was increased (Singh and Singh, 2019c). Increased liver weight as well as hepatocellular hyperthrophy was reported for both dose groups, and hepatic lipid peroxidation was increased. Serum cholesterol was decreased at the highest dose. Blood glucose was increased, whereas testicular glucose, lactate and lactate dehydrogenase were decreased (Singh and Singh, 2019d).

In summary, the most sensitive endpoint after gestational exposure to PFNA was increased liver weight in both maternal and offspring CD-1 mice, and a reduction in postnatal weight gain in F1, with a LOAEL of 1 mg/kg bw per day, with corresponding concentration in serum from the dam at term of 20 μ g/mL. Delay in development was seen at 3 mg/kg bw per day, and at 5 mg/kg bw per day there was an increase in neonatal mortality. A 90-day male reproductive study reported decreased sperm

production, decrease in cholesterol, steroidogenic enzymes and testosterone, as well as decreased number of pups in the next generation, with a NOAEL and LOAEL of 0.2 and 0.5 mg/kg bw per day, respectively. Effects on male reproduction parameters were also reported by NTP in rats at somewhat higher exposure levels, and it was noted that 28 days is shorter than one spermatogenic cycle and too short to fully assess male reproductive parameters.

Harris and Birnbaum (1989a) dosed C57BL/6N mice with **PFDA** during GD 10 to 13 with 0, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, or 32.0 mg/kg bw per day and during GD 5 to 15 with 0, 0.03, 0.1, 0.3, 1.0, 3.0, 6.4, or 12.8 mg/kg bw per day. Toxicity was assessed in dams and fetuses on GD 18. Reduced maternal body weight gain and reduced fetal viability was observed in the two highest dose groups in both dosing regimes. After exposure during GD 5 to 15, a statistically significant increase in maternal liver weight could be observed in response to doses of 1 mg/kg per day and higher. No effects were observed at 0.3 mg/kg bw per day and below. There was a dose-related reduction in live fetal body weight, being statistically significant at 0.1 mg/kg bw per day, not significant at 0.3 mg/kg per day, but again significant at 1 mg/kg bw per day. In conclusion, effects in offspring were seen at doses that also indicated maternal toxicity, with the exception of reduction of live fetal body weight, being reduced already at 0.1 mg/kg bw per day.

In the 28-days study in rats conducted by NTP (NTP 2019a), PFDA was dosed at 0, 0.156, 0.312, 0.625, 1.25 and 2.5 mg/kg bw per day. Reproductive tissue parameters were reported in animals in the three higher dose groups and controls, and showed lower number of sperms in cauda epididymis in the two highest dose groups (significant in the highest dose group, 30% decrease). Also the bw was significantly decreased at these doses. This was accompanied by significantly lower epididymal weights (10% and 23%), and the number of sperm per g epididymis was unaltered. Serum testosterone decreased with dose, becoming statistically significant (75% reduction) at the highest dose, at which also testis weight was reduced (11%), but with unaltered spermatid counts. There were also multiple histopathological findings in testis at this dose. In females, cyclicity was disrupted at the highest dose, with prolonged time in diestrus. Serum testosterone in females increased with dose (41% to 141%), becoming significant from 0.312 mg/kg bw.

In a study on **PFUnDA**, a combined repeated dose (see Section 3.3.3.2), and reproductive/developmental toxicity approach according to OECD guideline 422 was used (Takahashi et al., 2014). Here only the effects on reproductive and developmental endpoints are reported. Male and female Crl:CD(SD) rats (12 per group) were dosed for 42 days with 0, 0.1, 0.3 and 1 mg/kg bw per day per gavage from 14 days prior to mating for 42 days in males and until PND 5 in females. The following reproductive and developmental parameters were assessed: copulation index, fertility index, gestation index, number of pregnant animals, gestation length, numbers of corpora lutea and implantation sites, implantation index, number of litters, number of live pups at PND 0 and 4, live birth index, sex ratio, viability index, as well as body weights of male and female pups at PND 0 and PND 4. The only statistically significant change observed in response to the treatment was a reduction in body weight of pups of both sexes on PND 0 and PND 4 at 1 mg/kg bw per day. Increased liver weight was observed at 1 mg/kg bw per day in the repeated dose part of the study (see Section 3.3.3.2.1).

Kato et al. (2015b) performed a combined repeated dose (see Section 3.3.3.2) and reproductive toxicity study in rats with **PFDoDA** at 0, 0.1, 0.5, 2.5 mg/kg bw per day, according to OECD guideline 422. Here only the effects on reproductive and developmental endpoints are reported. Twelve males per dose were orally treated for 42 days starting 14 days prior to mating. Twelve females per dose were treated starting 14 days before mating, throughout gestation until 6 days of nursing. Reproductive effects were only detected at the highest dose. Amongst them were decreased spermatid and spermatozoa counts in males, as well as a continuous diestrus in females. At this dose 7 out of 12 animals died in late pregnancy, 4 did not deliver live pups. In summary, gestational exposure to PFDoDA induces maternal and reproductive effects with a NOAEL of 0.5 mg/kg bw per days, which is higher than the NOAEL observed for repeated dose toxicity of 0.1 mg/kg bw per day (see Section 3.3.3.2).

Shi et al. (2009a) investigated the effect of PFDoDA exposure on pubertal transition in female Sprague-Dawley rats orally treated from PND 24 to PND 52 with 0, 0.5, 1.5 and 3 mg/kg bw per day. Body weight was decreased at 3 mg/kg bw per day. No effects were observed for absolute and relative weights of uteri and ovaries, age and weight at vaginal opening and on estrus cyclicity. At 3 mg/kg bw per day cholesterol levels were increased and estradiol levels were decreased, while LH and FSH levels were

unchanged. Authors in addition performed an extensive gene expression analysis of molecules involved in steroid metabolism and regulation of steroid metabolism. The most sensitive response was observed for 17 β -HSD mRNA expression, which was found to be upregulated at the lowest dose tested.

Shi et al. (2009b) investigated the effect of PFDoDA exposure on pubertal transition in male Sprague-Dawley rats, by treating 21-day-old animals orally for 110 days with 0, 0.02, 0.05, 0.2 and 0.5 mg/kg bw per day. Body weight was decreased at 0.5 mg/kg bw per day. No effects were observed for absolute and relative weights of testis, prostate, seminal vesicle and vas deferens. At 0.5 mg/kg bw per day, testosterone levels were decreased, while LH and FSH and total cholesterol levels were unaffected. Authors in addition performed an extensive gene expression analysis of molecules involved in steroid metabolism and regulation of steroid metabolism. The most sensitive response was observed for StAR, which was down-regulated at 0.02 mg/kg bw per day at the mRNA level and at the protein level at 0.05 mg/kg bw per day.

Chen et al. (2019) investigated the effect of PFDoDA on rat Leydig cell development in puberty of 21-days-old male Sprague-Dawley rats orally treated for 14 days with 0, 5, 10 mg/kg bw per day. Body weight and absolute testis weight was decreased at 10 mg/kg bw per day. No effects were observed for Leydig and Sertoli cell number. Levels of serum testosterone, LH and FSH were decreased at 5 mg/kg bw per day. In addition to the described changes in hormone levels, authors base this suggestion on the down-regulation of Leydig cell protein levels of LHCGR, SCARB1, STAR, CYP11A1, SIRT1 (5 mg/kg bw per day) and CYP17A1, HSD11B1, PGC1- α (10 mg/kg bw per day). In addition, a reduced viability and reduced mitochondrial membrane potential of isolated Leydig progenitor cells in culture is described in response to 10 μ M of PFDoDA.

In summary, exposure of rats to PFDoDA prior to and during gestation induced maternal and reproductive effects (continuous diestrus and fetal loss) with a NOAEL of 0.5 mg/kg bw per day. Male reproductive effects (decreased spermatid and spermatozoa counts) were seen at a similar NOAEL of 0.5 mg/kg bw per day, which is higher than the NOAEL of 0.1 mg/kg per day observed for repeated dose toxicity in the same experiment.

Hirata-Koizumi et al. (2015) also assessed **PFTeDA** in a combined repeated dose and reproductive/developmental toxicity study according to OECD guideline 422 (see Section 3.3.3.2). Here only the effects on reproductive and developmental endpoints are reported. Male and female CrI:CD(SD) rats (12 per group) were dosed from 14 days prior to mating with 0, 1, 3 and 10 mg/kg bw per day by gavage for 42 days in males and until PND 5 in females. In females, centrilobular hepatocyte hypertrophy was observed at 10 mg/kg bw per day. In males a decreased absolute weight of the seminal vesicles was observed at 1 mg/kg bw per day. No significant changes regarding reproductive parameters were observed. Investigations comprised estrus cyclicity, copulation index, fertility index, gestation index or length, number of corpora lutea, number of implantation sites, number of delivered pups as well as number of live pups at PND 1 and PND 4. However, on PND 1 and 4, the body weights of both male and female pups were significantly lower in the group with highest exposure.

In the paper described above (Hirata-Koizumi et al. 2015), the authors also report on a combined repeated dose and reproductive/developmental toxicity study with **PFHxDA** following the same experimental design, with dose levels of 0, 4, 20 and 100 mg/kg bw per day per gavage. The authors investigated the same reproductive parameters as specified for PFTeDA (see above) and observed no effects on reproductive/developmental parameters. Hepatic changes (centrilobular hepatocellular hypertrophy) were observed in the dams in the highest dose group. The NOAEL for developmental and reproductive effects was 100 mg/kg bw per day, the highest dose tested.

Hirata-Koizumi et al. (2012) performed a combined repeated dose (see Section 3.3.3.2) and reproductive toxicity screening study with **PFODA** according to OECD guideline 422. Here only the effects on reproductive and developmental endpoints are reported. Twelve male and female CrI:CD(SD) rats were dosed by gavage starting 14 days prior to mating at 0, 40, 200 and 1,000 mg/kg bw per day. Males were dosed for 42 days including the mating period and females until lactation day 5. In dams, hepatic changes (centrilobular hepatocellular hypertrophy and necrosis), reduced feed consumption and reduced body weight gain were seen at 1000 mg/kg bw per day. For offspring, reduced numbers of implantation, reduced total number of born pups and number of live pups occurred at 1,000 mg/kg bw per day. The NOAEL was 200 mg/kg bw per day for both maternal and developmental toxicity.

3407 3.3.3.3.4 PFSA_s

3408 Appendix F Table F.4³¹ lists the identified reproductive and developmental toxicity studies for PFBS and
 3409 PFHxS. No studies were identified for PFHpS and PFDS₂.

3410 A two-generation reproduction study with **PFBS** according to OECD guideline 416 was conducted in
 3411 Sprague-Dawley rats (Lieder et al., 2009b). Parental males and females (n=29-30 per sex per group)
 3412 were exposed to 0, 30, 100, 300, 1000 mg/kg bw per day for seven weeks prior to mating. Exposure of
 3413 parental females was continued through gestation and lactation. F1 offspring (n=29-30) was dosed
 3414 from weaning at PND 22 onwards. F2 generation was only exposed in utero and during lactation. The
 3415 experiment was terminated at PND 21 of the F2 generation animals. There were no treatment-related
 3416 findings on reproductive toxicity endpoints. In parental animals and in adult F1 offspring animals,
 3417 increased liver weight, increased hepatocellular hypertrophy in males and increased incidence of mild
 3418 microscopic findings in the medulla and papilla of the kidney were detected from 300 mg/kg bw per
 3419 day. Delayed preputial separation was observed in F1 males in the lowest and highest dose group, but
 3420 this was not significant after adjustment for body weight. No relevant treatment-related developmental
 3421 changes were found in the study. The NOAEL for reproductive and developmental effects was the
 3422 highest dose tested (1000 mg/kg bw per day).

3423 In the 28-day study conducted by NTP (NTP 2019b) PFBS at doses up to 1000 mg/kg bw per day had
 3424 no effect on sperm parameters, testosterone levels or estrous cyclicity in rats.

3425 Feng et al. (2017) orally exposed 30 pregnant female ICR mice per dose group to PFBS at 0, 50, 200
 3426 and 500 mg/kg bw per day from GD 1 (as assessed by the formation of a vaginal plug) until GD 20.
 3427 Mean PFBS levels on GD 20 in serum sampled 12 h after the last dosing in 10 dams per dose group
 3428 were 1.73, 74, 332 and 721 ng/mL. On PND 21 all offspring were weaned. Only female offspring animals
 3429 were followed up. Offspring from 10 dams (50 offspring) per dose group were assessed for perinatal
 3430 survival and growth, pubertal onset and ovarian and uterine development. Another group of offspring
 3431 was assessed for the hormones representing the hypothalamic, pituitary gland and thyroid, as well as
 3432 gonadal axis (n=30 PND 1 offspring, n=30 PND 30 offspring and n=10 PND 60 offspring from 10 dams).
 3433 In the two higher dose groups, PFBS exposure resulted in a dose-related decrease in body weight from
 3434 PND 1 to adulthood, delayed eye opening, delayed vaginal opening and occurrence of the first estrus
 3435 and prolongation of diestrus phase, which was matched by decreased serum levels of estradiol and
 3436 progesterone, as well as elevated levels of LH. The absolute and relative weight of the ovaries and the
 3437 number of ovarian follicles, as well as the absolute and relative uterus weight were reduced. Regarding
 3438 thyroid hormones in offspring, decreased T3 and T4 levels were observed throughout the observation
 3439 period (PND 1, PND 30 and PND 60), whereas increases in TSH were seen only on PND 30. In dams on
 3440 GD 20 there was a decrease in T3 and T4 and an increase in TSH in the two higher dose groups, while
 3441 serum levels of estradiol and progesterone remained unchanged. Authors suggest that the observed
 3442 hypothyroxinemia may relate to general growth deficits observed. The NOAEL for developmental effects
 3443 was 50 mg/kg bw per day, resulting in a serum level of 74 ng/mL in the dam at GD 20.

3444 **In summary**, reproductive toxicity was not reported in rats exposed to PFBS up to 1000 mg/kg bw per
 3445 day. Delay in development and decrease in body weight gain were seen in mice exposed during
 3446 gestation, with a NOAEL of 50 mg/kg bw per day (74 ng/mL serum in the dam at GD 20).

3447 **PFHxS** was investigated in rats in a combined repeated dose toxicity study with the reproduction/
 3448 developmental toxicity screening setting adapted from OECD guideline 422 (Butenhoff et al., 2009).
 3449 Doses of exposure were 0, 0.3, 1, 3 and 10 mg/kg bw per day for n=15 animals per sex and dose group
 3450 in the main study, as well as three animals per group for determination of levels of PFHxS in blood and
 3451 liver. Exposure per gavage was initiated 14 days prior to mating until termination at PND 22 or presumed
 3452 GD 25 for females and study day 42 for males. No reproductive or developmental effects became
 3453 apparent and no treatment related effects were observed in dams. For effects and serum concentrations
 3454 in males, see (see Section 3.3.3.2).

3455 Ramhøj et al. (2018) performed reproductive toxicity studies with PFHxS alone and in combination with
 3456 an endocrine disruptor mix in Wistar rats. Only results with PFHxS alone are described here. Pregnant

³¹ Results from NTP (2019b) were not included in the table, since the test substance was administered to adult non-pregnant rats.

rats (16-20 per dose group) were exposed to PFHxS at doses of 0, 0.05, 5, 25 mg/kg bw per day by gavage from GD 7 to PND 22, except for the day of delivering offspring. In F1 females and males an increased liver weight was observable at 5 and 25 mg/kg bw per day, respectively. A pronounced reduction of T4 levels occurred at 5 mg/kg bw per day and was detectable at different investigated time points for both dams and offspring. PFHxS exposure mildly decreased the body weight of female (5 mg/kg bw per day) and male (25 mg/kg bw per day) pups. No other relevant effects on anogenital distance, nipple retention or organ weights were identified.

Potential reproductive and/or developmental toxicity of PFHxS was also evaluated in CD1 mice (Chang et al., 2018), using oral gavage at 0, 0.3, 1, and 3 mg/kg bw per day (n = 30 animals per sex, and a toxicokinetic experiment with 12 mice per group). F0 males and females were treated from 14 days prior to cohabitation for at least 42 days (males) and throughout mating, gestation, until PND 21 (dams). F0 dams were sacrificed on PND 22. After weaning (PND 22), F1 animals were directly dosed for 14 days. Evaluated parameters included clinical observations, mating and estrus cyclicity, functional observational batteries, related to general animal health and behavior, motor activity, in response to noise, blood parameters, organ weights, organ histology by microscopy, reproductive parameters including sperm parameters, TSH levels and gene expression. Results for other endpoints than reproductive ones are for males reported in Section 3.3.3.2. In F0 there was an increased mean and relative liver weight at 1 mg/kg bw per day. Regarding reproductive parameters, a reduced litter size (without impact on the born pup to implant ratio) was observed at 1 and 3 mg/kg bw per day, but without clear dose-response. In F1 animals an increased relative liver weight in females and males, as well as an increased thyroid weight in males was observed at 3 mg/kg bw per day.

In the 28-day study conducted by NTP (NTP 2019b) PFHxS at doses up to 10 mg/kg bw per day in males and up to 50 mg/kg bw in females had no effect on sperm parameters, testosterone levels or estrous cyclicity in rats.

In summary, the most sensitive reproductive endpoint for PFHxS exposure was reduced litter size at 1 mg/kg bw per day in mice (51.5 µg/mL serum on GD 18 in dams) with a NOAEL of 0.3 mg/kg bw per day (16.8 µg/mL serum on GD 18 in dams). At 1 mg/kg bw per day, increased liver weight was seen in the dams. In general, gestational exposure to PFHxS produces effects in offspring animals at doses which were equal to or higher than those inducing responses in parental animals (see also Section 3.3.3.2).

3.3.3.3.5 Other PFASs

Appendix F Table F.5 lists the available reproductive and developmental studies for 8:2 FTOH and EtFOSE. No studies were identified for PFOSI, 8:2 monoPAP, 8:2 diPAP and FOSA.

The potential maternal and developmental toxicity of **8:2 FTOH** was assessed in time-mated female Crl:CD (SD)IGS BR rats (Mylchreest et al., 2005). 22 animals per group were orally gavaged with 0, 50, 200, 500 mg/kg bw per day from GD 6 to 20. In dams, exposure to 500 mg/kg bw per day resulted in mortality, a decreased body weight and body weight gain, which were not observed at 200 mg/kg bw per day. A slight reduction in food consumption was in some time periods detectable at 200 mg/kg bw per day. In offspring animals, slight increases in delayed skull ossification became apparent at 200 mg/kg bw per day, but was not considered a reliable indicator of developmental toxicity by the authors, since a common observation and within historical control values. Increased skeletal variations, due to delayed ossification, became apparent at 500 mg/kg bw per day, and the NOAEL for developmental and maternal toxicity was 200 mg/kg bw per day.

Toxicokinetic experiments in rodents have indicated that 8:2 FTOH is rapidly metabolized into PFOA and PFNA and any toxicity may not be attributable to the parent compound (see Appendix C, Section C.4).

A developmental toxicity study with **EtFOSE**, which was designed as a teratology study with termination of the experiment prior to the end of gestation, was performed with pregnant Crl:CD BR rats and with pregnant rabbits (Case et al., 2001). For both animal species a dose range finding was performed prior to the teratology study. In the dose finding studies eight pregnant rats per group were orally dosed with 0, 1, 5, 10, 20, 25 and 35 mg/kg bw per day of EtFOSE from GD 6 to GD 17. Developmental toxicity studies were performed with 25 pregnant rats in a dose range of 0, 1, 5, 10 and 20 mg/kg bw per day

from GD 6 to GD 17. Rats were sacrificed at GD 20. With rats, a maternal and a developmental NOEL for EtFOSE of 5 mg/kg bw per day was determined. This was based on reduced maternal weight and weight gain during pregnancy and reduced fetal weight, which occurred at doses of 10 mg/kg bw per day and higher and which was most probably caused by reduced food consumption. Fetal effects did not occur at doses below those of maternal toxicity. No indications for selective developmental toxicity was found in rat fetuses.

In the dose range finding study with rabbits, five pregnant rabbits were orally dosed with EtFOSE at 0, 1, 5, 10, 25, 50 and 75 mg/kg bw per day from GD 7 to 20. In developmental toxicity studies (22 pregnant does per dose), animals were orally dosed with 0, 0.1, 1, 2.5 and 3.75 mg/kg bw per day during GD 7 to 20. Rabbits were sacrificed at GD 29. The authors established a maternal NOEL of 0.1 mg/kg bw per day based on a transient reduction in maternal body weight during GD 7 to 13 in response to 1 mg/kg bw per day. A NOEL of 1 mg/kg bw per day was derived based on spontaneous abortions at doses of 2.5 mg/kg bw per day and higher. Like for the rats, no selective developmental toxicity could be observed in rabbits.

3.3.3.3.6 Summary

The most sensitive developmental effect observed was impaired development of the mammary gland in mice, after exposure of the dams late in gestation or the offspring in utero or via lactation. The impairment persisted beyond sexual maturity. Only PFOA has been investigated in relation to this outcome, with LOAELs of 0.01 to 0.00045 mg/kg bw per day, depending on the dosing period. These NOAELs resulted in a similar serum concentration in pups on PND 21, which corresponded to a maternal LOAEC around 66 ng/mL serum.

In addition, developmental toxicity studies in rodents were identified for the 10 PFCAs (PFBA, PFHxA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, PFHxDA and PFODA), for three PFASs (PFBS, PFHxS and PFOS), and for the two other PFASs (8:2 FtOH and EtFOSE). The effects most often observed were increased fetal and/or neonatal mortality and reduction in fetal weight and/or postnatal growth. In general, developmental toxicity occurred at similar or slightly lower doses than those exerting maternal toxicity. The LOAELs and NOAELs were generally orders of magnitude higher than for the developmental effects on mammary gland seen in response to PFOA.

Reproductive parameters were investigated in a comparative way in rat for PFHxA, PFOA, PFNA, PFDA, PFBS, PFHxS and PFOS in the 28-days studies by NTP (2019a, b), although noting that 28 days is shorter than one spermatogenic cycle, and too short to fully assess male reproductive parameters. There were no consistent findings for female reproductive parameters. Effects on male reproductive parameters, including atrophy of the testicular interstitium, accompanied by reduced serum testosterone levels and epididymal and testicular weights, as well as degenerative changes and spermatid retention in seminiferous tubules, have been reported for PFNA and PFDA.

3.3.3.4 Neurotoxicity

3.3.3.4.1. PFOS and PFOA

In 2018 the EFSA CONTAM Panel concluded that both PFOS and PFOA exert developmental neurotoxic effects in rodents at doses of 0.1–0.3 mg/kg bw per day or higher. The analysis of behaviour showed that the most frequent alterations observed are related to locomotor activity. PFOS exposure mostly decreased spontaneous activity, while PFOA increased it. In several neurodevelopmental exposure studies, a sex-related difference has been observed with males being more sensitive than females. The CONTAM Panel has not identified relevant new neurotoxicity studies in experimental animals.

3.3.3.4.1. PFASs other than PFOS and PFOA

The literature search from 2000 onwards identified the following studies.

In vivo neurotoxicity studies were identified for PFDA, PFHxS and PFDoDA and are listed in Appendix G Table G.1. All studies were single exposure studies.

In the paper by Johansson et al. (2008), NMRI mice were exposed at PND10 by gavage to 1.4 or 21 $\mu\text{mol/kg bw}$ (0.72 or 10.8 mg/kg bw) **PFDA**, PFOS or PFOA. The effects on behaviour were investigated at the age of 2 and 4 months by monitoring spontaneous activity in the open field, and by recording the activity in the elevated plus maze in order to assess anxiety-like behaviour. With the exception of a decrease in spontaneous activity in the first 20 min of testing in the lower dose group at 2 months of age, the authors did not find significant differences between PFDA-exposed mice and controls.

Viberg et al. (2013) exposed NMRI mice to 1.4, 14, or 21 $\mu\text{mol/kg bw}$ (0.61, 6.1 or 9.2 mg/kg bw) **PFHxS** at PND10 via gavage. Spontaneous locomotor activity was assessed in 2- and 4-month-old mice, both males and females. In addition, the increase in locomotor activity stimulated by nicotine (80 $\mu\text{g/kg bw, s.c.}$) was assessed at the age of 4 months. At the age of 2 months, the mice exposed to the highest dose (both males and females) displayed globally lower levels of activity, and decreased habituation. Male mice exposed to the lowest dose displayed hyperactivity in the beginning of the testing period. When the animals were tested at the age of 4 months, the authors describe a similar pattern of alterations as at 2 months. Upon stimulation by subcutaneous administration of nicotine, the normal increase in activity was blunted in the animals exposed to the highest dose. In a study by Lee and Viberg (2013) NMRI mice were exposed to 14 or 21 $\mu\text{mol/kg bw}$ (6.1 or 9.2 mg/kg bw) PFHxS at PND10 via gavage. The mice were killed at 24 h and 4 months after exposure, and the levels of CAMK-II, GAP-43, synaptophysin, Tau, and BDNF were measured in the frontal cortex and in the hippocampus. In the hippocampus, the lower dose increased the expression levels of CAMK-II and Tau, while the higher dose increased the expression of CAMK-II, synaptophysin, and Tau in both male and female mice. No alterations in protein expression were detected in the hippocampus 4 months after exposure. In the cerebral cortex, the expression of GAP-43 was decreased 24 h after exposure in both males and females. Four months after exposure, only male mice displayed an increase in Tau expression, while females showed no change in protein expression.

Kawabata et al. (2017) compared the level of **PFDoDA** with those of PFOA and PFDA in rats 9 days after a single oral dose (50 mg/kg bw). The PFDoDA level in the brain was $44.4 \pm 2.0 \mu\text{g/g}$, higher than that in the serum ($24.4 \pm 1.0 \mu\text{g/mL}$). Conversely, the brain concentrations of PFOA and PFDA were low (<0.8 and $4.7 \pm 0.4 \mu\text{g/g}$), one-tenth of those in the serum. Behavioral analyses were performed to assess cognitive function alterations induced by PFOA, PFDA, and PFDoDA. The novel object recognition test performed 5–6 days after exposure revealed a significant decrease in the discrimination index in PFDoDA-exposed rats, whereas there were no significant alterations in PFDA- and PFOA-treated rats. The effects of PFDoDA were also assessed by the elevated-plus maze test, the Y-maze test, open-field test, and forced swim test. PFDoDA induced alterations in the elevated-plus maze test, but not in the Y-maze test, open-field test, and forced swim test. The results show that PFDoDA easily distributes in the brain and causes cognitive behavioral alterations.

3.3.3.4.3 *In vitro* studies

Three *in vitro* studies have been identified for PFHxS, PFNA, PFUnDA.

Berntsen et al. (2017) compared the cytotoxicity of PFASs with different length of the perfluorinated carbon chain and functional group attached. Two PFSA, chain length C6 and C8, and four PFCAs, chain length C8–C11, were studied using primary cultures of rat cerebellar granule neurons (CGNs). Toxicity potencies were determined by evaluating cell viability by trypan blue and MTT assays after exposure to PFUnDA, PFDA, PFOS, PFNA, PFOA or PFHxS at concentrations determined by preliminary range-finding experiments. The following NOEC - LOEC values were identified: PFHxS 300 – 400 μM ; PFOS 10 – 20 μM ; PFOA 200 – 300 μM ; PFNA 20 – 40 μM ; PFDA 10 – 20 μM ; PFUnDA 10 – 15 μM . Different PFAS doses, producing equipotent effects after 24 h exposure, were applied to study the dynamics of viability changes after 10, 30, 60, 90, 120 and 180 min, as well as 6, 12, 18 and 24 h. The following concentrations were used: 550 μM PFHxS, 60 μM PFOS, 500 μM PFOA, 100 μM PFNA, 40 μM PFDA and 30 μM PFUnDA. The onset of reduction in viability occurred relatively quickly (30–60 min) for PFOS, PFDA and PFUnDA as compared to PFHxS, PFOA and PFNA (12–24 h). Vitamin E exerted a slight protective effect against PFOA, PFNA and PFOS-induced reduction in viability, suggesting the occurrence of oxidative stress. At cell membrane level, PFHxS, PFOS and PFUnDA aggregated in large hotspots, whereas PFOA, PFNA and PFDA had a scattered distribution pattern. In summary, PFAS toxicity

increased with increasing length of carbon chain, and a sulfonate functional group induced greater toxicity than a carboxyl group.

In a follow-up study, Berntsen et al. (2018) showed that excitotoxicity induction by PFOS in CGNs is likely due to over-activation of the N-methyl-D-aspartate receptor (NMDA-R) and excess influx of Ca^{2+} . Channel blockers (memantine and MK-801) also showed some effects against PFHxS-induced reductions in viability. No substantial involvement of the NMDA-R in PFOA-, PFNA- and PFDA-induced effects on viability were observed.

Fang et al. (2018) showed that in PC12 cells PFNA (0, 10 20, 50, 100 μM , 24 h) dose-dependently decreased the viability ($\geq 20 \mu\text{M}$ PFNA), increased the malondialdehyde content (100 μM PFNA) and the intracellular calcium level ($\geq 50 \mu\text{M}$ PFNA), and decreased the total antioxidant capacity ($\geq 50 \mu\text{M}$ PFNA). Additionally, an upregulation of the Ca^{2+} -calmodulin-dependent protein kinase II (CaMKII) expression and decrease in Bcl-2 expression and increased Bax were observed.

3.3.3.4.4 Summary

In summary, as reported before for PFOS (EFSA CONTAM Panel, 2018), PFHxS and PFDA have been shown to decrease locomotor activity in rodents. Data from one study indicate that PFDoDA, in contrast to PFDA and PFOA, can efficiently transfer into rat brain and causes cognitive behavioral changes.

3.3.3.5 Immunotoxicity

In general, effects on the immune system include among others, changes in weights of lymphoid organs (thymus, spleen), cellularity of these organs, expression of interleukins and total immunoglobulin levels. Such parameters may indicate effects on the immune system. There are also more direct parameters that gauge the functionality of the immune system, such as antibody responses to T-cell dependent antigens. The antibody response to a T-cell dependent antigen in experimental animals is a prime parameter to indicate immunotoxic effects in experimental animals (Luster et al., 1993). A model that has mostly been used is to sensitize rodents by i.v. or i.p. injection of sheep erythrocytes (SRBCs), followed by enumerating specific antibody producing cells in a blood-agar plate, by counting the plaques formed. These plaques are formed by lysis of erythrocytes by IgM produced by plasma cells, in conjunction with complement that is also in the system. This so-called plaque forming assay (PFC) is a model for vaccination to a T-cell dependent antigen in humans. An alternative way to measure the antibody response to the PFC assay is to measure the antibodies in serum by Enzyme-Linked Immunosorbent Assay (ELISA). Other antigens, than SRBCs used in experimental studies include ovalbumin, keyhole limpet hemocyanin (KLH) or TNP-LPS (trinitrophenol coupled to lipopolysaccharide). In order to achieve antibody production specific for a particular antigen, different components of the immune system are required, i.e. antigen processing by phagocytes, antigen presentation by antigen presenting cells, induction of T-helper lymphocytes, maturation of B cells into plasma cells, and eventually production of different isotypes of specific antibodies. Different antigens depend to a different extent on the various components of the immune system. For instance, particulate antigens such as sheep erythrocytes require more processing than soluble antigens. KLH is more T-cell dependent than TNP-LPS. Also, the duration of the sensitization schedule is a determinant of the eventual outcome. Hence, effects of an exogenous factor on antibody production may depend on the antigen and immunization schedule used. A decrement in antibody titres may not only signify decreased protection to the specific pathogen, but also signifies a decrement in functionality of the immune system, potentially resulting in reduced resistance to infectious diseases (van Loveren et al., 2001; WHO/IPCS, 2012). An approach that directly probes the efficacy of the immune system is the use of experimental infections. A reduced resistance to an infectious agent may represent the ultimate consequence of a suppressed immune functionality.

3.3.3.5.1 PFOS

Studies already assessed in 2018

In the previous opinion (EFSA CONTAM Panel, 2018), several animal studies that have investigated effects of **PFOS** exposure on the immune system were reviewed. The studies on **PFOS** are briefly summarised below (Table 20, see also Appendix H). The studies had different study design, used different strains of mice or rats, applied different doses of **PFOS** and investigated different parameters

that may shed light on effects on the immune system. Also duration was different and studies are presented according to short-term or longer-term exposure.

Qazi et al. (2009a,b, 2012) carried out a number of short-term exposure studies and observed that after **PFOS** administration, body weight, thymus, spleen and epididymal fat weights were reduced compared to controls. A significant reduction in the number of total white blood cells and lymphocytes, but not neutrophils, was observed in the circulation, in addition to reduced numbers of macrophages in the bone marrow, but not the spleen or peritoneal cavity. Production of TNF- α and IL-6 by macrophages isolated from animals treated with PFOS was modestly increased, while *ex vivo* stimulation with LPS enhanced production of TNF- α and IL-6 by peritoneal and bone marrow (but not splenic) macrophages taken from PFOS exposed animals. These results may indicate immunomodulatory activity but cannot rule out that the effects were secondary to a general toxic effect as also effects on body weights were noted. It was noted that after 10 days of cessation of exposure, body weight had recovered, whereas relative weights of liver, thymus, and spleen remained largely unchanged, while numbers of lymphocytes of the B-cell lineage did not recover, hence were still reduced.

3672 Table 20: Studies on immunological effects of PFASs in rodents

Species	Strain	Sex	Route	duration	PFAS	NOAEL	LOAEL	NOAEC	LOAEC	Immune treatment	days before sacrifice	immune endpoint (*)	Effect	Reference
				(days)		(mg/kg perday)			(ng/mL)					
mouse	B6C3F1	male	Gavage	28	PFOS	0.000166	0.00166	18	92	SRBC	5 days	PFCs	↓	Pedem-Adams et al., 2008
mouse	B6C3F1	female	Gavage	28	PFOS	0.00331	0.0166	123	666	SRBC	5 days	PFCs	↓	
mouse	B6C3F1	female	Gavage	21	PFOS	0.005	0.025	189	670	Influenza	None	survival	↓	Guruge et al., 2009
mouse	B6C3F1	male	Diet	28	PFOS	0.25	--	11600	--	SRBC	5 days	serum IgM, PFCs	--	Qazi et al., 2010
mouse	B6C3F1	male	via dams, gavage	GD 1-17	PFOS	1	5	NR	NR	SRBC	4 days	PFCs	↓	Keil et al., 2008
mouse	B6C3F1	female			PFOS	5	--	NR	--	SRBC	4 days	PFCs	--	
mouse	C57BL/6	male	Gavage	60	PFOS	0.00833	0.0833	674	7132	SRBC	4 days	PFCs	↓	Dong et al., 2009
mouse	C57BL/6	male	Gavage	60	PFOS	0.0167	0.0833	2360	10750	SRBC	7 days	serum IgM	↓	Dong et al., 2011
mouse	C57BL/6	male	Gavage	60	PFOS	0.0833	0.4167	8210	24530	None		TNF-α, IL-6	↑	Dong et al., 2012
mouse	C57BL/6	male	Gavage	7	PFOS	--	5	--	110460	SRBC	5 days	PFCs	↓	Zheng et al., 2009
mouse	C57BL/6	male	Gavage	7	PFOS	--	5	--	97250	None		non-specific IgM	↓	Zheng et al., 2011
mouse	BALB/c	female	Gavage	21	PFOS	--	20	--	NR	Ovalbumin	14 and 7 days (two injections)	serum IgM	↓	Vetvicka and Vetvickova, 2013
					PFOA	--	20	--	NR				↓	
mouse	CD-1(ICR)BR	male	Gavage	29	APFO	1	10	32000	225000	SRBC	5 days	serum IgM	↓	Loveless et al., 2008
mouse	C57BL/6	female	Gavage	15	PFOA	1.88	3.75	NR	74913	SRBC	5 days	serum IgM	↓	DeWitt et al., 2008
mouse	C57BL/6	female	Water	15	PFOA	7.5	30	NR	NR	SRBC	5 days	serum IgM	↓	DeWitt et al., 2016
rat	SD	male	Diet	28	PFOS	--	0.14	470	950	None		serum IgG1	↓	Lefebvre et al., 2008
rat	SD	female	Diet	28	PFOS	7.58	--	43200	--	None		--	--	Lefebvre et al., 2008
rat	CD(SD)IGS BR	male	Gavage	29	APFO	30	--	223000	--	SRBC	5 days	serum IgM	--	Loveless et al., 2008

NR: not reported; PFCs: plaque forming colonies in spleen cells producing anti SRBC antibodies; * In case serum IgM is mentioned as well as the time between injection of antigen and sacrifice, authors looked for antigen-specific IgMs.

Wang et al. (2011) reported the outcome of short-term exposure studies that are in line with those of Qazi (2009a,b, 2012), i.e. significantly decreased thymus and spleen weights, compared with controls. These effects were associated with increased thymus and spleen atrophy. Immune effects were noted at doses that also affected body weights. In line with the latter were results from a study by Zhang et al. (2013d), who also reported induction of apoptosis in splenocytes and thymocytes isolated from mice exposed to **PFOS**.

Also, Vetvicka and Vetvickova (2013) found effects on the immune system after short-term exposure to **PFOS**. As in the studies by Qazi et al. (2009a,b, 2012) and Wang et al. (2009), a significant decrease in cellularity of both the spleen and thymus was observed. In addition, significantly inhibited phagocytosis (by peripheral blood neutrophils) and NK splenic activity was observed, as well as suppressed T-lymphocyte proliferation but not B-cell proliferation. Also in this study, effects on immune parameters were noted while also body weights were depressed.

Whereas these studies have indicated some effects of short-term exposure to **PFOS** on general parameters of the immune system, another approach is to investigate the functionality of the immune system, as indicated above. Zheng et al. (2009, 2011) achieved this by sensitizing animals to sheep erythrocytes. While effects on the cellularity in spleen and thymus were noted as in other studies, as well as effects on cytokine expression, the antibody response to the sheep erythrocytes as measured by plaque-forming activity, was reduced at lower exposure doses that did not affect body weights or other parameters of general toxicity. It is noteworthy that in contrast to IgM, IgG was increased rather than decreased. In addition to effects on antibody production, also lymphocyte proliferation and NK-cell activity, both parameters of the functionality of the immune system, were reduced at the lowest dose tested.

In addition to short-term studies, also studies with a longer exposure time were performed, i.e. 21, 28 or 60 days, as well as during development.

Fair et al. (2011) observed in a 28-days exposure study with adult female B6C3F1 mice variable effects on several non-functional parameters of the immune system, with increased IL-6 production by B cells as the most sensitive parameter, observed at 0.1 mg/kg (TAD). Mollenhauer et al. (2011), observed in a 28-days exposure study variable effects on various non-functional parameters of the immune system, but only at the higher dose at which also effects on body weights were noted.

In a 28-day exposure study by Qazi et al. (2010), applying only one dose of PFOS, no effects on circulating lymphocytes, the number of cells in the thymus or spleen, the number of plasma cells secreting anti-SRBC IgM, the levels of IgM, or of IgG directed to SRBCs were noted, nor were effects on IgM antibody response to TNP recorded.

In contrast, Vetvicka and Vetvickova (2013) who administered PFOS to mice also for 21 days and sensitized the animals to ovalbumin, found significantly reduced NK splenic activity and antibody responses.

Lefebvre et al. (2008) performed a 28-day exposure study with Sprague-Dawley rats and applied two concurrent study protocols: one in which animals were only exposed to **PFOS**, the other where animals were also sensitized to keyhole limpet haemocyanin (KLH), to investigate antibody production and delayed-type hypersensitivity to KLH. In the study performed according to the first protocol, significant trends in the elevation of levels of total IgG, IgG2a and IgG2c were reported in males (and IgG2c also in females), whereas serum IgG1 levels were significantly reduced in the male dose groups that did not show effects on body weight. In the study performed according to the second protocol, there was a trend towards increasing concentrations of IgG specific for KLH in males, but none of the doses showing a significant difference.

Dong et al. (2009) investigated the effects of PFOS on immunotoxicity in male C57BL/6 mice after longer term exposure. **PFOS** (potassium salt, purity 98%) was administered via oral gavage (in deionised

water in 2% Tween 80) to groups of 10 mice at dose levels of 0, 8.3, 83.33, 416.67, 833.33 or 2,083.33 µg/kg bw per day for 60 days (to achieve total added doses (TAD) of 0.5, 5, 25, 50 or 125 mg/kg bw). A dose-related decrease in specific IgM antibody production as measured by the PFC assay was observed at doses of 5 mg/kg bw TAD and higher. The level of PFOS in serum at the 5 mg/kg bw TAD dose was determined to be 7,132 ng/mL. These functional effects on the immune system were noted at doses not causing other toxicity. It should be noted that, in contrast to the other studies reported here that used the antibody response to SRBC as a read out of functionality of the immune system, Dong et al. (2009) applied a shorter sensitization schedule for the PFC assay (4 days instead of 5 or 6), which may result in a lower PFC response at the time of harvesting, as demonstrated by the lower numbers of plaques counted as compared for instance with the study by Peden Adams et al. (2008), presented below.

In a follow-up study by Dong et al. (2011), with doses of (TAD) 0.5, 1, 5, 25 and 50 mg/kg bw for 60 days, antibody responses to SRBC were measured using another approach, i.e. an ELISA to specific antigens of SRBC. SRBC specific serum IgM levels were decreased at doses of 5 mg/kg bw (TAD) and higher. Specific IgG, IgG1 and IgE levels were significantly elevated at the highest dose of 50 mg/kg bw (TAD). The serum level at the 5 mg/kg bw dose was 10,750 ng/mL.

In a study by Peden-Adams et al. (2008), **PFOS** (potassium salt, purity > 98%, doses of 0, 0.166, 1.66, 3.31, 16.6, 33.1 or 166 µg/kg bw per day) were administered to B6C3F1 mice (groups of 5 males or females) via oral gavage (0.5% Tween 20 in Milli-Q water) for 28 days. The TAD over 28 days was 0, 0.005, 0.05, 0.1, 0.5, 1 or 5 mg PFOS/kg bw (reported as free ion doses). There were no signs of overt toxic effects on body weight or organ weight of spleen, thymus, liver, kidney or gonads following PFOS exposure, nor were the cellularity and viability of spleen and thymus affected. NK-cell activity was significantly increased by 2- to 2.5-fold in males exposed to ≥ 0.5 mg/kg bw TAD but not in females. The specific IgM SRBC response, as measured by plaque forming colonies, was suppressed in males (52–78%) and females (50–74%) at ≥ 0.05 and ≥ 0.5 mg/kg bw TAD, respectively. In males, T-cell CD4/CD8 subpopulations in the thymus were not affected by PFOS whereas the numbers of all T-cell subpopulations were altered in the spleen at ≥ 0.1 mg/kg bw TAD (CD4-/CD8+ and CD4-/CD8- were increased whilst CD4+/CD8 and CD4+/CD8+ were decreased). In females, the numbers of T-cell populations were minimally affected by PFOS. Splenic CD4-/CD8+ cells were decreased at ≥ 0.1 mg/kg TAD, whereas CD4+/CD8 were decreased at 0.1 and 0.5 mg/kg bw TAD only. The effects on sensitization to SRBC was corroborated in a separate experiment, where female mice were dosed with 0.344 mg PFOS/kg bw per day for 21 days (TAD, 7 mg/kg bw) and, 7 days before termination, animals were injected iv with TNP. Serum levels of TNP-specific IgM were significantly suppressed (62%) compared with controls. The authors determined a LOAEL of 0.05 mg/kg bw TAD for males and 0.5 mg/kg bw TAD for females, i.e. a LOAEL in males of 1.66 µg/kg bw per day, corresponding to a measured serum level of 91.5 ng/g. The NOAEL of 0.166 µg/kg bw per day resulted in a NOAEC serum level of 18 ng/mL. Peden-Adams et al. (2008) mention that the study was repeated with similar results. Upon request from EFSA, data on both studies were provided to EFSA. It was stressed that the results were obtained by a standardised protocol (Peden-Adams, 2020, personal communication) and that for the results in the second study, shown in the paper, samples were blinded for the person scoring the PFCs. In addition, data were provided on an unpublished study with C57BL/6 mice (WT and PPARα mutant) where a similar protocol was applied. The results suggest that the B6C3F1 strain is more sensitive than the C57BL/6 strain used by Dong et al. (2009), which may be another reason for the lower doses of PFOS causing the effect on the antibody titres in the study by Peden Adams et al (2008) compared to Dong et al, (2009) (see Appendix H, Table H.3 and Figures H.1 to H.3).

Keil et al. (2008) measured antibody responses to SRBCs using the PFC in the offspring following maternal exposure to **PFOS**, thereby establishing a NOAEL of 0.1 mg/kg per day for decreased NK activity and 1.0 mg/kg per day for the antibody response to SRBCs. Also, these authors used a shorter immunisation protocol of 4 days. It is noteworthy that in the study by Keil et al. (2008) there was a considerable sex difference, in that an effect was found in males, but not in females. This was found

also by the publication of the same group presented above (Peden-Adams et al., 2008), be it to a lesser extent.

Guruge et al. (2009) administered **PFOS** to B6C3F1 mice for 21 days prior to inoculation with mouse-adapted influenza virus A/PR/8/34 (H1N1) and examined the animals for signs of morbidity and mortality for a further 20 days. The survival rates on day 20 after virus infection were dose-dependently reduced, being significant at 25 µg/kg bw per day.

New studies on PFOS

Since the deadline for inclusion of studies in the previous EFSA opinion (EFSA CONTAM Panel, 2018) two additional studies have been published.

For **PFOS**, Suo et al. (2017) used a mouse infection model using *Citrobacter rodentium*. Wild-type mice on a C57Bl background (4 per group), 6-8 weeks old, were exposed to 2 mg PFOS/kg bw per day for a period of 7 days, followed by exposure by gavage of 10^{10} *C. rodentium* in 200 µL phosphate buffered saline (PBS). PFOS exposure was continued until the end of the experiment, i.e. day 18. Intestinal lamina propria lymphocytes were harvested in the course of the exposure and infection. Whereas early on, PFOS exposure inhibited the outgrowth of the pathogen, which coincided with enhanced production of IL22. In the later phase enhanced bacterial counts were noted, accompanied by increased display of inflammatory cytokines, reduced mucus production, dysbiosis, and increased levels of *E. coli*.

Lee et al. (2018) investigated the effect of PFOS exposure on allergic inflammation in ICR mice in an ovalbumin model. Sensitized mice, 6 weeks of age, were intraperitoneally sensitized to ovalbumin with adjuvant on day 0 and 7. On days 9, 11, and 13 they were orally exposed to relatively high doses of 50-150 mg PFOS/kg bw, and subsequently challenged i.p. with ovalbumin on day 14. PFOS aggravated the allergic symptoms, i.e. induced hypothermia, and increased serum histamine, TNFα, IgG1 and IgE. The authors did not evaluate other toxic effects of PFOS exposure in these animals, which reduces its validity.

3.3.3.5.2 PFOA

In the previous opinion (EFSA CONTAM Panel, 2018), also several studies on effects of **PFOA** on the immune system were reviewed.

In two oral exposure studies, DeWitt et al. (2008; 2009) performed in female C57BL/J mice, in which PFOA (98% purity) were administered by oral gavage for 10 days, IgM synthesis after immunisation to sheep red blood cells (SRBCs) was significantly decreased, with a NOAEL for this parameter at 1.88 mg/kg per day. Mice were also immunized to ovalbumin, and also IgM responses to this antigen were suppressed with a NOAEL at 1.88 mg/kg bw per day. The same group repeated studies in 2016 (DeWitt et al, 2016), studies were repeated using a T-cell independent antibody response (to i.v. DNP sensitization) and also using this endpoint, a NOAEL of 1.88 mg/kg bw per day was found. Similar studies were performed by Vetvicka and Vetvickova (2013), who administered PFOA female BALB/c mice, for either 10 or 21 days, and significantly reduced antibody responses to ovalbumin and the formation of IgM directed against tri-nitrophenyl (TNP) were observed at doses of 20 mg/kg bw per day (lowest dose tested).

Another important study was performed by Loveless et al. (2008). These authors administered PFOA (100% pure, 0, 0.3, 1, 10, 30 mg/kg bw per day) by oral gavage to groups of male CD-1 mice and 10 male CD rats for 29 days. Rats and mice were given an i.v. dose of SRBCs on day 23 or 24, respectively, and sacrificed on day 28. Immune effects included reduced IgM titres to SRBCs (at 10 and 30 mg/kg bw per day), decreased spleen and thymus weights and numbers of cells in these. In rats, unlike mice, no effects on SRBC-IgM titres were reported. The NOAEL for immunotoxicity in this study was 1 mg/kg bw per day based on suppression of the anti-SRBC IgM response.

3.3.3.5.3 PFASs other than PFOS and PFOA

In two studies by Fang et al. (2008, 2009), indications of immunotoxic activity were noted. In the first study, **PFNA** was administered via oral gavage (vehicle not stated) to groups of 6 male BALB/c mice at 0, 1, 3 or 5 mg/kg bw per day for 14 days. Levels of serum adrenocorticotrophic hormone and cortisol were increased at 5 mg/kg bw per day and at 3 and 5 mg/kg bw per day, respectively. Body weight was reduced from 3 mg/kg bw per day. Absolute and relative thymus and spleen weights, as well as cell cycle parameters in thymus and spleen were significantly influenced from 3 mg/kg bw, while in the thymus, T-cell subpopulations were altered at only the highest dose. At the higher doses also markers for apoptosis were noted in the spleen. Whereas these effects might have been secondary to effects on body weights and hormonal alterations, in the spleen, a significant decrease in the percentage of F4/80+, CD49+ immature T-cells was seen at all dose levels and a significant decrease in CD11c+CD8+ at 1 mg/kg bw per day only. In spleen cells, IL-4 was reduced at all dose levels in a dose-related manner. Whereas in this study no functional parameters of the immune system were included, the data suggest that effects on the immune system are evident at the lowest dose tested, i.e. 1 mg/kg bw per day.

In a similar study, PFNA (0, 1, 3 or 5 mg/kg bw per day) was administered via oral gavage (vehicle 0.5% Tween 20) to male Sprague Dawley rats for 14 days; 6 rats/dose level were analyzed. The results largely confirmed the mouse study, i.e. decreased body weights were noted from 3 mg/kg bw per day, while immune effects were noted at 1 mg/kg bw per day, albeit in a bit less pronounced fashion than in mice. The effects at the lower dose pertained to thymus weight that was increased at 1 mg/kg bw per day and decreased at 3 and 5 mg/kg bw per day, while an increase in apoptotic cortical lymphocytes and tangible macrophages (which had ingested apoptotic cells) was noted.

In two studies by Rockwell et al. (2013, 2017), male and female 8-week-old C57Bl mice (n=5) were administered a single dose of 0.1 mmol/kg PFNA by i.p. injection (0.46 mg/kg bw). In a sub-study, mice were given 1 mg/kg LPS i.p., 28 days after treatment with PFNA. 28 days after i.p. injection of PFNA, spleen and thymus atrophy persisted. A decreased spleen weight was still noted; percentages of CD4 and CD8 cells were still up, at the cost of percentages of CD19 and CD14 cells. The TNF α response in serum to LPS treatment was markedly increased. The study supports the immunotoxicity of PFNA after i.p. injection, as well as its persistence over time. Although the mice were administered PFNA intraperitoneally, the study supports the immunotoxicity of PFNA after oral administration as observed by Fang et al. (2008, 2009).

Frawley et al. (2018) reported on the effects of **PFDA** on the immune system (see also section 3.3.3.2.1 on effects following repeated exposure), when applied to female rats and mice for a period of 28 days. Female Harlan Sprague–Dawley rats were exposed to 0, 0.125, 0.25, 0.5, 1 or 2 mg PFDA/kg bw per day by oral gavage for 28 days. Rats treated with the two highest doses showed decreased body weight gain and these dose groups were excluded from further evaluation. Treatment-related hepatocyte necrosis and hepatomegaly was observed in rats treated with 0.5 mg PFDA/kg per day, while effects on liver weights were found at 0.125 mg/kg body weight. No effects on spleen or thymus weight were noted, except on one group treated with 0.125 mg/kg bw, which was sensitized to SRBC. Female B6C3F1/N mice were exposed once per week to 0, 0.3125, 0.625, 1.25, 2.5 or 5 mg PFDA/kg bw by gavage for 4 weeks. Body weight was not affected. Increased liver weight and hepatomegaly was observed following exposure to 0.625 mg PFDA/kg bw per week and higher, while splenic atrophy was observed at 5 mg PFDA/kg bw per week. At higher doses in mice, total numbers of spleen cells were decreased, while also subpopulations of spleen cells were altered at lower doses, i.e. from 1.25 mg/kg bw, whereas in PFDA-exposed rats no changes were observed in leukocyte subpopulations. The phagocytosis of sheep red blood cells (SRBC) by fixed tissue macrophages in liver was measured in rats after i.v. administration of ⁵¹Cr labelled SRBCs. The vascular half-life for ⁵¹Cr-SRBC was not significantly altered, but the specific activity of fixed tissue macrophages in the liver was decreased by 24–39% at

0.25–0.5 mg PFDA/kg per day. The PFDA-induced effects on the functionality of the immune system (i.e. humoral- and cell-mediated immunity, host resistance) were limited.

In summary, the literature database, including the publications that appeared after the publication of the EFSA CONTAM opinion (2018), if any, support the notion that **PFOS** exposure, possibly more than PFOA, causes immunosuppression, as evidenced by decreased antibody responses to sensitization to an antigen, and that suppressed immune functionality may lead to reduced resistance to infection. Pachkowsky et al. (2019) derived a reference dose of 1.8×10^{-6} mg/kg per day for PFOS, based on the study by Dong et al. (2009). They preferred to derive a PoD from the Dong et al. (2009) study rather than the Peden-Adams et al. (2008) study, because of the longer exposure duration in the former. They did not, however, take into consideration that in the Dong et al. (2009) study a shorter, and perhaps suboptimal sensitization schedule was used, rendering the parameter tested less sensitive. The CONTAM Panel concluded that the study by Peden-Adams et al. (2008), showing effects at the lower serum levels, is the critical study for immune effects in animals.

PFOA effects in mice are similar to those of PFOS in mice, with both structural and functional parameters influenced, but effects were observed at higher doses compared to PFOS; the NOAEL for immunotoxicity of PFOA was 1 mg/kg bw per day based on suppression of anti-SRBC IgM titres in mice.

The information on other PFASs is rather limited.

Immune effects of **PFNA** indicate a LOAEL of 1 mg/kg bw per day, but this was not based on similar outcome parameters as measured for PFOS and PFOA (i.e. the functional parameter antibody production specific for sheep red blood cells was not measured).

PFDA did not influence antibody responses at the concentrations tested, but did cause other immune effects, i.e. effects on fixed liver macrophages, at a dose of 0.25–0.5 mg PFDA/kg per day.

3.3.3.6 Genotoxicity

3.3.3.6.1. PFOS and PFOA

In 2018 the EFSA CONTAM Panel concluded that for PFOS and PFOA the available genotoxicity data are inconclusive. No evidence for a direct genotoxic mode of action for PFOS and PFOA was identified. There has been some evidence for oxidative stress induced by both PFOS and PFOA (EFSA CONTAM Panel, 2018). Three new studies and two recent NTP reports have been identified since the literature deadline for this EFSA CONTAM Panel 2018 opinion on PFOS and PFOA.

PFOS

Chen et al. (2016) used a transgenic fish model, the λ transgenic medaka, to evaluate the potential mutagenicity of PFOS in liver tissue following a subchronic exposure (0, 6.7, 27.6, 87.6 $\mu\text{g/L}$ in water) for 30 days. The dose-dependent increase in mutation frequency at the *cII* target gene (2-fold at the maximal dose) was due to +1 frameshift mutations.

Treatment of rats with PFOS (0, 0.6, 1.25 and 2.5 mg/kg bw per day) by gavage for 30 days at 48 h intervals significantly increased both micronucleus frequency and DNA single strand breaks (SSBs) as assessed by the comet assay as well as the number of apoptotic cells in the liver (Eke et al., 2017).

In the 2019 NTP study, no mutagenic activity was seen for PFOS (NTP, 2019b) in *E. coli* (WP2 uvrA/pKM101, 100–10,000 $\mu\text{g/plate}$) or *S. typhimurium* (TA100 or TA 98, 50–5,000 $\mu\text{g/plate}$). In a 28-day study in female rats, PFOS (5 mg/kg bw per day) significantly increased the frequency of micronucleated polychromatic erythrocytes. Since this increase was within the historical control range, NTP judged the result to be equivocal. No increase in micronucleated erythrocyte frequency was seen in males. A significant dose-dependent decreases in the percentage of polychromatic erythrocytes in the peripheral blood of both sexes was observed.

PFOA

In the 2019 NTP study, no mutagenic activity was seen for PFOA (NTP, 2019a) in *E. coli* (WP2 uvrA/pKM101, 100-10,000 µg/plate) or *S. typhimurium* (TA100 or TA 98, 50-5,000 µg/plate).

In mice after a 5-week administration of PFOA (0, 0.2, 1, 5 mg/kg bw per day in drinking water) no evidence of treatment related genotoxicity was observed. This was assessed by measurements of SSBs by comet assay in liver and testis and by micronuclei in reticulocytes and spleenocytes (Crebelli et al., 2019). No evidence of lipid peroxidation and oxidative stress (decreased antioxidant capacity) was observed at the highest PFOA dose associated with marked liver hypertrophy and signs of cell injury (elevated ALT and AST).

No increase in micronucleated reticulocytes were observed in female rats administered PFOA (6.25-100 mg/kg bw per day) for 28 days. In male rats a significant increase in micronucleated reticulocytes was seen over the administered doses (0.625-10 mg/kg bw per day). Since this increase was within the historical control range NTP questioned the biological significance. No changes were observed in the percentage of immature erythrocytes in peripheral blood of both sexes.

In summary, the new studies do not change the conclusion made in 2018 (EFSA CONTAM Panel, 2018).

3.3.3.6.2 PFASs other than PFOS and PFOA

Appendix I, Table I.1, lists available *in vitro* genotoxicity studies identified in peer reviewed papers for relevant PFASs other than PFOS and PFOA. In addition, a report from industry about PFBS was available.

PFBS did not induce mutations in a bacterial reverse mutation assay or chromosome aberrations in the Chinese Hamster Ovary-W-B1 cell line (NICNAS, 2005). PFBS mutagenicity was judged to be equivocal in *S. typhimurium* TA 98, but negative in TA 100 and *E. coli* WP2 uvrA/pKM101 (NTP, 2019b). No induction of SSBs as measured by comet assay was observed in HepG2 cells (Eriksen et al., 2010). No increase of micronucleated erythrocytes was seen in peripheral blood samples from male and female rats administered PFBS (62.5-500 mg/kg bw per day) for 28 days. A significant decrease in polychromatic erythrocytes was seen in the peripheral blood of both sexes (NTP, 2019b).

PFBA and **PFHpA** did not induce mutations in bacteria and was negative in the micronuclei test in V79 cells (Buhrke et al., 2013).

PFDODA did not induce mutations in bacteria and was negative in the micronuclei test in V79 cells (Buhrke et al., 2013). No induction of SSBs as measured by comet assay was observed in HepG2 cells (Wielsøe et al., 2015). In Japanese medaka fish exposed to water levels of 100, 500 and 2,500 µg/L PFDODA (for 1, 4, 7, 14, 21 and 28 days) a significant time- and concentration-dependent increase of SSBs in liver DNA and erythrocyte abnormalities including micronuclei was observed (Ayanda et al., 2018).

PFUnDA did not induce SSBs as measured by comet assay in HepG2 cells (Wielsøe et al., 2015).

PFNA did not induce mutations in bacteria and was negative in the micronuclei test in V79 cells (Buhrke et al., 2013; NTP, 2019a). Three studies reported increased SSBs, including Fpg sensitive sites and 8-OHdG in HepG2 cells (Eriksen et al., 2010; Wielsøe et al., 2015) and TK6 cells (Yahia et al., 2014). In the green mussel (*Perna viridis*) PFNA (water levels 0, 0.01 – 1000 µg/L, 7 days) increased SSBs as measured by comet assay (Liu et al., 2014b). A 28 days PFNA administration to female (1.56-6.25 mg/kg bw per day) and male (0.626-2.5 mg/kg bw per day) rats did not increase micronucleated reticulocytes. Severe toxicity to the bone marrow was observed in male rats (at 2.5 mg/kg bw per day), while none was evident in the females (NTP, 2019a).

PFDA did not induce mutations in bacteria and was negative in the micronuclei test in V79 cells (Buhrke et al., 2013; NTP, 2019a). In HepG2 cells PFDA was negative in the Comet assay (Eriksen et al., 2010). In the green mussel (*Perna viridis*) PFDA (water levels 0, 0.01 – 1000 µg/L, 7 days) exerted genotoxicity

as assessed by the alkaline comet assay (Liu et al., 2014b). A 28-day PFDA administration did not affect the frequency of micronucleated reticulocytes in female rats (0.156 – 1.25 mg/kg bw per day). However, an increase in micronuclei was seen in male rats at the highest administered PFDA dose (2.5 mg/kg bw per day) which induced severe toxicity to the bone marrow (NTP, 2019a) see Section 3.3.3.2.1 and Appendix E table E.1 for further details on this study.

PFHxA did not induce mutations in bacteria and was negative in the micronuclei test in V79 cells (Buhrke et al., 2013; Loveless et al., 2019, NTP, 2019a). Two studies reported no genotoxic effects (SSBs including Fpg sensitive sites and chromosomal aberrations) after PFHxA treatment of HepG2 cells (Eriksen et al., 2010) or human peripheral blood lymphocytes (Loveless et al., 2019). In a 28-day study no increase in micronucleated reticulocytes was observed in peripheral blood of male and female rats administered PFHxA (31.5–500 mg/kg bw twice daily), whereas the percentage of circulating immature erythrocytes was markedly increased (NTP, 2019a).

PFHxS induced SSBs as measured by comet assay in HepG2 cells (Wielsøe et al., 2015). No increase of micronucleated erythrocytes was seen in peripheral blood samples from male and female rats administered perfluorohexane sulfonate potassium salt (PFHxSK) (males: 0.625-10 mg/kg bw per day, females: 3.12-60 mg/kg bw per day) for 28 days. PFHxSK exerted significant decreases in polychromatic erythrocytes in the peripheral blood of males (NTP, 2019b).

For PFASs relevant to this opinion no further genotoxicity studies in experimental animals and published in peer reviewed papers were identified.

The CONTAM Panel concluded that for PFASs other than PFOS and PFOA the number of studies and data are limited. Structural similarity for PFHxS and PFOS as well as for PFNA and PFOA and some evidence for oxidative stress induced by PFHxS and PFNA (See Appendix I, Table I.1) might indicate that, as reported for PFOS and PFOA before, (EFSA CONTAM Panel, 2018), for PFHxS and PFNA a direct genotoxic mode of action is unlikely.

3.3.3.7 Long-term toxicity and carcinogenicity

Previous long-term/carcinogenicity studies showed that PFOS and PFOA are tumor promoters in rodent liver and that PFOA also may induce Leydig cell tumours in rats (EFSA 2008 and EFSA CONTAM Panel, 2018). The CONTAM Panel has not identified relevant new carcinogenicity studies for PFOS and PFOA in experimental animals.

Klaunig et al. (2015) treated SD rats with 0, 2.5, 15, or 100 mg of **PFHxA**/kg bw per day (males) and 0, 5, 30, or 200 mg of PFHxA/kg bw per day (females) by gavage for 104 weeks. There were no effects on body weights, food consumption, hematology, hormone parameters, several functional observational endpoints, or motor activity. In females a dose-dependent decrease in survival and histological changes in the kidney (papillary necrosis) (at 200-mg/kg bw per day) could be observed. Other changes included decreases in triglyceride serum levels for males at 2.5 mg/kg bw day and for females decreases in serum LDL/VLDL and increases in urinary volume were noted at 200 mg/kg bw per day. There was no indication for carcinogenicity of PFHxA at any of the dosages tested.

Benninghoff et al. (2012) used a two-stage chemical carcinogenesis model in trout to study whether **PFNA**, **PFOS**, **PFOA**, **PFDA** or **8:2FTOH** are complete carcinogens or tumor promoters. Hepatocarcinogenesis was initiated with aflatoxin B1 (AFB1). Both, PFNA and PFDA, were applied via the diet 5 days per week for 6 months starting with a concentration of 2000 mg/kg diet (approximately 50 mg/kg bw per day), which was reduced to 200 mg/kg diet (approximately 5 mg/kg bw per day). 8:2 FTOH was applied at 2000 mg/kg diet throughout the experiment. PFNA and PFDA enhanced incidence and size of liver tumors when compared with AFB1-initiated animals fed a control diet. In the groups, receiving PFNA or PFDA only, there were no tumors or a few liver adenomas, respectively. This indicates a tumor promoting capacity of both compounds in this animal species. However, incidence and size of

liver tumors in AFB1-treated trouts fed diets containing 8:2 FTOH were unaltered when compared with AFB1-initiated animals fed control diet. Thus 8:2 FTOH exerted no liver tumor promoting activity in trout. Also, no tumors were found in animals treated with 8:2 FTOH only.

No long-term toxicity or carcinogenicity studies were identified for the other PFASs under consideration in this assessment.

In summary, there is a long-term study for PFHxA only, providing no evidence for any carcinogenicity of this PFAS. PFNA and PFDA showed a liver tumor promoting capacity in a trout two-stage model of hepatocarcinogenesis, while 8:2FTOH failed to do so. For the remaining PFASs in this opinion information on their carcinogenic potency is missing.

3.3.4. Observations in humans

In this section the body of evidence on epidemiologic studies in humans is presented and assessed. Under the various subheadings (per outcome) the basic order is chronological, but if a specific cohort is re-examined later, then this breaks the chronological order, so that several studies of a specific outcome in the same cohort are placed together. For clarity the type of study design is also emphasized in the review below.

Regarding the four outcomes (increased serum cholesterol, impaired antibody response after vaccination, increased serum ALT, and decreased birth weight) that were considered potential critical effects in the opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018), key studies published after the deadline of the literature review for the PFOS and PFOA opinion (EFSA CONTAM Panel, 2018), were also considered. These will be commented in Section 3.4.1 on Critical effects, where the evidence regarding PFOS and PFOA is discussed again.

3.3.4.1 Fertility and pregnancy outcomes

3.3.4.1.1 Birth weight

In the opinion on PFOS and PFOA, (EFSA CONTAM Panel, 2018), 13 prospective studies (maternal pregnancy or pre-pregnancy serum) and four cross-sectional studies (cord blood) were reviewed that had examined associations between PFOS and/or PFOA and birth weight. Only studies with more than 100 participants were included in that review. In addition, two large studies from the C8 cohort were reviewed that examined associations between retrospectively modelled exposure to PFOA and birth weight. Among the reviewed studies, relatively modest but consistent inverse associations with birth weight were observed for both compounds. No clear associations with low birth weight (LBW <2,500g) or small for gestational age (SGA) were observed in six studies addressing those outcomes. Also, no association was observed between PFOA and birth weight in the two studies from the C8 cohort relying on modelled exposures. While acknowledging that the association between PFOS and PFOA and birth weight might be partly confounded by physiological changes in pregnancy (Verner et al, 2015; Savitz, 2007), and the lack of association with LBW or SGA, the CONTAM Panel still concluded that there may well be an association between exposure to PFOS and PFOA and birth weight".

Since the publication of the previous Opinion on PFOS and PFOA in 2018 (EFSA CONTAM Panel, 2018), several papers on PFASs and birth weight have been published. These include both original research papers and meta-analyses. Below, these publications are reviewed first, for all compounds. Then results on PFASs other than PFOS and PFOA are summarised for those papers previously included in the Opinion (EFSA CONTAM Panel, 2018). Finally, a review of published findings from meta-analyses is given prior to a short summary.

New publications not reviewed in the EFSA CONTAM Panel 2018 opinion

In a study from the Danish National birth cohort, Meng et al. (2018) examined associations between maternal concentrations (median) of PFOS (30.1 ng/mL), PFOA (4.6 ng/mL), PFHxS (1.0 ng/mL), PFNA (0.5 ng/mL), PFHpS (0.4 ng/mL) and PFDA (0.2 ng/mL) and birth weight, gestational age, LBW and preterm delivery (<37 weeks of gestation). First trimester serum samples from 3,535 women were analyzed for PFOS and PFOA, and from 2,120 women for the other PFASs. This included 1,400 women that had previously been described in another publication (Fei et al., 2007). In this updated analysis, PFOS, PFOA, PFNA and PFHpS (but not PFHxS and PFDA) were significantly and inversely associated with birth weight and gestational age as continuous outcomes. For birth weight the mean decrease in birth weight ranged from 50 to 100 g across quartiles of exposure. For all compounds no significant associations with LBW were observed.

In a cohort study of 424 mother-infant pairs from China, Wang et al. (2019a) examined associations between cord blood concentrations (median) of PFOS (0.7 ng/mL) and PFOA (2.0 ng/mL) with birth weight and length. No associations were observed for any of the assessed outcomes.

In the UK, Marks et al. (2019) examined associations between maternal serum concentrations (median) of PFOS (14 ng/mL), PFOA (3 ng/mL), PFHxS (2 ng/mL) and PFNA (0.4 ng/mL) with birth size in 457 male infants whose mothers had been recruited into the ALSPAC birth cohort. Serum samples were drawn between week 12 and 33 of gestation. Maternal PFOS concentrations were significantly and inversely associated with birth weight, birth length and head circumference. Non-significant inverse associations were observed for the other compounds.

In a study of 2,071 singleton and 1,040 twin infants born to mothers in New York State, Bell et al. (2018) examined associations between concentrations (median) of PFOS (1.7 ng/mL) and PFOA (1.1 ng/mL) quantified in newborn bloodspots and measures of birth size. No consistent associations were observed.

Manzano-Salgado et al. (2017a) examined in 1,202 Spanish pregnant women, associations between first trimester serum (mean) PFHxS (0.6 ng/mL), PFOS (6.1 ng/mL), PFOA (2.4 ng/mL) and PFNA (0.7 ng/mL) with birth weight, LBW, and SGA. No consistent associations were observed for any of the outcomes examined.

In a cohort of 638 pregnant women from Denmark, providing blood samples between gestation weeks 8 to 16, Lind et al. (2017) examined associations between maternal serum concentrations (median) of PFOS (8.1 ng/mL), PFOA (1.7 ng/mL), PFHxS (0.3 ng/mL), PFNA (0.7 ng/mL) and PFDA (0.3 ng/mL) with birth weight. No consistent associations with birth weight as continuous outcome were observed for individual PFASs. Maternal concentrations of PFOA were, however, significantly higher among those few cases (n=13) who gave birth to LBW infants (<2,500 g).

Buck Louis et al. (2018) examined associations between several PFASs and birth weight in a cohort of 2106 healthy pregnant women recruited across 12 US cities. The median concentrations of individual PFAS (all in ng/mL) were 0.06 for N-methylperfluoro-1-octane-sulfonamidoacetic acid (N-MeFOSAA), 0.25 for PFDA, 0.03 for PFDoDA, 0.01 for PFDS, 0.02 for PFHpA, 0.71 for PFHxS, 0.76 for PFNA, 1.99 for PFOA, 5.13 for PFOS and 0.18 for PFUNDA. In this study PFOA was inversely associated with birth weight while for other PFASs no association were observed with birth weight or length. Several PFAS were inversely associated with upper thigh-length. Although of interest the interpretation of such inverse association is unclear in the absence of consistent inverse association with birth weight and length.

In a study of 693 mother-child pairs from four European birth cohorts, Govarts et al. (2018) examined the association between PFOS (median: 2 ng/mL) and PFOA (median: 0.5 ng/mL) in cord blood and small for gestational age (SGA). PFOS was not associated with SGA [OR: 0.82 (95%CI: 0.74, 0.91) per interquartile range increase in exposure] while the corresponding estimate for PFOA showed a borderline significant increased risk of SGA [OR: 1.64 (95%CI: 0.97, 2.76)].

Summary of existing meta-analyses not reviewed in the 2018 EFSA CONTAM Panel opinion

Steenland et al. (2018) published a meta-analysis based on 24 studies (n = 5,772) that had measured serum concentrations of PFOA. In addition to these studies they also included 4,142 women from the C8 cohort where exposure to PFOA had been retrospectively modelled (not included in the other meta-analyses). Based on the 24 studies with measured concentrations, a -11 g decrease (-17, -4) in birth weight was observed for every 1 ng/mL increase in PFOA. Adding the modelled exposures, the corresponding estimate was non-significant [-1.0 g decrease (95% CI -2.4, -0.4)]. The authors also concluded that studies based on serum samples drawn early compared to late in pregnancy showed weaker associations. This difference was interpreted as evidence of bias due to confounding by physiological changes during pregnancy. The conclusions by Steenland et al. (2018) are largely in line with the conclusions on a consistent association between PFOA and birth weight in the previous opinion (EFSA CONTAM Panel, 2018) on PFOS and PFOA, where the conclusion was based on studies with measured concentrations only, as the modelled concentrations in the C8 studies did not take the inter-pregnancy interval in mothers into consideration when modelling their exposure, which is a strong determinant of pregnancy concentrations for parous women (Bach et al., 2018). The updated study from the Danish National birth cohort by Meng et al. (2018) with serum samples drawn early in pregnancy and reporting around 50 - 100 g reduction in birth weight is, however, not in line with the conclusion by Steenland et al. (2018) that samples drawn early in pregnancy show much weaker associations.

In contrast to the meta-analyses by Steenland et al (2018), another previous review on PFOA only included studies using quantified levels of PFOA in serum or plasma. Johnson et al. (2014) used the Navigation Guide methodology to evaluate the evidence for an association between developmental exposure to PFOA and fetal growth. Nine studies met the inclusion criteria. Based on these studies each 1-ng/mL increase in serum PFOA concentration was associated with a 19 g decrease in birth weight (95% CI: -30, -8). Three years later Negri et al. (2017) published another meta-analysis of 12 studies with maternal plasma/serum concentrations of PFOA and eight studies with plasma/serum concentrations for PFOS. The regression coefficient for decrease in birth weight per ~3-fold (1-Ln) increase in PFOS and PFOA was -46 g (95% CI -80, -12,) and -27 g (95% CI -51, -4), respectively.

Associations with PFASs other than PFOS and PFOA in papers reviewed for only PFOS and PFOA the EFSA CONTAM Panel 2018 opinion

Kwon et al. (2016) examined associations between cord blood concentrations (median) of PFHxS (0.4 ng/mL), PFNA (0.2 ng/mL), PFDA (0.1 ng/mL), PFUnDA (0.3 ng/mL), PFDoDA (0.1 ng/mL) and PFTrDA (0.4 ng/mL) and birth weight in 268 Korean infants. Serum levels of PFHxS, PFNA, PFDA and PFUnDA were all significantly and inversely associated with birth weight.

Wang et al. (2016) examined associations between maternal serum concentrations (medians) of PFNA (1.6 ng/mL), PFDA (0.4 ng/mL), PFUnDA (3.4 ng/mL), and PFDoDA (0.4 ng/mL) and birth weight among 223 Taiwanese mothers and their term infants. Serum samples were drawn in the third trimester. Among female infants (n = 106) all four PFASs were significantly and inversely associated with birth weight, while no associations were observed for male infants (n=117).

Maisonet et al. (2012) examined associations between maternal serum concentrations (median) of PFHxS (2 ng/mL) with birth size in 447 female infants whose mothers had been recruited into the ALSPAC birth cohort. Serum samples were drawn mainly between weeks 10 and 28 (IQR) of gestation. Maternal PFHxS concentrations were significantly and inversely associated with birth weight and birth length.

Using maternal serum sampled in gestation week 9–20, Bach et al. (2016) studied associations between serum concentrations (medians) of PFHxS (0.5 ng/mL), PFHpS (0.2 ng/mL), PFNA (0.8 ng/mL), PFDA (0.3 ng/mL) and PFUnDA (0.3 ng/mL) in 1,507 primiparous women from Aarhus, Denmark. No significant associations were observed.

Robledo et al. (2015) examined the association between pre-pregnancy maternal serum concentrations of (means) 2-(n-ethyl-perfluorooctane sulfonamide) acetate (Et-PFOSA-AcOH) (0.1 ng/mL), 2-(n-methyl-perfluorooctane sulfonamide) acetate (Me-PFOSA-AcOH) (0.3 ng/mL), PFDeA (0.5 ng/mL), PFNA (1.6 ng/mL) and PFOSA (0.1 ng/mL) and birth weight and ponderal index in 243 US mothers. Analyses were performed for male and female infants separately. Only PFOSA was significantly and inversely associated with birth weight in male infants (n = 113).

Chen et al. (2012) examined the associations between cord blood concentrations of PFNA (mean 2.4 ng/mL) and PFUnDA (10.6 ng/mL) among 429 mother-infant pairs from Taiwan. Non-significant associations were observed for both compounds.

Hamm et al. (2010) studied associations between maternal concentrations during pregnancy of serum PFHxS (mean 2.1 ng/mL) and birth weight in 252 Canadian mother-child pairs. No association was observed.

Lenters et al. (2016) studied associations between PFHxS, PFNA, PFDA, PFUnDA, and PFDoDA and birth weight in a combined cohort of 1,250 mothers from Greenland (n = 513), Poland (n = 180) and Ukraine (n = 557) who gave birth at term ($\geq$ week 37 of gestation). With the exception of PFHxS ($\sim$ 3.5 ng/mL), median concentrations were below 1 ng/mL. No significant associations were observed.

Shi et al. (2017) examined associations between cord blood concentrations (medians) of PFHxS (0.2 ng/mL), PFNA (0.2 ng/mL), PFDA (0.01 ng/mL) and PFUnDA (0.06 ng/mL) and birth weight and height in 170 Chinese infants. No significant associations were observed.

Summary

Six new studies on PFOS and PFOA have been published after the 2018 Opinion (Wang et al., 2019a; Marks et al., 2019, Bell et al., 2018, Meng et al., 2018, Buck Louis et al., 2018, Govarts et al., 2018, Manzano-Salgado et al., 2017a, Lind et al., 2017). Several but not all studies observed no association. For example, the large (n = 3,535) study by Meng et al. (2018) observed consistent inverse associations between PFOS and PFOA and birth weight. In that study, samples were drawn in early pregnancy (first trimester), which should minimize the risk of confounding by physiological changes in pregnancy (Steenland et al., 2018). The difference between the study by Meng et al. (2018) and some other studies are the 2 to 4-fold higher serum levels, as women were recruited around the year 2000 when environmental contamination was at peak levels. Taking into consideration these differences in serum levels, the additional studies reviewed on PFOS and PFOA do not contradict the previous conclusion from the 2018 Opinion (EFSA CONTAM Panel, 2018) that "*there may well be a causal association between PFOS and PFOA and birth weight*". That conclusion is also in agreement with most meta-analyses that have been conducted to date.

Concerning PFASs other than PFOS and PFOA, concentrations were generally much lower compared to PFOS and PFOA and inconsistent associations with birth weight were observed.

3.3.4.1.2 Preterm delivery

In the Opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018) many of the studies reviewed above in relation to birth weight also examined associations with preterm delivery (gestational age less than 259 days). Overall, no consistent associations were observed for PFOS and PFOA in these studies. This was also the case for other PFASs (see Appendix J for details on these studies).

Since the publication of the previous Opinion (EFSA CONTAM Panel, 2018), only one large (n = 3,535) study by Meng et al. (2018) has reported results on preterm delivery. In that study maternal serum PFAS concentrations in early pregnancy showed relatively consistent associations with preterm delivery. Despite this report, and in line with the conclusions from the previous Opinion, the evidence for an association between PFASs and preterm delivery is still limited.

4183 *3.3.4.1.3 Time to pregnancy*

4184 In the Opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018), six studies reporting results on time to
 4185 pregnancy were reviewed. Based on that review the Panel's conclusion was that "*there is insufficient*
 4186 *evidence to suggest that exposure to PFOS/PFOA may adversely affect fecundity*". The same conclusion
 4187 was reached when reviewing these same studies for PFASs other than PFOS and PFOA (see Appendix
 4188 J).

4189 *3.3.4.1.4 Miscarriage*

4190 In the Opinion on PFOS and PFOA five studies reporting results on miscarriage were reviewed. Based
 4191 on that review the Panel's conclusion was that there is "*limited evidence to suggest that PFOS or PFOA*
 4192 *are associated with increased risk of pregnancy loss*". The same conclusion was reached when reviewing
 4193 these same studies for PFASs other than PFOS and PFOA (see Appendix J).

4194 *3.3.4.1.5 Hypertension in pregnancy - Preeclampsia*

4195 In the Opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018), six studies reporting results on
 4196 pregnancy hypertension were reviewed. Five of those studies were from the same study population, the
 4197 C8 cohort. The CONTAM Panel's conclusion was that "*there is insufficient evidence to suggest that PFOS*
 4198 *or PFOA are associated with pregnancy induced hypertension or preeclampsia*". No new studies have
 4199 since been identified for PFOS, PFOA or other PFASs.

4200 **3.3.4.2 Developmental effects**4201 PFOS and PFOA

4202 In the Opinion on PFOS and PFOA, 30 studies on 18 cohorts were identified with PFOS/PFOA
 4203 measurements in serum of pregnant mothers, in cord blood or in breast milk and various follow up
 4204 outcomes in children between 6 months and 22 years (EFSA CONTAM Panel, 2018). Most studies
 4205 examined associations between prenatal or perinatal exposure to PFOS/PFOA and later
 4206 neurobehavioural developmental effects. The CONTAM Panel concluded that support for associations
 4207 between prenatal exposure to PFOS or PFOA and early life neurobehavioural development or overweight
 4208 was considered insufficient (EFSA CONTAM Panel, 2018).

4209 PFASs other than PFOS and PFOA

4210 For PFASs other than PFOS and PFOA, 17 publications on 14 longitudinal studies were identified with
 4211 PFHxS/PFNA measurements in serum/plasma of pregnant mothers or in cord blood and follow-up of
 4212 various outcomes in children between 6 months and 22 years. Most studies have examined associations
 4213 between prenatal or perinatal exposure to PFHxS/PFNA and later neurobehavioral development and
 4214 overall, they do not provide support for any association. Also, for overweight/obesity and timing of
 4215 puberty no consistent associations were found.

4216 Fewer studies examined associations between these outcomes and prenatal exposure to PFASs other
 4217 than PFOS, PFOA, PFHxS and PFNA, and no significant associations were found.

4218 A description of the individual studies can be found in Appendix J.

4219 The CONTAM Panel concludes that there is insufficient evidence to suggest that exposure to PFAS may
 4220 adversely affect neurobehavioural development or overweight.

4221 **3.3.4.3 Neurotoxic outcomes**4222 PFOS and PFOA

In the Opinion on PFOS and PFOA, eight cross-sectional studies, five in children and three in adults, assessing various neurobehavioural, neuropsychiatric and cognitive outcomes were reviewed (EFSA CONTAM Panel, 2018). Consistent adverse associations were not found with serum levels of PFOS or PFOA. Several studies found inverse associations ('protective effect').

PFASs other than PFOS and PFOA

Three cross-sectional studies assessed different endpoints relevant to neurotoxicity in children. One of them was a large cross-sectional study from the C8 cohort (Stein and Savitz, 2011) which showed a statistically significant association between serum PFHxS and diagnosis of ADHD (based on interview, including medications) in children and adolescents. Same-direction non-statistically significant associations between were also reported for PFNA (and PFOS). There was no association between PFHxS and learning problems which would indicate that, should the association be causal, it would be based on aspects of ADHD that do not affect learning. In contrast to PFOA where contaminated drinking water was the main source of exposure in this cohort, the serum PFHxS levels were not higher than in other populations. Another study showed an association with behavioral changes at age 5 but not at age 7. The third study was very small (N=83).

Three cross-sectional studies were performed in adults. They reported possible inverse ("protective direction") associations for PFNA and PFHxS.

The CONTAM Panel concludes that there is insufficient evidence to suggest that exposure to PFASs may adversely affect neurobehavioural, neuropsychiatric and cognitive outcomes.

3.3.4.4 Immune outcomes

3.3.4.4.1 Asthma and allergies in children and adults

PFOS and PFOA

In the opinion on PFOS and PFOA, four prospective studies and six cross-sectional studies were reviewed that had examined associations between PFOS and PFOA with asthma and allergies (EFSA CONTAM Panel, 2018). The CONTAM Panel concluded that "*there is not much evidence to suggest that PFOS or PFOA are associated with asthma and allergies in children and adults*". Since then, five new prospective studies on asthma and allergies have been published. The associations reported in these new studies were reviewed for all PFASs and the studies previously reviewed for PFOS and PFOA were reviewed for other PFASs as well (See Appendix J).

In summary, new studies on PFOS and PFOA showed no or inconsistent associations with asthma and allergies for both prenatal and postnatal exposures. These studies do not change the conclusion of the previous opinion on PFOS and PFOA.

PFASs other than PFOS and PFOA

Concerning PFASs other than PFOS and PFOA, prospective studies in children with prenatal exposure assessment have produced inconsistent results (Timmerman et al., 2017; Averina et al., 2019; Wang 2011). Results of studies focusing on prenatal exposures have also been inconclusive (Manzano-Salgado et al., 2019; Impinen et al., 2018, 2019; Timmerman et al., 2017; Goudarzi et al., 2016; Okada et al., 2014). Two well-conducted cross-sectional studies by Dong et al. (2013) and Quin et al. (2017) from the same study population in Taiwan, suggest that several PFASs may be positively associated with asthma in children. Three other cross-sectional studies in adolescents and adults (Tao et al., 2008; Buser and Scinicariello, 2016 and Stein et al., 2016) found no association. One possible explanation for a positive association with asthma being observed in a cross-sectional study is that asthmatic cases might be differently exposed through altered behaviour because of their underlying disease condition

4266 In conclusion, the available evidence is insufficient to suggest that exposure to PFASs are associated
4267 with allergy and asthma in children and adults.

4268 3.3.4.4.2 Vaccination response

4269 In the previously published opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018), a total of six
4270 studies were reviewed. Since then, three additional publications on two cohorts have been published
4271 (Grandjean et al., 2017a, 2017b, Zeng et al 2019). All these studies are reviewed below for all PFASs,
4272 including also PFOS and PFOA.

4273 Grandjean et al. (2012) examined associations between both pre- (gestation week 32) and postnatal (5
4274 years) serum concentrations of PFASs and offspring antibody concentrations against tetanus and
4275 diphtheria following booster vaccination at age 5 years (cohort 3, n=456-587, 1997-2000). Post-natally,
4276 serum PFASs and pre-booster antibody concentrations were measured at a mean age (SD) of 5.0 (0.1)
4277 years. Serum antibody response was then measured ~4 weeks later after booster vaccination and at
4278 offspring age 7.5 (0.1) years. The median concentrations for antibody titres to tetanus were 0.22 IU/mL
4279 at 5 years pre-booster, 35 IU/mL at 5 years post-booster and 1.6 IU/mL at 7.5 years. For diphtheria the
4280 corresponding numbers were 0.12, 13.0 and 0.68 IU/mL, respectively. Associations between offspring
4281 PFAS concentrations at age 5 pre-booster with antibody titres at age 5- post-booster and 7.5-years
4282 post-booster can be interpreted as a short- and long-term influence on the efficacy of the booster
4283 vaccination, respectively. The strength of this study is that it is not purely observational and has an
4284 interventional component. The large increase in antibody concentration is initiated through vaccination
4285 and this increase is examined in relation to baseline PFASs concentrations. The interpretation of
4286 associations reported between maternal PFAS concentrations and offspring antibody concentrations
4287 during childhood are, however, more challenging, as several vaccinations are administered from birth
4288 at various timepoints. Furthermore, among breastfed infants, maternal PFAS concentrations are, due to
4289 exposure through breastfeeding, strong determinants of offspring concentrations during the first few
4290 years of life. Several associations were explored in this study and the results are summarised below:

4291 Association between maternal PFAS concentrations and antibody concentrations at ages 5 (pre- and
4292 post-booster) and 7.5:

4293 **PFOS:** Mean concentration in maternal serum was 27.3 ng/mL. Each 2-fold increase in maternal
4294 PFOS concentrations was associated with -39% (95%CI: -55, -17) and -21% (95%CI: -38, 1)
4295 decrease in diphtheria antibody concentrations at 5 years pre- and post-booster, respectively.
4296 Non-significant but inverse direction associations were observed for tetanus antibody
4297 concentrations.

4298 **PFHxS:** Maternal concentrations of PFHxS (mean: 4.4 ng/mL) were not associated with
4299 antibody concentrations to tetanus or diphtheria at age 5 years pre- and post-booster.

4300 **PFOA:** Maternal concentrations of PFOA (mean: 3.2 ng/mL) showed a non-significant inverse
4301 association with antibody concentrations to diphtheria at age 5 years pre- and post-booster
4302 while the associations for tetanus were in opposite directions at pre- and post-booster, neither
4303 of them being significant.

4304 **PFNA:** Similar to PFOA, maternal concentrations of PFNA (mean: 0.6 ng/mL) showed a non-
4305 significant inverse association with antibody concentrations to diphtheria at age 5 years pre-
4306 and post-booster, while the associations for tetanus were centered around the NULL.

4307 **PFDA:** Maternal concentrations of PFDA (mean: 0.3 ng/mL) were significantly and inversely
4308 associated with antibody concentrations to diphtheria (around 20% decrease per 2-fold
4309 increase) at age 5 years pre- and post-booster. No association was observed for tetanus.

4310 **Combined exposures:** Structural equations were used to evaluate the associations for
4311 combined exposure to PFOS, PFHxS and PFOA during pregnancy and in relation to offspring

antibody response to diphtheria and tetanus at age 5.0 years pre-booster and at age 7.5 years post-booster. A 2-fold increase in maternal concentrations during pregnancy was significantly associated with -48% (95%CI: -68, -16) and -42% (95%CI: -66, -1) decrease in serum antibody response to diphtheria at age 5 pre-booster and age 7.5 post-booster, respectively. No associations were observed for tetanus.

Offspring PFAS concentrations at age 5 and offspring antibody concentrations at ages 5 and 7.5 years:

PFOS: Each 2-fold increase in offspring PFOS concentrations at 5 years pre-booster (mean 16.7 ng/mL) was associated with -29% (95%CI: -46, -6) and -24% (95%CI: -44, 4) change in post-booster antibody response to tetanus at ages 5-year and 7.5 years, respectively. The corresponding estimates for diphtheria were -16% (95%CI: -32, 4) and -28% (-46, -3), respectively.

PFHxS: At age 5 years pre-booster, two-fold offspring concentrations of PFHxS (0.6 ng/mL) were significantly associated with -19% (95%CI: -30, -7) lower tetanus antibody concentration at 5 years post-booster and -20% (95%CI: -32, -6) lower concentration at age 7.5 years. A non-significant -10% lower concentration was observed for diphtheria for these two timepoints.

PFOA: At 5 years of age, pre-booster offspring concentrations of PFOA (4.1 ng/mL) showed a weak but inverse association with antibody response to tetanus and diphtheria post-booster at age 5 years (6-13% decrease). At age 7.5 years the association for both antibody titres to diphtheria and tetanus was, however, strongly significant, corresponding to around ~25% decrease per 2-fold increase in PFOA.

PFNA: At 5 years pre-booster, each 2-fold increase in offspring PFNA concentrations (mean: 1.0 ng/mL) was associated with around 15-20% decrease in antibody response to diphtheria and tetanus at age 5- and 7.5-years, although formal significance was not always reached.

PFDA: At 5-years pre-booster, each 2-fold increase in PFDA (mean: 1.0 ng/mL) concentrations was associated with around 10-20% decrease in antibody response to diphtheria and tetanus at 5- and 7.5-years post-booster, although formal significance was reached only for tetanus.

Combined exposures: Structural equations were used to evaluate the associations for combined exposures to PFOS, PFHxS and PFOA at offspring age 5 years (pre-booster) in relation to offspring antibody response to diphtheria and tetanus at age 5 years pre-booster and at age 7.5 years post-booster. A 2-fold increase in offspring serum levels at age 5 years pre-booster showed a non-significant inverse association with antibody concentrations age 5 years pre-booster. A two-fold increase in combined exposure at age 5.0 years pre-booster was, however, significantly associated with a -44% (95%CI: -66, -11) and -55% (95%CI: -73, -25) decrease in serum antibody response to diphtheria and tetanus at age 7.5, respectively.

Low antibody levels At age 5 years pre-booster, a 2-fold increase in PFOS concentrations was associated with 1.6 (95%CI: 1.1, 2.3) higher odds of being below a protective level (0.1 IU/mL) against diphtheria. The corresponding estimates for PFOA was OR 1.2, 95%CI: 0.8-1.7. Slightly elevated but non-significant OR were observed for tetanus. At age 7.5 years concentrations of PFOS and PFOA at 5 years were associated with 2.4 (95%CI: 0.9, 6.4) and 3.3 (95%CI: 1.4, 7.5) higher odds of being below protective levels against diphtheria. Similar elevated odds were reported for tetanus at age 7.5.

Co-exposures: Concerning possible confounding by other co-exposures, PCBs in maternal samples and offspring samples at age 5.0 years showed a weak correlation with individual PFASs. Adjustment for these co-exposures had no impact on the effect estimates. With respect to individual PFASs, the correlation between the five substances at offspring age 5 years ranged between 0.2 and 0.8. The strongest correlation was observed between PFNA and PFDA, while for PFOS and PFOA the correlation was ~0.5. Other pair-wise correlations were weaker. The authors performed benchmark dose (BMD)

analyses for each of the five PFASs in serum of the 5-year-old children in relation to antibody response at 5 and 7.5 years. The results were reported with and without mutual adjustment for PFOS and PFOA (Budtz-Jorgensen and Grandjean, 2018). In short, the modelling showed that both PFOS and PFOA, in statistical terms, were associated with antibody concentrations independent of each other (not confounded).

In summary, combined serum levels of PFOS, PFHxS and PFOA during pregnancy and at age 5 years pre-booster vaccination showed a strong inverse association with serum antibody titres to diphtheria and/or tetanus at age 7.5 years. Use of combined exposure is supported by evidence suggesting that the associations observed for each PFAS are at least partly independent (Budtz-Jorgensen and Grandjean, 2018). For individual PFASs at age 5 years, the associations with antibody concentrations to tetanus and diphtheria at 5 and 7.5 years post-booster were consistently inverse. Associations for tetanus were generally weaker and less consistent compared to diphtheria. A lack of formal significance for some of the associations reported is not unexpected, given the different concentrations of individual PFASs. Higher odds of being below protective levels for diphtheria at age 5.0 and 7.5 years suggest that the associations observed are of clinical relevance.

Looker et al. (2014) examined, among 411 US adults, associations between serum concentrations of PFOA and PFOS and antibody response following influenza vaccination. Participants had been exposed to PFOA contaminated drinking water and were enrolled into the C8 project in 2005–2006. They were again followed-up and recruited for this study in 2010. Median serum concentrations of PFOA and PFOS in 2010, at baseline prior to vaccination, were 31.5 and 9.2 ng/mL, respectively. After vaccination (after 21 ± 3 days), seroconversion for influenza type B, A/H1N1 and A/H2N3 was 62%, 84% and 65%, respectively. Mean antibody concentrations for influenza type B post vaccination were significantly lower among those in the highest compared to the lowest quartile of serum PFOA levels. Non-significant decreases in antibody titres were also observed across quartiles for A/H1N1 and A/H2N3. After adjustment for age and sex, the association between PFOA and antibody titres to influenza Type B weakened and non-significant, while the inverse association for A/H2N3 became more pronounced, showing a significant risk of not attaining the antibody threshold considered to offer long-term protection. No association was observed for PFOS.

Kielsen et al. (2016) recruited 12 healthy adult volunteers from Denmark, aged 23 to 67, and examined associations between serum concentrations of several PFASs with changes in antibody concentrations to diphtheria and tetanus following booster vaccination in an exploratory study. Serum samples were drawn at baseline prior to booster vaccination. Baseline concentrations (medians) were 0.4 ng/mL for PFHxS, 9.5 ng/mL for PFOS, 0.12 ng/mL for PFHpA, 1.7 ng/mL for PFOA, 0.7 ng/mL for PFNA, 0.3 ng/mL for PFDA, 0.21 ng/mL for PFUnDA and 0.04 ng/mL for PFDoDA. Changes in antibody concentration were quantified at days 2, 4, 7, 10, 14 and 30 after booster vaccination. Concentrations of both diphtheria and tetanus antibodies increased by more than ten-fold and reached a plateau by day 10. The outcome measure was, as a result, defined as the individual change in serum antibody titres between days 4 and 10 post-booster. Baseline concentrations of PFHxS, PFOS, PFNA, PFDA, PFUnDA and PFDoDA were significantly and inversely associated with the rate of increase in diphtheria antibody concentrations between 4 to 10 days. A non-significant inverse association was observed for PFOA ($p=0.25$) and PFHpA ($p=0.75$). The percent reduction in diphtheria antibody concentrations ranged between ~8 to ~18% for a 2-fold increase in PFAS concentrations. However, for tetanus, only PFUnDA and PFDoDA were significantly inversely associated with the rate of increase in antibody response, corresponding to ~10% reduction in antibody concentrations per 2-fold increase in exposure.

Stein et al. (2016b) examined the association between serum concentrations (means) of PFOS (5.2 ng/mL), PFHxS (1.1 ng/mL), PFOA (2.3 ng/mL) and PFNA (0.8 ng/mL) with immune response following vaccination against influenza (FluMist). A total of 78 subjects from the US, aged 18–49, provided a baseline blood sample and were then immunised. Blood samples were then drawn after 48–72 h and again, at least 30 days after vaccination. A total of 13 immune outcomes were explored including

cytokine, chemokine and immunoglobulin concentrations in serum. Overall, no significant associations were observed between baseline concentrations of these four compounds with the outcomes explored. When interpreting these results, it is worth noting that the FluMist generated a limited systemic response to the vaccination. More precisely, seroconversion was only observed in 7 and 19 out of 78 participants as measured by haemagglutinin inhibition and immunohistochemistry, respectively. Such limited response to the vaccination is a clear limitation, making any association with antibody response difficult to detract.

In a cohort of 101 infants from Germany, Abraham et al. (2020) examined the association between plasma concentrations of PFHxS, PFOS, PFOA and PFNA and antibodies to diphtheria, tetanus and haemophilus influenza type b (Hib). Mothers and their children were recruited in 1997-1999 when the infants were between 341 and 369 days old. Of these, 21 were formula fed (≤ 2 weeks of breastfeeding) and 80 were breastfed for > 4 months. When combining exclusive and partial breastfeeding into "equivalent to exclusive breastfeeding" the median duration was 7.4 months. Mean levels of PFASs in plasma from, respectively, non-breastfed and breastfed infants were for PFOA 3.8 and 16.8 ng/mL, for PFOS 6.8 and 15.2 ng/mL, for PFHxS 1.7 and 2.1 ng/mL and for PFNA 0.2 and 0.6 ng/mL. For the mothers, the mean concentrations in plasma among those who did not breastfeed ($n=21$) and those who breastfed ($n=80$) were for PFOA 4.9 and 3.2 ng/mL, for PFOS 17.2 and 14.1 ng/mL, for PFHxS 1.8 and 1.0 ng/mL and for PFNA 0.4 and 0.3 ng/mL. Higher concentrations in plasma among breastfed infants and lower concentrations among mothers who breastfed is explained by lactational transfer of PFASs from the mother to the baby. This transfer into breast milk is more effective for PFOA compared to PFOS, which also explains the differences in PFOS/PFOA ratio between mothers and infants (see human TK section).

Concentrations of PFOA in infant plasma were significantly and inversely correlated with antibody concentrations to diphtheria ($r=-0.23$, $p=0.02$), tetanus ($r=-0.25$, $p=0.01$) and Hib ($r=-0.32$, $p=0.001$). Analyses were adjusted for time since last vaccination and for tetanus also the number of vaccinations. Adjustment for other co-contaminants quantified in infant blood, including PCBs, dioxins (I-TEQ), organochlorine pesticides, mercury, cadmium and lead did not influence these associations. Adjustment for duration of exclusive breastfeeding had no relevant influence. The NOAECs for PFOA, estimated by dividing exposure into quintiles, ranged between 18.9 and 19.4 ng/mL, depending on the type of antibody titers. In terms of effect size the mean reduction in antibody response when comparing the highest to lowest quintile of PFOA exposure was -57%, -53% and -78% for diphtheria, tetanus and Hib, respectively. Associations for PFOS, PFHxS and PFNA were not significant. Upon request from EFSA, the authors provided analyses of the associations with the sum of PFOA, PFNA, PFHxS and PFOS (see Appendix K). Similar as for PFOA, the sum of the 4 PFASs was significantly and inversely correlated with tetanus and Hib, while the correlation for diphtheria was borderline significant.

Since the first study from the Faroe Islands (Grandjean et al., 2012), reviewed above, two additional studies using partly the same data have been published. One study was based on later follow-up of the children, while the other examined associations with PFAS concentrations at earlier age. Both these studies, and others reviewed in this section, are of more observational character as the timing of exposure assessment is not standardised and assessed in relation to administration of the booster vaccination.

Using the same cohort as in the first study (Grandjean et al., 2012; cohort 3, $n=587$, 1997-2000), additional follow-up was conducted when the children were 13 years of age (Grandjean et al., 2017a). In that study, associations between offspring PFAS concentrations at ages 7.5 and 13 years were examined in relation to serum antibody titres to tetanus and diphtheria at age 13 years. At age 7.5 years, serum concentrations (means) were 15.3 ng/mL for PFOS, 4.4 ng/mL for PFOA, 0.5 ng/mL for PFHxS, 1.1 ng/mL for PFNA and 0.4 ng/mL for PFDA. These median concentrations were quite similar to those observed at age 5 (Grandjean et al., 2012). Serum PFOS concentrations at age 7.5 were inversely associated with antibody concentrations to diphtheria at age 13 corresponding with around -

24% (95%CI: -43, -2) decrease per 2-fold increase in serum levels. The corresponding estimate for PFDA was -22% (95%CI: -34, -6). For PFOA, PFHxS and PFNA a modest inverse association was observed, being far from significant ($p>0.20$). After exclusion of subjects having received booster vaccinations between age 7.5 to 13, slightly stronger associations were observed. At age 13 years, serum PFAS concentrations were approximately halved compared to those at that age 7.5. At age 13 years, the cross-sectional analyses showed a modest and non-significant inverse association for all five compounds. After exclusion of those having received booster vaccinations, the association for PFOA became significant, showing a -25% (95%CI: -43, -3) decrease per 2-fold increase in exposure, while the associations for the other four compounds were still inverse but non-significant. For tetanus antibodies, the associations were, however, in the opposite direction for some of the compounds, suggesting an increase in antibody concentration at 13 years with higher exposure at ages 7.5 and 13. One methodological limitation of this study, as explained by the authors, is that children and adolescents that visit the emergency room for cuts or other injuries, frequently receive a tetanus booster for protection, which adds considerable variability to the outcome measures. Around 46% of the subjects in that study were suspected of having received a booster vaccination against tetanus between ages 7.5 and 13 years, which makes it difficult to evaluate the association for tetanus with the same precision as for diphtheria.

Another study aimed at examining the effect of PFASs on antibody response in young children (Grandjean et al., 2017b), using partly the same data (cohort 3, $n=587-532$, 1997-2000) along with another comparable cohort recruited 10 years later (cohort 5, $n=275-239$, 2007-2009). The aim of this study was to explore associations with serum PFAS concentrations at birth, age 18 months and 5 years in relation to serum antibody concentrations at age 5 years prior to booster vaccination at that age. In Cohort 5 maternal blood samples were drawn around birth, while in Cohort 3 serum PFAS concentrations at birth were estimated based on maternal samples drawn in gestation week 32.

For tetanus, both cohorts individually and combined showed a consistent inverse association between serum PFAS concentrations at birth, age 18 months and 5 years and serum antibody concentrations at 5 years (pre-booster). Formal significance was, however, only consistently reached for PFOA.

For diphtheria, serum PFAS concentrations at birth showed an inverse association with serum antibody concentrations at 5 years (pre-booster) in both cohorts. In combined analyses of both cohorts, significant associations were observed for PFOS, PFOA and PFNA. For PFAS levels at 18 months and 5 years, the associations with antibody concentrations at 5 years (pre-booster) were inconsistent, with cohort 3 showing in general inverse associations, while cohort 5 frequently showing positive associations. In cohort 3, serum PFOS and PFOA concentrations were ~ 17 and 4 ng/mL. Due to phase-out of long-chain PFASs, environmental concentrations had decreased continuously and for cohort 5 the concentrations were 2-3 fold lower (~ 5 ng/mL for PFOS and 2 ng/mL for PFOA). Decreasing and lower exposures in cohort 5 may well explain these inconsistent findings.

Modelled exposures: The authors also estimated offspring serum concentrations of PFOS and PFOA at months 3, 6 and 12 based on maternal serum concentrations at birth and information on duration of breastfeeding. Associations with serum antibody concentrations to tetanus and diphtheria at age 5 years pre-booster were then explored. The strongest associations were generally observed for predicted concentrations at 3 months of age, while the associations became increasingly weaker at ages 6 and 12 months.

Granum et al. (2013) examined in a sub-set ($n=50$) of children from the Norwegian Mother and Child Cohort study the associations between maternal serum concentrations (medians) of PFOS (5.5 ng/mL), PFHxS (0.3 ng/mL), PFOA (1.1 ng/mL) and PFNA (0.3 ng/mL), measured at delivery with serum antibody concentrations in offspring that had all followed a routine vaccination program, where vaccines against tetanus and Hib were given at ages 3, 5, and 12 months, and vaccination against measles and rubella

at 15 months. Maternal serum concentrations were for all compounds significantly and inversely associated with offspring antibody concentrations to rubella at age 3 years (n=50). Similar conclusions were reached when each compound was mutually adjusted for the other. Non-significant inverse associations were observed for Hib, tetanus and measles antibodies.

Stein et al. (2016a) examined cross-sectionally associations between concentrations (means) of PFHxS (2.5 ng/mL), PFOS (20.8 ng/mL), PFOA (4.1 ng/mL) and PFNA (0.8 ng/mL) and serum antibody concentrations to measles, mumps and rubella among 1,188 12- to 19-year-old US children from the US National Health and Nutrition Examination Survey (NHANES). Among seropositive individuals (96%) a 1-ng/mL increase in concentrations of PFOS, PFHxS and PFOA was associated with, respectively, -13% (95%CI: -20, -6), -6% (95%CI: -11, -2) and -9% (95%CI: -16, -3) lower serum antibody concentrations to rubella. PFOS and PFOA showed slightly weaker but significant inverse associations with antibody concentrations to Mumps (~6-7% decrease per 1-ng/mL increase). Non-significant, but inverse associations were observed for PFHxS and non-significant associations were observed for measles for all four compounds.

Finally, Zeng et al. (2019) examined in 201 Chinese mother-child pairs associations between several PFASs measured in cord blood and antibodies to hand, foot and mouth disease (CA16 and EV71) both in cord blood and at 3 months of age. PFAS cord-blood concentrations were (medians) PFOA (1.2 ng/mL), PFOS (3.2 ng/mL), PFDA (0.1 ng/mL), PFDoDA (0.05 ng/mL), PFHxS (4.0 ng/mL), PFNA (0.2 ng/mL), PFUnDA (0.1 ng/mL) and sum of all PFASs (10.6 ng/mL). Except for PFHxS and PFDoDA, all PFASs quantified (including the sum) were inversely associated with antibodies to hand, foot and mouth disease in cord blood. Similar inverse associations were observed at 3 months, but formal significance was not reached for several compounds. Cord blood concentrations of individual PFASs (including the sum) were, however, associated with increased odds of having serum antibodies below protective levels in both cord blood and in offspring serum drawn at 3 months. The only exception from this pattern was PFHxS.

Summary

Results from six vaccination response studies in children and adults have been reported to date (Grandjean et al., 2012; Granum et al., 2013; Kielsen et al., 2016; Stein et al., 2016b, Looker et al., 2014; Abraham et al., 2020). Three of these studies show, for several PFASs, relatively strong inverse associations with antibody response following booster vaccination to tetanus and diphtheria in both children (Grandjean et al., 2012; Granum et al., 2013; Abraham et al., 2020) and adults (Kielsen, 2016). One study showed some, but more modest inverse associations with antibody titres to influenza in adults (Looker et al., 2014). The null findings by Stein et al. (2016b) on influenza vaccination do not contradict these results, as most subjects did not respond to the vaccination. Associations with antibody titres falling below protective levels were also reported (Grandjean et al., 2012; Looker et al., 2014). Concerns have been raised previously that findings on vaccination response among the Faroese children (Grandjean et al., 2012) could be confounded by other seafood contaminants. However, stability of those findings after adjustment for PCBs (Grandjean et al., 2012) and replication of findings in infants (Abraham et al., 2020) and a small group (12) of Danish adults (Kielsen et al., 2016), where exposure to seafood-borne contaminants differs, make such confounding less likely. Consistent findings from observational studies in other study populations provide further support for an adverse association between several PFASs and vaccination response in children and adolescents (Zeng et al., 2019; Stein et al., 2016a). The two recent studies from the Faroe Islands are in line with other studies showing associations in early infancy (Grandjean et al., 2017b) and at adolescent age (Grandjean et al., 2017a). It is, however, difficult to conclude from these studies if one age period is more sensitive than another.

Overall, different compounds appear to show significant findings across different studies. Such inconsistencies are not unexpected given the differences in concentrations and mixture composition. As a result, it is difficult to make strong conclusions if one compound is much more potent than the other.

Based on more detailed analyses from the Grandjean et al. (2012) study, it seems likely that both PFOS and PFOA may affect antibody response independently (Budtz-Jorgensen and Grandjean, 2018).

In line with the conclusion from the opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018), additional studies published since then strengthen the conclusion that both PFOS and PFOA are associated with reduced antibody response to vaccination. Given the lower concentration of other PFASs the evidence for association is weaker.

3.3.4.4.3 Clinical Infections

In the previously published opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018), a total of five studies were reviewed. Since then three other studies have been published. Given the relevance of this outcome for the opinion, all these studies are reviewed below for all PFASs (including PFOS and PFOA).

In the previously mentioned study by Granum et al. (2013), maternal concentrations of PFHxS, PFOS, PFOA and PFNA were also examined in relation to common cold in the offspring, that was assessed by parental report at ages 1, 2 and 3 years (n = 99). The authors observed a significant positive association between the total number of reported episodes of common cold in the offspring up to 3 years of age (n = 99) and maternal PFHxS, PFOA and PFNA, but not PFOS. For PFOA and PFHxS a positive association with a number of reported episodes of gastroenteritis was also observed.

In the previously mentioned study on asthma and allergies by Impinen et al. (2019), associations between maternal PFHxS, PFHpS, PFOS, PFOA and PFUnDA concentrations and offspring risk of infections at ages 0-3 years and 6-7 years, assessed by parental report, were also examined. Although positive associations with certain types of infections were observed for individual PFASs (pseudocroup, bronchitis/pneumonia at age 0-3, and Diarrhea/gastric flu at age 7), inverse associations were also observed (such as for episodes of common cold at age 0-3 years). Overall, the results of this study are inconclusive.

Dalsager et al. (2016) examined associations between maternal serum concentrations (median) for PFHxS (0.3 ng/mL), PFOS (8.1 ng/mL), PFOA (1.7 ng/mL), PFNA (0.7 ng/mL) and PFDA (0.3 ng/mL) and infections in children among 649 mother-child pairs from Odense, Denmark. Maternal serum samples were drawn prior to week 16 of gestation and episodes of infection were recorded every 2 weeks over 12 months from birth, using mobile phone text messages. Both the number of days and information on symptoms (11 different categories) were recorded. Among the 649 participants, 354 reported symptoms ranging from rare events such as blood in stools (n=4) to temperature over 38.5 degrees (n=283) and runny nose (n=337). Maternal PFOS concentrations in the upper tertile (>10.2 ng/mL) compared with the lowest (<6.9 ng/mL) were significantly associated with increased proportion of days with fever (incidence rate-ratio: 1.7 (95% CI: 1.2, 2.2), p-trend=0.001). No association was observed for the other compounds. An increased odds ratio of experiencing days with fever above the median was found for PFOS (OR: 2.4 (95% CI: 1.3, 4.1)) and for PFOA (OR: 2.0 (95% CI: 1.1, 3.6), but no such association was observed for PFHxS, PFNA and PFDA. For other symptoms than fever, different PFASs showed no associations with other symptoms including coughing, nasal discharge, diarrhoea or vomiting.

In a cohort of 1,558 mother-offspring pairs, Goudarzi et al. (2017) examined the association between maternal concentrations (means) of PFHxS (0.3 ng/mL), PFOS (5.5 ng/mL) PFHxA (<0.1 ng/mL), PFHpA (<0.1 ng/mL), PFOA (2.7 ng/mL), PFNA (1.1 ng/mL), PFDA (0.5 ng/mL), PFUnDA (1.4 ng/mL), PFDoDA (0.2 ng/mL), PFTTrDA (0.3 ng/mL) and PFTeDA (<0.1 ng/mL) and prevalence of offspring infectious diseases up to 4 years of age. Samples were drawn in gestation week 28 to 32. At the 4-year follow-up, mothers filled out a questionnaire that included questions on common offspring infectious diseases that had occurred up to 4 years of age, including otitis media, pneumonia, varicella, and respiratory syncytial virus. Associations between maternal PFASs and total infection diseases, defined as cases with at least one of these four common infectious diseases up to 4 years of age, were then explored. Maternal

PFOS was associated with increased propensity for total infections up to 4 years of age [OR of 1.6 (95%CI 1.2, 2.2), when comparing those with >6.7 ng/mL vs. <2.7 ng/mL]. No associations were observed for the other compounds.

In a cohort of 641 mother-child pairs from Norway, Impinen et al. (2018) examined the association between cord blood concentrations of (medians): PFHxS (0.2 ng/mL), PFOS (5.2 ng/mL), PFOSA (0.4 ng/mL), PFOA (1.6 ng/mL), PFNA (0.2 ng/mL) and PFUnDA (0.1 ng/mL) and the number of episodes of common cold infections in the offspring from 0 to 2 years, and number of episodes of respiratory tract infection in the offspring from 0 to 10 years. The number of episodes was based on parental report at follow-up at offspring age 2 and 10 years, respectively. PFUnDA was positively associated with the number of episodes of infections in the offspring until age 2 years. No associations were observed for other PFASs. PFOS, PFOA, PFOSA, PFNA and PFUnDA (but not PFHxS) were, however, all strongly and significantly associated with the number of episodes of respiratory tract infection until 10 years of age.

In the previously reviewed study by Looker et al. (2014) on vaccination associations, self-reported occurrence of recent (< 12 months) respiratory episodes of colds or influenza in adults (n=411) were also examined. No associations were observed for PFOS and PFOA (median levels 31.5 and 9.2 ng/mL).

Fei et al. (2010) examined in a subset of participants from the Danish National Birth Cohort (n=1,400, 1996–2002), associations between maternal concentrations of PFOS and PFOA and risk of hospitalisation due to infectious diseases, up to 2008. Information on hospital admission was extracted from nationwide registries. Mean concentrations for PFOS and PFOA in maternal serum, drawn in gestation week 4–14, were 35 and 5.6 ng/mL, respectively. At the end of follow-up, 26% of the offspring had been hospitalised at least once. Offspring age at the end of follow-up ranged from 6 to 11 years. In this study, no associations were observed between maternal concentrations of PFOS and PFOA and offspring hospitalisation. The authors noted that they were only able to examine associations with resistance to infections in general, and the study was not designed to examine if prenatal exposure could predict occurrence of specific infections.

Okada et al. (2012) also found no association between second trimester maternal serum concentrations (median) of PFOS (5.2 ng/mL) and PFOA (1.3 ng/mL) and offspring otitis media (parental report) up to 18 months of age (n = 343).

Summary

Based on the results from the vaccination studies reviewed above, findings on increased propensity of infections would seem plausible. Overall four studies on infection in children found some positive associations between maternal concentrations of PFASs with offspring's later risk of infections (Granum et al., 2013; Dalsager et al., 2016; Goudarzi et al., 2017; Impinen et al., 2018). Two studies in children reported no such association (Impinen et al., 2019; Fei et al., 2010). In general, associations were most frequently reported for PFOS, but as for the vaccination studies, differences in concentration and mixture composition makes it difficult to evaluate the evidence for each compound independently. One study in adults also reported no association with infections (Looker et al., 2014). All but two of these studies (Dalsager et al., 2016; Fei et al., 2010) were based on self-reported episodes of infection, which are prone to bias. Since those reporting such infections should have been blinded to the exposure, such bias is likely to be non-differential. Overall, there is some evidence to suggest that exposures to PFASs are associated with increased propensity of infections but more studies with objective measures of infections (not self-reports) are needed.

3.3.4.5 Endocrine effects

3.3.4.5.1 Thyroid function and disease

PFOS and PFOA

In the opinion on PFOS and PFOA, six studies on associations with thyroid disease and twenty studies on associations with thyroid hormones were reviewed (EFSA CONTAM Panel, 2018). Some were performed in children and some in adults. Most of them were cross-sectional. It was concluded that there is insufficient support for associations between PFOS or PFOA and thyroid disease or changes in thyroid hormones.

PFASs other than PFOS and PFOA

Most studies examined associations with PFHxS and PFNA, and the nineteen studies are tabulated in Appendix J, where the individual studies are reviewed.

Regarding thyroid disease, one study found an association between PFHxS and thyroid disease (as defined by TSH levels outside a certain range), but only in women and not consistent with results when TSH was used as a continuous variable. No associations were reported between PFNA and thyroid disease.

Associations between serum levels of PFHxS and/or PFNA and thyroid hormones were investigated in seventeen cross-sectional studies

In summary, the available evidence is insufficient to suggest that exposure to PFASs are associated with thyroid disease or changes in thyroid hormones.

3.3.4.5.2 Male fertility and puberty

In the opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018) a number of cross-sectional studies on semen quality and sex hormones in men were reviewed. In summary those studies did not provide support for serum PFOS/PFOA as predictors of adverse effects on semen quality or reproductive hormones. Some of these studies also examined other PFASs than PFOS and PFOA, as described in appendix J. In addition, one additional study reporting results on PFOS and PFOA have been published after the PFOS/PFOA opinion.

In summary, the available evidence is insufficient to suggest that pre- or postnatal exposures to PFASs are associated with effects on pubertal development or male fertility.

3.3.4.5.3 Female fertility, menstrual cycle and puberty

In the opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018) a number of studies on semen quality and sex hormones in men were reviewed. In summary those studies provided insufficient evidence that exposure to PFOS or PFOA is related to menstrual cycle length, menopause or puberty development. Likewise, the studies reviewed in this opinion (Appendix J) do not provide consistent associations between pre- or postnatal exposures to PFASs and female reproduction outcomes or puberty.

3.3.4.6 Metabolic outcomes

3.3.4.6.1 Blood lipids

PFOS and PFOA

In the opinion of PFOS and PFOA 26 publications in 16 study cohorts on associations between PFOS and/or PFOA and serum lipids were reviewed (EFSA CONTAM Panel, 2018). Some were performed in occupational cohorts, but most of them in the general population. Most were cross-sectional but there were also some longitudinal studies. It was concluded that it is likely that associations between serum PFOS and PFOA levels and serum cholesterol are causal and that an increase in cholesterol was considered adverse.

After the Opinion on PFOS and PFOA some new studies examined associations with blood lipids. Convertino et al. (2018) in a before-after study with no external control group examined 49 patients

with advanced solid tumors, who failed standard therapy. The patients received oral doses of between 50 and 1200 mg weekly for six weeks of ammonium perfluorooctanoate (APFO) as a possible chemotherapeutic agent. The serum PFOA concentrations were about 10,000 – 150,000 ng/mL after the first dose and about 150,000 – 500,000 ng/mL after six weeks. Overall there was a significant, but slight, decrease of total and LDL cholesterol with increasing dose and serum levels of PFOA. The authors concluded that at these serum PFOA levels the decrease in total cholesterol (and LDL), was consistent with results in animal models, and the effects of PPAR α -agonists such as clofibrate in humans. Additionally, the authors noted that these changes of total cholesterol (TC) and LDL at very high serum PFOA levels contradict the epidemiological findings of increasing cholesterol with increasing serum PFOA at much lower serum PFOA concentrations.

However, the median TC in the study with cancer patients seemed to increase at the lowest serum PFOA categories, although there were only few patients in each category. Moreover, the small sample size, the singularity of the exposure conditions (extremely high level, non-dietary medicinal administration, etc), the unique characteristics of the population under study and the lack of a control group hinders both the internal and the external validity of this study. Thus, the CONTAM Panel noted that this report cannot shed additional light on effects in humans at the much lower PFOA intake levels occurring from normal diet or contaminated drinking water.

Graber et al. (2019) examined the association between PFAS and self-reported physician-diagnosed "high" cholesterol in 105 adults exposed to contaminated drinking water in New Jersey, MA, USA. The geometric means of serum PFOS and PFOA were 5.4 and 3.0 ng/mL. After adjustment for potential confounders, there were no significant associations between PFOS/PFOA and self-reported high cholesterol.

Dong et al. (2019) studied cross-sectional associations between serum levels of PFAS and cholesterol in six US NHANES surveys 2003 – 2014, including about 9000 adults and 3000 adolescents. The median PFOS (10.9 ng/mL) and PFOA (3.0 ng/mL) levels in adults were only slightly higher than in adolescents. After adjustment for a number of potential confounders, there were significant linear associations between serum PFOS/PFOA and total cholesterol. BMD was estimated using a "hybrid approach" where the outcome was "high" cholesterol and the BMR was 10%. BMDLs for PFOS and PFOA were estimated to be 24 and 5.6 ng/mL.

Jain and Ducatman (2019) studied associations between (log-transformed) PFAS and (log-transformed) serum lipids cross-sectionally in 3629 adults from NHANES surveys 2005 - 2014. The aim was to examine if associations differed by gender and obesity (BMI >30). After adjustment for a number of potential confounders, serum PFOA (but not PFOS) was positively associated with total and LDL cholesterol in obese males, but not in the other three groups (women and non-obese males). Serum PFOS (but not PFOA) was positively associated with LDL cholesterol in obese females, but not in the other three groups. The authors propose that PFAS exposure also contributes to liver steatosis and/or that increased serum cholesterol from PFAS is more likely to occur in individuals with steatosis.

Donat-Vargas et al. (2019) studied associations between serum PFAS and blood lipids in a longitudinal study of 187 individuals from a population-based cohort followed up for 10 years. The median PFOS and PFOA levels were 20 and 2.9 ng/mL at baseline and 15 and 2.7 ng/mL at follow-up. Total cholesterol decreased from 5.5 to 5.4 mmol/L, but there was no significant association between changes of PFOS/PFOA and cholesterol within individuals at the prospective follow-up of 172 individuals, not taking cholesterol-lowering medications. There was, however, a significant inverse association between the change of PFOS and the change of triglycerides.

Lin et al. (2019) studied associations between baseline serum PFAS and blood lipids cross-sectionally, and in a longitudinal follow-up of 888 prediabetic adults followed up for 15 years, half of which had an intervention aiming at lifestyle changes. The median serum PFOS and PFOA levels were 27 and 4.9 ng/mL at baseline in the late 1990s. At baseline there were significant positive associations between

log-transformed serum PFOA and serum cholesterol (total, LDL, VLDL, non-HDL) as well as the risk of hypercholesterolemia, while the associations with PFOS were statistically significant only for VLDL and non-HDL cholesterol. The prospective risk of hypercholesterolemia was associated with PFOA in the subgroup without lifestyle intervention (N=361), with a hazard ratio of 1.4 (95% CI 1.1 – 1.9), when comparing individuals with PFOA >4.9 ng/mL (median) and PFOA ≤4.9 ng/mL, while the risk was not increased in the intervention group. For PFOS the corresponding risk was not significantly increased (HR 1.2, 95% CI 0.9 – 1.6). There were also some positive associations between PFOS/PFOA and serum triglycerides.

These new cross-sectional studies in general population samples are consistent with the large number of previous studies finding positive associations between serum levels of PFOS and/or PFOA and serum cholesterol. More interesting are the two new longitudinal studies, one of which did not find any association between the change of PFOS/PFOA and change of cholesterol (Donat-Vargas et al. 2019) in 172 individuals, and the other one finding an association between baseline PFOA and prospective risk of hypercholesterolemia in 361 prediabetics (Lin et al., 2019).

Associations between PFOS/PFOA and cholesterol has been reviewed again after external comments to the previous opinion. Further details are presented in section 3.4. As illustrated there, the CONTAM panel now considers the uncertainty regarding causality is larger than what was stated in the previous opinion.

PFASs other than PFOS and PFOA

Nelson et al. (2010) performed a cross-sectional study of the association between PFOS, PFOA, PFNA and PFHxS and blood lipids, body weight and insulin resistance in 860 adults from US NHANES 2003 – 2004, 20–80 years of age, not taking cholesterol-lowering medication. Median serum levels were PFOS: 20 ng/mL, PFOA: 3.8 ng/mL, PFNA: 1.0 ng/mL, and PFHxS: 1.8 ng/mL (all four >LoD in >98% of samples). Total cholesterol (TC) and non-HDL cholesterol (total minus HDL) in serum were determined in all participants and LDL cholesterol in half of them. A number of potential confounders were adjusted for. TC was about 7% higher (14 mg/dL higher) in the fourth quartile (Q4) of PFNA than in the first quartile (Q1); the difference in PFNA medians between Q4 and Q1 was 1.6 ng/mL. The result for non-HDL cholesterol and LDL showed similar results, but for LDL the association was not statistically significant. PFNA was moderately correlated with PFOS and PFOA (Spearman $r = 0.5$), which were also associated with cholesterol (almost as strong as for PFNA). PFHxS was inversely associated with cholesterol (statistically significant with PFHxS as a continuous variable, and almost so when analysed by quartiles).

Lin et al. (2011) studied the association between PFNA (median 1.7 ng/mL) and PFUnDA (median 7.1 ng/mL) and metabolic outcomes including blood lipids (HDL, LDL, total cholesterol, triglycerides) in a cross-sectional study of 287 young people from Taiwan. No significant associations were found with blood lipids in adjusted models.

Fisher et al. (2013) examined cross-sectional associations between PFHxS and plasma lipids, glucose homeostasis, and metabolic syndrome in 2700 fasting participants aged 18 – 74 years in the national Canadian Health Measures Survey (CHMS). The GM PFHxS level was 1.9 – 2.8 ng/mL in the different age groups. In analyses adjusted for age, sex, and a number of other potential confounders (and excluding participants taking cholesterol-lowering drugs) there was a positive association between PFHxS and total cholesterol, LDL, and TG, and a negative association with HDL. The OR for high cholesterol increased by PFHxS quartiles.

Fu et al. (2014) performed a small (N=133) cross-sectional study of the association between PFASs and blood lipids in a Chinese sample aged 0 – 80 (mean 30) years, coming for a health check-up. Analyses were adjusted for age, gender and BMI, but there was no information on use of lipid-lowering medications. Median serum levels were PFOS: 1.5 ng/mL, PFOA: 1.4 ng/mL, PFNA: 0.4 ng/mL. Other PFASs were <0.3 ng/mL or below the LoD in >25% of samples. There was a statistically significant

association between total cholesterol and LDL and PFNA. TC was about 6% higher (0.25 mmol/L higher) in the fourth quartile (Q4) of PFNA than in the first quartile (Q1); the difference in PFNA medians between Q4 and Q1 was 0.9 ng/mL. There were also positive associations between PFDA (median 0.2 ng/mL) and cholesterol and HDL. There were no associations between total cholesterol and PFHxA, PFHpA, PFUnDA.

In a cross-sectional study of 891 pregnant women from Norway, part of which had been selected for a study of subfertility, Starling et al. (2014) studied the association between PFASs and blood lipids. About 40% of invited women participated. A number of potential confounders were adjusted for. Mean levels for the four most abundant PFASs were PFOS: 13, PFOA: 2.3, PFHxS: 0.6, and PFNA: 0.4 ng/mL. Total cholesterol was significantly associated with PFOS, about 4 mg/dL (2%) higher per interquartile range (about 6 ng/mL). There was no significant association with the other PFASs and specifically for PFNA (difference between means of Q4 and Q1 about 0.5 ng/mL) there was no tendency towards a positive association. The Spearman correlation coefficient between PFOS and PFNA was 0.66.

Zeng et al. (2015) performed a cross-sectional study in 225 Taiwanese children 12 – 15 year old, serving as controls in a study of asthma (participation rate 72% of those approached). PFNA (median 0.8 ng/mL), PFBS, and PFTeDA were positively associated with total cholesterol in some adjusted models.

Seo et al. (2018) published a cross-sectional study on associations between several PFASs and blood lipids in 786 adults from Seoul, Korea, participating in a medical check-up. A significant positive association was found between PFNA (median 1.7 ng/mL) and total cholesterol, and a significant inverse association between PFHxS (median 7.1 ng/mL) and cholesterol. The authors reported that there were also positive rank correlations between PFDA (median 1.1 ng/mL) and PFUnDA (median 1.5 mg/mL), but no details were presented. All associations reported were unadjusted for age, sex, and other PFASs.

The study by Graber et al. (2019), mentioned above, examined the cross-sectional associations between PFHxS (GM 2.0 ng/mL) and PFNA (GM 3.5 ng/mL) and self-reported physician-diagnosed "high" cholesterol in 105 adults exposed to contaminated drinking water. After adjustment for potential confounders, there was a significant association (OR 1.16, 95% CI 1.03 – 1.29) between PFNA (but not PFHxS) and self-reported high cholesterol.

The study by Dong et al. (2019), mentioned above, found a significant linear association between serum levels of PFNA (median 1.0 ng/mL) and total and LDL cholesterol. No associations were found for PFHxS (median 1.6 ng/mL) or PFDA (0.2 ng/mL). BMD for PFNA was estimated using a "hybrid approach" where the outcome was "high" cholesterol (>95-percentile) and the BMR was 10%. The BMDL for PFNA and total cholesterol was estimated to be 2.0 ng/mL, i.e. lower than for PFOS/PFOA.

The study by Donat-Vargas et al. (2019), mentioned above, found no significant associations between changes of PFHxS, PFNA, PFDA, or PFUnDA and cholesterol within 172 individuals at the prospective follow-up for ten years. There were, however, significant inverse associations between the changes of PFNA, PFDA, PFUnDA and the change of triglycerides.

The study by Lin et al. (2019) mentioned above had median serum levels of PFHxS=2.3 ng/mL, PFNA=0.6 ng/mL, EtFOSAA=1.1 ng/mL, and MeFOSAA=1.0 ng/mL at baseline. At baseline there were significant positive associations between log-transformed serum PFNA and serum cholesterol (total, LDL, and non-HDL) as well as the risk of hypercholesterolemia, while this was not the case for PFHxS, EtFOSAA, or MeFOSAA. The prospective risk of hypercholesterolemia was significantly associated with PFNA in the subgroup without lifestyle intervention (N=361), with a hazard ratio of 1.2 (95% CI 1.0 – 1.3) per doubling of PFNA at baseline, while the risk was not increased in the intervention group. There were also some positive associations between PFAS and serum triglycerides.

The study by Jain and Ducatman (2019) mentioned above found that serum PFNA (GM 1.0 ng/mL) was positively associated with total and LDL cholesterol in obese males and females, but not in non-obese individuals. Serum PFDA (GM 0.25 ng/mL) was positively associated with total and LDL cholesterol in

obese females, but not in the other three groups. No such associations were found for PFHxS (GM 1.6 ng/mL), PFUnDA (GM 0.12 ng/mL), or MePFOSAA (GM 0.18 ng/mL). PFHxS, PFDA, and PFUnDA were also positively associated with HDL cholesterol. The authors propose that PFAS exposure also contributes to liver steatosis and/or that increased serum cholesterol from PFAS is more likely to occur in individuals with steatosis.

Thus, stratified by each examined PFAS, the findings regarding associations between various PFASs and total cholesterol were as follows:

- For PFHxA, Fu et al. (2014) and Zeng et al. (2015) found no association with TC.
- For PFHpA, and PFBA, Fu et al. (2014) found no association with TC.
- For PFNA, Nelson et al. (2010), Fu et al. (2014), Zeng et al. (2015), Seo et al. (2018), Graber et al. (2019), Dong et al. (2019), Lin et al. (2019), and Jain and Ducatman (2019) found a positive association with TC, while Starling et al. (2014) found no association.
- For PFDA, Fu et al. (2014) and Jain and Ducatman (2019) found a positive association with TC, while Starling et al. (2014), Dong et al. (2019), and Donat-Vargas et al. (2019) found no such association.
- For PFUnDA, Fu et al. (2014), Starling et al. (2014), Donat-Vargas et al. (2019), and Jain and Ducatman (2019) found no association with TC.
- For PFDoDA, and PFTeDA, Zeng et al. (2015) found no association with TC.
- For PFHxS, Nelson et al. (2010) and Seo et al. (2018) found an inverse association with TC, while Fisher et al. (2013) found a positive association, and Starling et al. (2014), Zeng et al. (2015), Graber et al. (2019), Dong et al. (2019), Donat-Vargas et al. (2019), Lin et al. (2019), and Jain and Ducatman (2019) found no association.
- For PFHpS, Starling et al. (2014) found no association with TC.
- For PFBS, Zeng et al. (2015) found a positive association with TC.

Summary

There were 12 cross-sectional studies on associations between cholesterol and PFAS other than PFOS and PFOA, and mostly results were not consistent. The only exception is PFNA, where almost all studies (Nelson et al., 2010; Fu et al., 2014; Zeng et al., 2015; Seo et al., 2018; Graber et al., 2019; Lin et al., 2019, and Jain and Ducatman, 2019) found significant associations with TC, while Starling et al. (2014) did not. In most of these studies the median or GM PFNA level was <1 ng/mL. Several of the studies were large and adjusted for potential confounders. In most of the studies PFNA was moderately correlated with PFOS/PFOA (Pearson r 0.4 – 0.6). In the smallest studies, it is difficult to disentangle the possible impact of PFNA exposure from that of PFOS and PFOA, overall the data suggest that PFNA has an association with serum cholesterol which is independent from PFOS/PFOA.

3.3.4.6.2 Diabetes, Obesity and Metabolic Syndrome

PFOS and PFOA

In the opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018) 15 studies were reviewed on the associations between PFOS and or PFOA and glucose homeostasis or diagnosis of diabetes. Overall, the results did not indicate adverse effects on glucose homeostasis or increased risk of diabetes. Several studies also examined adiposity and overall, the results did not indicate an increased risk of overweight or obesity due to exposure to PFOS or PFOA. Thus, it was concluded that there is no evidence that PFOS or PFOA increases the risk of metabolic syndrome.

PFASs other than PFOS and PFOA

In summary, eight studies were reviewed on associations between PFASs other than PFOS and PFOA, and diabetes or metabolic syndrome, and the results are not consistent. Details on the individual studies are shown in Appendix J.

3.3.4.6.3 Liver

PFOS and PFOA

In the opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018), eleven studies on associations between PFOS/PFOA and liver enzymes, other liver biomarkers, or liver disease were summarized. Most studies on associations between serum PFOA and serum levels of the liver enzyme ALT at occupational exposure showed no significant associations. The association between PFOA and ALT was considered likely to be causal, but the adversity of the increase in the normal range was considered uncertain since the increase in ALT per unit of PFOS/PFOA was small and no association with liver disease had been shown. For PFOS, data was inconsistent. In the large C8 cohort an association was also found between PFOA and ALT above the reference range, and this was considered adverse. Since the increase in ALT was a potential critical effect (EFSA CONTAM Panel, 2018), the association has now been reviewed again.

After the publication of the opinion on PFOS and PFOA, several studies have been published on associations between PFOS/PFOA and markers of effects on liver cells and are summarized below.

Bassler et al. (2019) studied associations between PFOS (mean 27 ng/mL) and PFOA (mean 95 ng/mL) and two biochemical markers of hepatocyte apoptosis: Cytokeratin 18 M30 (CK18 M30) and CK18 M65 in a cross-sectional study of 200 participants in the C8 study, randomly selected among those 40 - 70 years of age, and without high alcohol consumption or history of hepatitis. The rationale for examining these biomarkers was the findings of hepatocyte steatosis and apoptosis in experimental studies, as well as associations with ALT in human epidemiological studies. In addition, several biomarkers of inflammation (cytokines and C3a) were examined. The rationale for this was that PFAS are immunosuppressors and may have anti-inflammatory properties. If so, the observed association with ALT would not be caused by inflammation, which is a common cause of liver damage. The results were consistent with the hypotheses. Positive associations were found between serum levels of PFOA and CK18 M65 and, especially, CK18 M30. For PFOS the positive trends were not statistically significant. For markers associated with inflammation, associations suggested anti-inflammatory effects. The authors' interpretation is that PFOA causes hepatocellular apoptosis, and maybe non-alcoholic fatty liver disease (NAFLD).

Salihovic et al. (2018) examined such associations longitudinally in a population-based study of 1016 old (70 years) individuals. They were followed up at 75 and 80 years of age. The initial mean levels of PFOS and PFOA were 13.2 and 3.3 ng/mL. For ALT there was a statistically significant association between the change of PFOS/PFOA and the change of serum ALT.

Nian et al. (2019), reported cross sectional findings regarding associations between PFOS/PFOA and liver biomarkers in 1605 adult government employees or residents in Shenyang, China (see also Bao et al., 2017). The median PFOS and PFOA concentrations in serum were 24 and 6.2 ng/mL. After adjustment for potential confounders, serum ALT was positively associated with PFOA and PFOS, although for PFOS not when individuals taking lipid-lowering medications were excluded. There was also a positive association between serum PFOS and ALT outside the reference range, but only for branched PFOS.

Jain (2019) examined associations between PFOS (GM 6.0 ng/mL), and PFOA (GM 2.1 ng/mL) and liver enzymes (ALT, AST, GGT) in 2883 individuals from NHANES 2011 – 2014 with the specific aim to study if associations were more pronounced among obese (BMI ≥ 30) participants. Statistically significant (but modestly sized) associations were found between log-transformed serum levels of PFOA (but not PFOS) and log-transformed ALT in the 1082 obese individuals but not in the others. The authors' interpretation

is that obese individuals already have a tendency towards fatty liver (NAFLD) and therefore are more susceptible regarding an impact of PFASs.

In summary, the conclusion in the opinion on PFOS/PFOA (EFSA CONTAM Panel, 2018) regarding associations between serum ALT and exposure to PFOA is in agreement with these newer studies. Of special interest is the study by Salihovic et al. (2018) because the longitudinal design of examining "change of ALT versus change of PFAS" is superior to cross-sectional studies and minimises the potential confounding by individual factors. While previous studies were not consistent regarding associations between PFOS and ALT (EFSA CONTAM Panel, 2018), the study by Salihovic et al. (2018) finds a significant association between PFOS and ALT, and suggests that also PFOS exposure might increase serum ALT. The study by Bassler et al. (2019) provides more insight in the mechanisms behind the associations.

PFASs other than PFOS and PFOA

Lin et al. (2010) examined associations between PFNA and PFHxS and liver function biomarkers (ALT, GGT and total bilirubin) in 2200 adults from US NHANES surveys 1999-2004. The mean serum PFNA was 0.8 ng/mL and for PFHxS it was 2.0 ng/mL. In adjusted models there were no significant positive associations between log-transformed PFNA or PFHxS and liver enzymes, but there was a tendency towards a positive association with total bilirubin for both PFNA and PFHxS.

Gleason et al. (2015) studied associations between serum PFNA and PFHxS and liver function biomarkers (ALT, AST, GGT, ALP and bilirubin) in about 4300 individuals from US NHANES surveys 2007 – 2010. The median levels of PFNA and PFHxS were 1.4 ng/mL and 1.8 ng/mL. A number of potential confounders were adjusted for. Significant positive associations were found between ln-transformed PFNA and ln-transformed levels of ALT, GGT, and bilirubin, and between ln-transformed PFHxS and ln-transformed ALT. There was also a positive trend for PFNA when calculating odds ratios of having levels of ALT higher than the 75th percentile of levels in the NHANES surveys.

Rantakokko et al. (2015) studied serum levels of PFHxA, PFNA, PFDA, PFUnDA, and PFHxS in extremely obese subjects undergoing bariatric surgery. There were some inverse associations (less inflammation) between PFNA, PFDA, and PFHxS levels and grade of histologic inflammation in the liver. There was however, also a positive association between PFHxA and ALT at 12 months after surgery.

Salihovic et al. (2018) examined associations between PFHpA, PFNA, PFDA, PFUnDA, and PFHxS (apart from PFOS and PFOA) and markers of effects on liver cells, including ALT, ALP, bilirubin, and GGT. The associations were examined longitudinally in a population-based study of 1016 70 year-old individuals. They were followed up at 75 and 80 years of age. The initial median levels were PFHpA: 0.05, PFNA: 0.7, PFDA: 0.3, PFUnDA: 0.3, and PFHxS: 2.1 ng/mL. For ALT there was a statistically significant association (trend) over the three examinations between the changes of these PFASs and the change of serum ALT. Many of the p-values were very low; the highest one (for PFHxS) was 0.01, which was not statistically significant if applying a Bonferroni adjustment for multiple comparisons. For most PFASs there were also significant positive associations between PFASs and ALP, and significant inverse associations with bilirubin.

The above-mentioned study by Jain (2019) examined associations between PFHxS (GM 1.3 ng/mL), and PFNA (GM 0.8 ng/mL) and liver enzymes (ALT, AST, GGT) in 2883 individuals from NHANES 2011 – 2014 with the specific aim to study if associations were more pronounced among obese (BMI ≥30) participants. Statistically significant (but modestly sized) associations were found between log-transformed serum levels of PFHxS/PFNA and log-transformed ALT in the 1082 obese individuals but not in the others. The authors' interpretation is that obese individuals already have a tendency towards fatty liver (NAFLD) and therefore are more susceptible regarding an impact of PFASs.

The study by Nian et al. (2019), mentioned above, reported findings regarding associations between PFNA and PFDA, and liver biomarkers in 1605 adult government employees or residents, in Shenyang,

China. The median serum concentrations were 2.0 (PFNA) and 0.9 (PFDA) ng/mL. After adjustment for potential confounders, serum ALT was positively associated with PFNA and PFDA, but the magnitude was limited; 6.2% and 3.1% increase for a 2.7-fold (1 ln) increase in PFNA and PFDA, respectively. For PFNA there was also a significant association with prevalence of abnormal (outside the reference range) ALT (OR 1.5 per 2.7-fold increase of PFNA, 95% CI 1.2 – 1.9). Also, PFBA, PFHxA, PFUnDA, PFDoDA, PFTrDA, and PFHxS could be detected in >50% of individuals, but levels were relatively low, and had no significant association with serum ALT.

The study by Bassler et al. (2019) mentioned above, found significant positive associations between PFHxS and PFNA and CK18 M30, and for PFHxS also with CK18 M65, the two biochemical markers of hepatocyte apoptosis in 200 participants in the C8 study. This finding was similar to what was found for PFOA, and as for PFOA results for other biomarkers suggested anti-inflammatory effects. The authors' interpretation is that the class of PFAS causes hepatocellular apoptosis, and maybe non-alcoholic fatty liver disease (NAFLD).

In summary, there were several studies on associations between liver biomarkers and PFASs other than PFOS and PFOA, and the results indicate positive associations between PFHxS/PFNA and serum ALT. In particular the longitudinal study by Salihovic et al. (2018) provides support for this. The magnitude of the association was, however, modest in most of the studies and only one of them reported an association with ALT outside the normal range (as found for PFOA, see previous opinion (EFSA CONTAM Panel, 2018)).

In summary, the available evidence on associations between ALT and PFASs is insufficient to use as a basis for a health-based guidance value.

3.3.4.7 Kidney Function and Uric acid

PFOS and PFOA

In the EFSA opinion on PFOS and PFOA three studies on associations between PFOS/PFOA and estimated (based on serum creatinine) glomerular filtration rate (EFSA CONTAM Panel, 2018) were reviewed. In addition, seven studies examined associations with serum uric acid, a compound which usually increases when glomerular filtration rate (GFR) decreases. In summary, there were relatively strong associations between serum PFOS/PFOA and estimated GFR as well as serum uric acid. Taking into account that some reverse causality is plausible, that there may also be confounding by variables such as age, sex, height and weight, which are used in the eGFR equations, and that no significant association were shown between PFOS/PFOA and chronic kidney disease, the CONTAM Panel considered the evidence that PFOS/PFOA exposure causes reduced GFR insufficient. The possibility of reverse causality is very relevant also for other outcomes based on serum markers, which are eliminated by renal excretion, e.g. uric acid

PFASs other than PFOS and PFOA

In summary, five studies examined associations between PFASs other than PFOS/PFOA and estimated glomerular filtration rate and/or serum uric acid. Some associations were found, but not consistent between studies. As mentioned above for PFOS/PFOA, associations with eGFR may be affected by reverse causation and the variables used to calculate eGFR.

In summary, there is insufficient evidence to conclude that exposure to PFASs decrease GFR or increase uric acid in serum.

3.3.4.8 Carcinogenicity outcomes

In the opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018) a number of studies on cancer incidence or cancer mortality at occupational or environmental exposure were reviewed. In summary those studies provided limited evidence that exposure to PFOS or PFOA are related to increased cancer risk. It was

noted that this is in line with the conclusion from the recent IARC report on PFOA (IARC, 2016), which concluded that there was limited evidence for carcinogenicity. After the PFOS/PFOA opinion, some additional studies have been published on other PFASs. In addition, one study has been published reporting only results on PFOS and PFOA

In summary, taking into consideration the review of studies provided in the previous opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018), these three additional studies provide no evidence for a link between exposure to PFASs and cancer risk.

3.3.4.9 Cardiovascular disease and mortality

PFOS and PFOA

In the opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018), five cross-sectional and four longitudinal studies examining associations with cardiovascular outcomes (mortality, coronary heart disease, stroke, hypertension and atherosclerosis) were reviewed. Altogether, these studies did not show any clear association between PFOS/PFOA and cardiovascular disease. It was noted, however, that the longitudinal studies could not demonstrate a very small increase of the relative risk.

PFASs other than PFOS and PFOA

In summary, six studies report findings on cardiovascular disease in relation to exposure to other PFASs than PFOS and PFOA. The individual studies are reviewed in Appendix J. The CONTAM Panel notes that some recent studies (Bao et al. 2017, Huang et al. 2018, and Mastrantonio et al. 2018) suggest an association between exposure to PFAS and cardiovascular disease, but considers the evidence yet insufficient to use as a basis for a health-based guidance value.

3.3.4.10 Bone mineral density

PFOS and PFOA

In the opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018), two cross-sectional US NHANES studies examining associations with bone mineral density were reviewed. There were some inverse associations between PFOS/PFOA and bone mineral density, but only in subgroups and for some sites, with limited consistency between the two studies. The magnitudes of the associations were small and may be due to residual confounding and/or reverse causation. The latter would be mediated by higher PFOS/PFOA in women with early menopause, which in turn decreases bone mineral density.

PFASs other than PFOS and PFOA

One of the US NHANES studies reviewed in the opinion on PFOS and PFOA (EFSA 2018), namely a study by Khalil et al. (2016) examined associations also with PFHxS (mean 2.5 ng/mL) and PFNA (mean 1.9 ng/mL). The study was performed in 1914 individuals aged 12–80 years, and bone mineral density was measured by DXA in spine and femur. In addition, self-reported physician-diagnosed osteoporosis was examined. In post-menopausal women, significant inverse associations were found between serum PFNA and BMD in femur and spine. The odds ratios for self-reported osteoporosis in women were significantly increased in the upper two quartiles of PFHxS and in the highest quartile of PFNA. These analyses were based on only 77 cases of self-reported physician-diagnosed osteoporosis. With the findings from this single study the evidence on an impact of PFNA or PFHxS on bone mineral density is insufficient.

3.3.5. Mode of action

In animals the most commonly reported effects are those on the liver (increased weight, hypertrophy, increased fat content) and the levels of thyroid hormones, cholesterol and triglycerides, and liver transaminases in serum. In addition, some PFASs were shown to cause liver tumours. In addition, effects

on the immune system, as well as on the development of the mammary gland, were observed in various studies, often at lower levels than those causing effects on the liver and thyroid hormones (see sections 3.3.3.3 and 3.3.3.5). Certain effects seem associated with binding of PFASs to the PPAR α receptor, but the question is to what extent this mechanism can explain the adverse effects, as will be discussed below. In a separate section the effects of PFASs on thyroid hormone levels will be discussed but it is unclear if such effects are underlying the effects on immune response, mammary gland development and liver effects. In subsequent paragraphs, possible mechanisms behind the effects on liver (including lipid metabolism), mammary gland development and the immune system will be discussed, including data from knock-out (KO) animals and *in vitro* models. In particular PPAR α KO mice but also mice expressing human PPAR α have been used to study the role of PPAR α , and potential differences between rodents and humans. It should be realized that both such models and *in vitro* models do not necessarily represent the *in vivo* situation, also due to kinetic differences. The MoA section focuses on liver effects and important endpoints for the risk assessment and is not intended to discuss all effects observed for PFASs.

3.3.5.1. Receptor mediated effects

3.3.5.1.1 PFASs transactivate PPAR α

In vivo studies

Mechanistic studies showed that many PFASs are ligands of the nuclear receptor PPAR α , as demonstrated *in vivo* and *in vitro* (see below). When activated by ligand binding, PPAR α heterodimerizes with another nuclear receptor family member, the retinoid X receptor (RXR), the receptor for 9-cis-retinoic acid. The heterodimer complex attaches to peroxisome proliferator response elements, being present in the promoter or enhancer regions of genes regulated by PPAR α , such as enzymes of the peroxisomal β -oxidation pathway but also mitochondrial enzymes, as well as transporters. PPAR α plays an important role not only in lipid metabolism but also various other metabolic processes like lipoprotein metabolism, gluconeogenesis and bile acid metabolism. The activation of this nuclear receptor also appears to be the critical key-event in the induction of liver hyperplasia in rodents, as is given in detail in 3.3.5.1.2. A previous prevailing view has been that PPAR α is less important in human than in rodent liver, but it needs to be kept in mind that PPAR α plays a prominent role in regulation of lipid metabolism also in human liver (reviewed by Kersten and Stienstra, 2017).

Analyses of rodent liver tissue samples revealed that PFBA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFDoDA, PFBS, PFHxS, PFOS and 8:2 FTOH induce peroxisomal β -oxidation or at least the expression of essential enzymes of this biochemical pathway. There are few *in vivo* studies comparing the relative potencies of PFASs to induce peroxisomal β -oxidation and/or hepatomegaly. Daily *i.p.* administrations of four PFCAs to male rats for 5 days induced hepatic peroxisomal β -oxidation in the order of PFDA > PFNA > PFOA >>> PFHpA (Kudo et al., 2000). In female rats, PFDA was also slightly more potent than PFNA, but PFOA and PFHpA were inactive. This is likely to be related to differences in toxicokinetics between male and female rats (see Section 3.3.1.1). In a subsequent study, Kudo et al. (2006) compared daily *i.p.* treatments with PFHxA, PFHpA, PFOA and PFNA for 5 days in male and female mice. All compounds induced hepatomegaly and peroxisomal β -oxidation with PFNA and PFOA being most effective, followed by PFHpA and PFHxA.

NTP (2019a,b) treated male and female rats for 28 days by gavage with different daily doses of PFBS, PFHxS, PFOS, PFHxA, PFOA, PFNA, PFDA, as well as the PPAR α agonist Wyeth (WY)-14,643. Absolute and relative liver weight were increased by all compounds in both males and females, although with clear differences in the potencies. Based on the applied dose, PFOS, PFOA, PFNA, and PFDA were the most potent in males followed by PFHxA and WY-14,643, and PFBS and PFHxA. In females, PFDA was the most potent, followed by PFOS, PFNA and WY-14,643, PFOA, PFHxS, PFBS and PFHxA. The order changed when basing it on measured serum or liver levels. In females, e.g. PFOA treatment resulted in

relatively low serum levels. Most compounds also induced the liver mRNA levels of Acox1, as well as the enzyme activity (the latter only measured in males), and mRNA levels of CYP4a1, CYP2b1 and CYP2b2. An exception was PFHxS, which did not affect mRNA levels of Acox1 in females, and only had a weak effect on CYP4a1. Similar was true for PFHxA in males and females. In general, however, when studied, most endpoints are somehow affected by all PFASs, and differences seem merely due to kinetics and relative potencies.

In vitro studies

In vitro models may be suitable tools for studying effects mediated by PPAR α , although the PPAR α -expression and related processes in cell-lines and isolated hepatocytes may differ from the *in vivo* situation (Kersten and Stienstra, 2017). A further severe limitation is the frequent lack of information on the actual uptake of PFASs by the cultured cells, which may be very low (Rosenmai et al., 2018).

A number of studies investigated the relationship between the structure of PFASs and induction of genes involved in peroxisomal β -oxidation or other steps of lipid metabolism in *in vitro* assays. Naile et al. (2012) reported on the impact of PFBA, PFPeA, PFHxA, PFOA, PFNA, PFUnDA, PFDoDA, PFBS, PFHxS, and PFOS on transcript levels of 7 genes, 5 of them involved in fatty acid and cholesterol synthesis, in rat H4IIE hepatoma cells. The PPAR α -regulated peroxisomal 3-ketoacyl-CoA thiolase was induced most strongly by PFHxS followed by PFOA, PFPeA, PFHxA and PFDoDA, but was down-regulated by PFBA, PFBS, PFOS, PFNA and PFUnDA. However, overall the full picture of effects on gene expression did not show consistent patterns related to e.g. chain length or PFCAs vs. PFASs, and lacked clear dose response relationships across the wide range of concentrations applied. In addition, the results seem not consistent with the NTP-studies with rats, showing up-regulation of Acox1 by all PFASs tested and also increased activity of acyl-CoA oxidase (although with clear differences in potency).

Björk and Wallace (2009) showed that in primary rat hepatocytes, peroxisomal Acox and Cte/Acot1 genes were induced, in the order of PFOA > PFHxA > PFOS > PFHxS > PFBA = PFBS = PFPeA, when compounds were applied at 25 μ M for 24 h. In human primary hepatocytes or human HepG2 cells, no effect on expression of these genes could be observed when compounds were applied at concentrations up to 200 μ M. Cyp4A1/11, however, was induced by some PFASs in human primary hepatocytes. The authors speculated that - unlike to rat - no peroxisome proliferator responsive element (PPRE) has been identified within the 5'-flanking region of the human Cyp4A11 gene (Kawashima et al., 2000), indicating that the regulation of this gene differs between the two species. Björk and Wallace (2009) concluded that there is (1) a chain length-dependent increase in gene expression, (2) higher transcriptional activation by PFCAs than the corresponding PFASs, (3) absence of induction of expression of peroxisome-related fatty acid oxidation genes by the compounds in human liver cells.

Wolf et al. (2012) transfected COS-1 cells (monkey fibroblast-like cell line) with mouse or human PPAR α -luciferase reporter plasmids. In the case of mouse PPAR α , all tested compounds showed a response, but C_{20max} concentrations (the concentration showing 20% of the maximal activity) varied between 5 and 317 μ M. The transcriptional activation (based on C_{20max} values) was in the order of: PFNA (5 μ M) > PFOA (6 μ M) > PFHpA (11 μ M) > PFDA (20 μ M) > PFDoDA (33 μ M) > PFHxA (38 μ M) > PFPeA (45 μ M) > PFBA (51 μ M) > PFHxS (76 μ M) > PFOS (94 μ M) > PFBS (317 μ M). In the case of human PPAR α , C_{20max} values varied between 11 and 471 μ M: PFNA (11 μ M) > PFHpA (15 μ M) > PFOA (16 μ M) > PFHxA (47 μ M) > PFPeA (52 μ M) > PFBA (75 μ M) > PFHxS (81 μ M) > PFBS (206 μ M) > PFOS (262 μ M) > PFHxA (471 μ M) > PFDA and PFDoDA (no response). Overall, these studies show that PFASs are able to activate PPAR α with little difference between the mouse and human receptor, with similar order. In addition, the chain length determines the transcriptional activation of PPAR α , and the PFASs seem less potent than the PFCAs.

Activation of PPAR α was also assessed in NIH-3T3 cells (murine fibroblast cell line) transiently transfected with mouse PPAR α or PPAR γ (Rosenmai et al., 2016). The assay investigated the dependency of PPAR activation, assessed by a reporter gene, on the chain length for PFBA, PFPA,

PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA and PFDoDA. All PFCAs activated PPAR α around 2-fold at 100 μ M. In another study, Rosenmai et al. (2018) studied the PPAR α transactivating potency by transiently transfecting human HepG2 liver cells with a human-PPAR α reporter gene plasmid. They also studied intracellular concentrations of the PFASs by LC–MS/MS. The uptake of the PFASs by HepG2 cells was generally very low (0.04–4.1%). There was a trend that the intracellular concentrations of PFCAs increased with the chain length, but this was the reverse for the 3 PFASs, PFOS showing the lowest cellular concentrations of all PFASs tested. The PPAR α transactivating potencies of PFCAs were in a similar range ($\sim$ 1.5- to $\sim$ 3.5-fold), increased with chain length up to C8 (PFOA), and then decreased. PFOS and FOSA were inactive.

To conclude: in *in vitro* studies, a tendency towards elevated transcriptional activation of rodent and human PPAR α was found with increasing chain length of PFASs up to the length of C9 and a trend to lower activity for PFCAs with chain lengths of > C9. PFASs show lower potencies, and decreasing with chain length. In addition, compound-specific effects on the regulation of peroxisomal but also other genes and enzyme activities were reported but not always consistent with *in vivo* studies. This could be explained by the variable uptake of PFASs by cells which may be relatively low and differ between individual PFASs, as well as between the cell models applied.

3.3.5.1.2 Interaction of PFASs with nuclear receptors other than PPAR α

In vivo and *in vitro* studies also addressed the question whether further nuclear receptors may be involved in the effects observed with PFASs in the liver. Rosen et al. (2008) compared the transcript profiles of the livers of WT and PPAR α KO mice treated with PFOA (1 or 3 mg/kg bw per day) or the PPAR α agonist WY 14,643 (50 mg/kg bw per day) for 7 days. It was concluded that approximately 85% of the genes altered by PFOA were dependent on PPAR α ; the PPAR α -independent genes were often involved in lipid homeostasis and xenobiotic metabolism and it was speculated that this may be due to PFOA-induced activation of PPAR γ , CAR (constitutive activated/androstane receptor) or the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2). In a follow-up study, Rosen et al. (2017) treated male mice for 7 days by oral gavage with PFNA (1 or 3 mg/kg bw per day) or PFHxS (3 or 10 mg/kg bw per day), and compared the hepatic gene expression patterns with historical data obtained from PFOS, PFOA or WY-14,643. They found that roughly 11–24% of up- or down-regulated genes in PFAS-treated animals were independent of PPAR α , and argued that this might be due to suppression of STAT5B or transactivation of the constitutive activated receptor (CAR), estrogen receptor alpha (ER α), and/or PPAR γ . In the NTP studies (NTP, 2019a,b), all PFASs tested induced mRNA levels of Cyp2b1 and Cyp2b2 in livers of male and female rats, supporting the activation of CAR. There were clear differences in potencies. Also, WY-14,643 induced these genes but it is unclear if this implies that this compound is less specific as assumed, or whether there is an indirect mechanism.

In HEK239 cells, Li et al. (2019) determined the potency of PFASs to bind to human PPAR β/δ and to activate the receptor, applying a reporter-gene assay. The binding capacity was elevated with increasing chain length until PFDoDA (C12) and then decreased again. The three PFASs tested (PFBS, PFHxS, PFOS) showed stronger binding than their carboxylic acid (PFCA) counterparts, while 6:2 FTOH and 8:2 FTOH did not interact with PPAR β/δ . The transactivating potency of PFASs showed a similar inverted U-shaped curve peaking at PFTrDA. Bjork et al. (2011) deduced from the analysis of transcriptome patterns, induced by PFOS or PFOA in rat and human primary hepatocytes, that multiple receptor systems are activated by these compounds, such as PPAR α , CAR, PXR, and LXR α . The activation of PXR by PFOS and PFDA was shown by Bijland et al. (2011) and Ma et al. (2005), respectively.

3.3.5.1.3 Thyroid hormone levels and function

There is considerable evidence from animal studies that PFASs interfere with the thyroid hormone homeostasis. Observations in human epidemiological studies do not provide sufficient support for associations between the PFASs examined and thyroid disease or changes in thyroid hormones at serum

levels observed in people exposed occupationally or via food. For thyroid hormone function the levels of the free hormones (free thyroxine (fT4) and free triiodothyronine (fT3)), are particularly important. In the NTP studies (2019a,b), most PFASs reduced serum levels of fT4 and total T4, in some cases by more than 80%. Also, total T3 levels were often decreased but only in some cases there was also a decrease in serum TSH levels. Disturbance of thyroid hormone function may not only occur at the level of the two thyroid hormone receptors (TR α and TR β), but also at the level of biogenesis, distribution and metabolism of thyroid hormones. This potentially includes disturbances at the level of the sodium iodide symporter, hemoprotein thyroperoxidase, iodinases and thyroid hormone conjugating enzymes, the T4 distributor proteins thyroxine-binding globulin (TBG), transthyretin (TTR) and deiodinases (deiodinization of T4 in target organs to the more active T3) (Köhrle, 2008). Ultimately, disturbances on either of the listed levels of biosynthesis, distribution and function may result in variation of fT4 and fT3 levels and consequently in compromised functions of organs including adverse health outcomes.

Competition between the binding of T4 and PFASs to the human variant of TTR was assessed with a radioligand-binding assay (Weiss et al., 2009). The binding potency (IC₅₀) was reported to decrease in the order: PFHxS (0.7 μ M) > PFOS/PFOA (0.9 μ M) > PFHpA (1.6 μ M) > PFOSi (1.7 μ M) > PFNA (2.7 μ M) > PFHxA (8.2 μ M) > PFDA (9.0 μ M) > PFBS (19 μ M) > PFUnDA (22 μ M) > PFTeDA (29 μ M) > PFDoDA (47 μ M). Overall, TTR binding potencies of the most potent PFASs were 12.5–50 times lower than those of T4. Some shorter (PFBS) but also longer chain PFASs (PFDA and larger) were even less potent (> 100 times less potent than T4. A comparative binding study to human TTR and TBG was performed by Ren et al. (2016). The compounds tested comprised PFBA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, PFBS, PFHxS, PFOS and FTOH (8:2). All compounds tested, except for the telomer alcohol, were identified as TTR binders, again showing highest potencies for PFHxS, PFOS, PFOA (IC₅₀s of 0.6, 0.1 and 0.4 μ M). Only two compounds bound to TBG and at a very high concentration (PFTeDA and PFTeDA, IC₅₀s of 170 and 190 μ M).

In summary, the findings of the two studies above on binding of PFASs to the thyroid hormone distributor proteins TBG and particularly TTR may contribute to understanding of potentially altered thyroid hormone levels in relation to exposure to PFASs. However, there is some debate on the importance of TTR for humans since thyroid hormones also bind to TBG.

Ren et al. (2015) also investigated the binding affinities of 15 PFASs to the ligand binding domain of TR α . All PFASs tested, except PFBA and FTOH (8:2), could be identified as binders. However, most PFASs were more than 100-fold less potent than T3 (IC₅₀ = 0.3 μ M), with the exception of six compounds, with the following rank order of potency (IC₅₀): PFDA (6 μ M) > PFOS (16 μ M) > PFODA ~ PFTeDA ~ PFUnDA (18 - 20 μ M) > PFHxDA (24 μ M). In conclusion, these results show that PFASs are able to interfere with thyroid hormone function on the level of thyroid hormone receptors, with PFDA showing the most potent activity.

A study by Long et al. (2013) investigated the effect of PFASs on proliferation control of rat pituitary GH3 cells in the T-screen assay. T3 was used as a positive control. At 10⁻⁸ M, T3 induced a 12-fold increase in proliferation in comparison to solvent control. All PFASs significantly decreased GH3 cell proliferation, being dose-dependent for PFOS, PFHxS, PFNA, PFDA, and PFUnDA. PFASs also decreased T3 induced proliferation of GH3 cells. The other PFASs reduced growth only at higher doses. No inhibitory effect could be detected for PFOA. These results provide further evidence that PFASs are capable to interfere with thyroid hormone function at the level of the control of proliferation of pituitary cells.

The studies by NTP (2019a,b) showed that all seven tested PFASs induced transcription of Acox1, Cyp4a1, Cyp2b1 and 2b2, showing that they are activators of PPAR α and CAR. This may also result in an increase of glucuronosyltransferases involved in conjugation of thyroid hormones. Yu et al. (2009) showed increased transcription of hepatic UGT1A1 in SD rats treated with PFOS.

At this stage, it appears that the effects on thyroid hormone levels occur at relatively high doses, i.e. higher than dose resulting in effects on antibody response or mammary gland development.

3.3.5.2 Liver toxicity

3.3.5.2.1 Liver hypertrophy and hyperplasia

Hepatotoxic effects of PFASs are indicated by steatosis, lipid peroxidation, occurrence of necrotic hepatocytes and elevated liver transaminases in serum. The elevated relative liver weight in PFAS-treated rodents appears to be a combination of net gain of liver cells (hyperplasia) and increased volume of hepatocytes (hypertrophy) due to proliferation of peroxisomes, endoplasmic reticulum and steatosis. Most mechanistic studies on the induction of hepatic hypertrophy and hyperplasia used rodents, which were treated with clofibrate, ciprofibrate, fenofibrate, WY-14,643 or diethylhexylphthalate (DEHP) (Corton et al, 2017). These well-studied PPAR α -ligands are often referred to as peroxisome proliferators (PP). Such PPs induce an initial and transient wave of DNA replication in rodent liver, followed by a plateau of an elevated number of cells (hyperplasia), as long as the treatment with the ligand is continued. This hyperplasia is frequently associated with increases in cell size (hypertrophy) due to proliferation of peroxisomes and of the smooth endoplasmic reticulum, which occurs preferentially in hepatocytes close to the central vein in the liver lobule. However, hyperplasia due to steatosis does not occur in livers of rodents treated with PPs. Upon withdrawal of the PPAR α ligand, the liver quickly returns to its original mass and eliminates the excess of cells via apoptosis. Retreatment with PPAR α ligands rapidly stops the apoptotic activity in the liver (Bursch et al., 1984; James et al., 1998). Thus, the PPAR α -induced hyperplasia of rodent liver results from both an increased transient wave of cell replication and a concomitant suppression of cell elimination by apoptosis.

Considering the PFAS-induced hepatomegaly in more detail, Wolf et al. (2008) reported a dose-dependent increase in hepatocellular proliferation in PFOA-treated WT mice, which was associated with increased liver weight and hepatocellular hypertrophy. Signs of a mild hepatocellular steatosis were documented in livers from PFOA treated animals by light microscopy. Das et al. (2017) studied WT mice treated with 10 mg/kg bw per day of PFNA, PFOA or PFHxS. PFOA induced the largest increase in relative liver weight, followed by PFNA and PFHxS. The DNA content, given as $\mu\text{g}/\text{mg}$ liver tissue was decreased by all compounds, indicating considerable hepatocellular hypertrophy. However, considering the 2- to 3-fold elevated absolute liver weight in the PFAS-treated animals, there was an overall net gain in hepatic DNA content, indicating a net gain in liver cells, and thus liver hypertrophy - similar to observations with the PP WY-14,643. However, contrary to the latter PP, steatosis was evident in the PFAS-treated WT mice. Das et al. (2017) documented an about 2-to 3-fold elevated content of hepatic triglyceride content in the PFAS-treated WT mice, which may contribute considerably to the hepatocellular hypertrophy observed.

To understand the relevance of PP-induced hepatomegaly for humans, primates have been investigated. Cynomolgus monkeys were treated with the PP di-ethyl-hexylphthalate (DEHP) or clofibrate for 2 weeks, which did not elicit any effect on liver weight or replicative DNA synthesis in the organ (Pugh et al., 2000). A 15-months treatment with DEHP elevated liver weight in mice and rats but not in marmosets (Ito et al., 2007a). When marmosets received DEHP or clofibrate over a period of 13 weeks, again no increase in liver mass and hypertrophy of hepatocytes was found (Kurata et al., 1998). Hoivik et al. (2004) applied fenofibrate or ciprofibrate to cynomolgus monkeys for up to 15 days. Both compounds caused slight hepatocellular hypertrophy but no cell proliferation as determined by the number of mitoses and Ki-67 positive nuclei. To conclude, these data show convincingly that PPAR α agonists fail to induce cell replication and hyperplasia in liver of monkeys. Occasionally there are reports on hypertrophy of hepatocytes in the monkey liver.

Primary cultures of hepatocytes from rats and humans were used to study species differences in the response to PPs. Mono-ethyl-hexylphthalate (MEHP), the active metabolite of the DEHP, caused dose-dependent induction of DNA synthesis in rat but not in human hepatocytes (Hasmall et al., 1999, 2000).

Similar reports exist for monoisononyl phthalate (MINP), the primary and active metabolite of the PP diisononyl phthalate (DINP) (Shaw et al., 2002). Goll et al. (1999) compared effects of various PPs, including DEHP and some fibrates, in primary cultures of rat and human hepatocytes. An increase in DNA synthesis was reported for ciprofibrate, nafenopin, bezafibrate, clofibrate and DEHP in rat hepatocytes only. Ciprofibrate, nafenopin, fomesafen, 2-ethylhexanoic acid and methylclofenapate caused induction of DNA synthesis in primary rat hepatocytes, but none did so in human hepatocytes (Doull et al., 1998). To conclude, these *in vivo* and *in vitro* studies provide strong evidence that monkey and human liver cells are insensitive to PP-induced growth effects.

Several mouse models served to study the molecular mechanisms underlying the different response of human and rodent hepatocytes towards activated PPAR α . Lee et al. (1995) knocked out the ligand binding domain of murine PPAR α and introduced a truncated gene, being expressed in all cells of mice. When treated with typical PPs (clofibrate, WY-14,643), homozygous KO mice did not show the typical PP-mediated pleiotropic response in the liver, such as increases in peroxisomes, liver cell proliferation and hepatomegaly. Wolf et al. (2008) addressed the question whether PPAR α also mediates the induction of liver growth by PFOA. WT (CD-1 and Sv/129) and KO mice (Sv/129 background) were treated by daily gavage with the ammonium salt of PFOA (1, 3, or 10 mg/kg bw per day) for 1 week. In WT mice, PFOA induced a dose-dependent increase in hepatocyte hypertrophy, vacuoles, peroxisome proliferation and a dose-dependent increase in PCNA-positive nuclei, the latter reaching significance only at the highest dose. KO mice also showed a dose-dependent increase in hepatocyte vacuolation, but these were much larger than in WT mice. There was also an increased number of PCNA-positive nuclei, as in WT being significant at the highest dose only, and at that dose higher than the increase seen in WT animals treated with the highest PFOA dose or WY-14,643 (positive control). The authors stated that the elevated cell replication in the PFOA-treated KO mice was probably due to the physical accumulation of material, which may be interpreted as a regenerative and not as hyperplastic growth reaction of the liver. This assumption is based on the fact that hepatocytes were full of large electron-lucent, non-membrane-bound spaces throughout the hepatocellular cytoplasm. These "deposits" may have contributed also to the increased relative liver weight in the KO mice. Since Das et al. (2017) reported elevated hepatic lipid content in PFOA-treated KO mice, the CONTAM Panel assumed these deposits to be lipid droplets.

Das et al. (2017) used PPAR α KO mice exposed to 10 mg/kg bw per day of PFNA, PFOA or PFHxS for 7 days (see also above). In the KO animals, PFOA induced the largest increase in relative liver weight, followed by PFNA and PFHxS. The hepatic triglyceride content was elevated considerably in the untreated KO mice and was elevated even further in the PFAS-treated KO mice. Similar to the WT mice, the DNA content per mg liver tissue was decreased being indicative for hypertrophy. Considering the increased liver weight, there was also a net gain in liver cells in the KO mice, based on the increase of total DNA per liver, especially for PFOA and PFNA; this increase in total DNA content per liver was in the range of the DNA gain seen in the WT animals. The authors did not provide an explanation for this finding. However, in the study of Minata et al. (2010) PFOA was applied to KO mice for a longer period (4 weeks). At this time point, not only steatosis but also severe cholangiopathy, enhanced blood levels of biliary acids, hepatocellular damage and apoptotic cells (especially in bile ducts) became evident. An increase in serum bile acid levels was also observed in the NTP studies with rats (NTP 2019a,b) exposed to PFBS, PFOS, PFHxA, PFOA, PFNA and PFDA, but also WY-14,643, pointing at cholestasis. The concomitant development of hepatic fibrosis by proliferating fibroblasts and the infiltration of immune cells might have started to develop already after one week of PFOA treatment leading to the elevated total hepatic DNA content, observed by Das et al. (2017). The study of Minata et al. (2010) substantiates also the assumption that the elevated DNA replication, reported by Wolf et al. (2008) after a 7 days' PFOA treatment of KO animals, may be interpreted as regenerative DNA replication following severe hepatocellular and bile epithelial damage, especially in the highest dose group.

Das et al. (2017) also investigated the mechanism of PFAS-induced steatosis by microarray analyses of hepatic gene expression patterns of WT and KO mice treated with PFOA, PFOS, PFNA, PFHxS or WY-

14,643. In WT mice the PFASs and WY-14,643 induced genes involved in fatty acid uptake into hepatocytes as well as the synthesis of fatty acids, triglycerides and cholesterol. In KO animals almost none of the gene expression changes induced by WY in WT were evident, while the expression levels of some fatty acid and TG synthesis genes were retained or even elevated by PFAS treatment. This indicates that some genes involved in triglyceride and cholesterol synthesis may be regulated independent of PPAR α , explaining partly the pronounced steatosis seen in KO animals. These findings show that PFASs induce hepatocellular steatosis in rodents in a PPAR α -independent way, which may increase the volume of the hepatocytes (hypertrophy) and contribute to the elevated liver weight.

In order to create a more physiological model, Cheung et al. (2004) used KO mice and introduced the human PPAR α under the control of a liver-specific promoter for stable hepatic expression. Both the WT and humanized mice responded to treatment with the PP Wy-14,643 as proven by upregulated genes of peroxisomal β -oxidation. However, WT mice but not the humanized animals, showed hepatocellular proliferation. The relative liver weight was only slightly increased in the humanized mice probably due to induction of peroxisomal β -oxidation and the concomitant peroxisomal proliferation, contributing to the development of a hepatocellular hypertrophy. Yang et al. (2008) replaced the mouse PPAR α gene by a construct designated hPPAR α PAC containing only the human PPAR α . The hPPAR α PAC mice expressed the construct in the same tissues as WT animals. By a 2-weeks treatment with WY-14,643, hepatomegaly was induced in the transgenics but to a considerably lower extent than in WT mice. Likewise, cell replication was not induced in the humanized mice when exposed to WY-14,643, indicating that the hepatomegaly may be due to hypertrophic hepatocytes.

With regard to PFASs, Nakagawa et al. (2012) used the mouse model, developed by Cheung et al. (2004), and observed an increased absolute and relative liver weight in WT, KO and humanized mice, when treated with PFOA doses at 1 or 5 mg/kg bw per day for 6 weeks. The hepatomegaly appeared to be mainly due to enhanced intrahepatic storage of triglycerides, which was highest in the KO mice, followed by humanized mice, but in WT mice much less and only at the intermediate dose. At lower doses and shorter treatment (0.1 or 0.3 mg/kg bw per day, 2 weeks), Nakamura et al. (2009) observed increased liver weight and hepatic triglyceride levels only in WT mice.

In humanized PPAR α mouse models, hPPAR α is expressed in cells made up exclusively of mouse proteins, factors and (co-)regulators. It was argued that this might suppress the human phenotype. This objection was refuted by a study of Tateno et al. (2015). Liver of SCID mice were repopulated, thereby creating chimeric organs consisting of > 70% human hepatocytes. Treatment with the PP fenofibrate induced hepatocellular hypertrophy, cell proliferation and peroxisome proliferation exclusively in the murine but not in the human hepatocytes. It should be stressed that fenofibrate is a weaker PP than WY-14,643, which induced some proliferation in KO mice (Yang et al., 2008).

It should be noted that in the chimeric livers, used by Tateno et al. (2015), human hepatocytes were surrounded by murine mesenchymal liver cells, which might have disturbed epithelial-mesenchymal interactions in this organ. To address this question, and to gain a profound understanding of the MoA of PFASs, the following studies should be considered. Some groups proposed that non-parenchymal Kupffer cells, macrophages located in the liver sinusoids, are critical for growth induction by PPAR α ligands. When being activated, Kupffer cells start to produce and release cytokines, like tumor necrosis factor- α (TNF α) (Bojes et al., 1997; Rolfe et al., 1997). TNF α is able to induce replication and to suppress apoptosis in cultured rodent hepatocytes (Holden et al., 2000; Rolfe et al., 1997). Also *in vivo*, hepatocyte proliferation was antagonized by pretreating animals with antibodies blocking either TNF α (Bojes et al., 1997; Rolfe et al., 1997) or the TNF α receptor (West et al., 1999). TNF α is an important activator of NF- κ B. NF- κ B coordinates not only adaptive and innate immune responses, but is also involved in the regulation of apoptosis. In dependence of the context, NF- κ B triggers either pro- or anti-apoptotic pathways and is involved in the decision whether a cell may survive or die. Several PPAR α activators were found to enhance the secretion of TNF α and to activate NF- κ B in liver cells (Rose et al.,

5390 2000; Calfee-Mason et al., 2004). On the other hand, in purified hepatocyte cultures without Kupffer
5391 cells, PPAR α ligands failed to induce DNA replication (Hasmall et al., 2000; Parzefall et al., 2001).

5392 Several groups reported on elevated expression of cyclins and/or the proto-oncogene c-myc in rat or
5393 murine liver when treated with PPAR α ligands (Ma et al., 1997; Jolly et al. 2005; Urbanek-Olejnik et al.,
5394 2016). Shah et al. (2007) described molecular mechanisms underlying the differences in c-myc
5395 regulation by human and murine PPAR α . The microRNA let-7c destabilizes the mRNA of c-myc. In WT
5396 mice, induction of hepatocellular proliferation by activated PPAR α caused down-regulation of the let-7c,
5397 which elevated the expression of c-myc and enhanced hepatocellular proliferation. In contrast, PPAR α -
5398 humanized mice did not enhance c-myc expression via down-regulation of let-7c.

5399 A further interesting aspect is that human livers express the protein of a truncated form of PPAR α at
5400 relatively high levels (Thomas et al., 2015). The truncated PPAR α is unable to bind to peroxisome
5401 proliferator-responsive elements (PPRE) in the DNA, and acts as dominant negative regulator of the full-
5402 length PPAR α and functions as an endogenous inhibitor of proliferative genes. The absence of the
5403 truncated form in mice may contribute to species-specific differences in PP induced liver growth.

5404 These findings provide evidence that PPAR α -mediated pathways controlling peroxisomal β -oxidation are
5405 independent from those controlling the hepatocellular proliferation pathways. Structural differences
5406 between human and mouse PPAR α exist, leading to the differential susceptibility towards the PP-induced
5407 hyperplastic liver growth. PFASs appear to induce liver hyperplasia via PPAR α , similar to PPs, assuming
5408 that the observed increase in PCNA content and total DNA in KO mice is due to other processes following
5409 severe liver damage by PFOA treatment.

5410 *3.3.5.2.1 Hepatocellular cytotoxicity, steatosis and increased liver transaminases in serum*

5411 Several *in vitro* studies investigated the impact of chain length of PFASs on the cytotoxicity of the
5412 compounds in cells of hepatocellular origin. Buhrke et al. (2013) reported a positive correlation between
5413 chain length and cytotoxicity in human HepG2 cells, i.e. the toxicity (IC₅₀) was as follows: PFDoDA (7
5414 μ M) > PFDA (15 μ M) > PFNA (23 μ M) > PFOA (47 μ M) > PFHpA (128 μ M) > PFHxA (344 μ M) > PFBA
5415 (1000 μ M). There was no evidence for the induction of apoptosis by any of the compounds tested. Rand
5416 et al. (2014) studied the relative cytotoxicity of PFCAs in human liver epithelial (THLE-2) cells. The EC₅₀
5417 values ranged from 65 $\pm$ 41 μ M (PFDA) to 1361 $\pm$ 146 μ M (PFBA). Toxicity decreased with shorter chain
5418 length, as follows: PFDA > PFNA > PFOA > PFHpA > PFHxA > PFPeA > PFBA.

5419 To conclude, there is some association between the chain length of PFASs and cytotoxicity, which may
5420 be partly due to the higher cell membrane permeability of long-chain PFASs.

5421 For many PFASs, hepatotoxic effects were documented in *in vivo* experiments. In the studies performed
5422 by NTP (2019a,b), increased ALT levels were observed in male rats at dose levels of 500 mg/kg bw per
5423 day for PFBS, 0.625 mg/kg bw per day for PFOS, 500 mg/kg bw per day for PFHxA, 0.625 mg/kg bw
5424 per day for PFOA, 2.5 mg/kg bw per day for PFNA and 0.312 mg/kg bw per day for PFDA. In female
5425 rats, this occurred at dose levels of 250 mg/kg bw per day for PFBS, 2.5 mg/kg bw per day for PFOS,
5426 500 mg/kg bw per day for PFHxA, 50 mg/kg bw per day for PFOA, 3.12 mg/kg bw per day for PFNA
5427 and 1.25 mg/kg bw per day for PFDA. Also treatment with WY-14,643 resulted in increased ALT levels,
5428 but PFHxS showed no effects. PFBS and PFHxA showed the effects at much higher doses. Increases in
5429 serum levels of ALT and/or AST were also documented in previous studies in which rodents were treated
5430 with PFOS (1 mg/kg bw per day Han et al., 2018a,b), PFHxA (20 mg/kg bw per day, Loveless et al.,
5431 2009), PFNA (1 mg/kg bw per day, Fang et al., 2015), PFUnDA (1 mg/kg bw per day, Takahashi et al.,
5432 2014), or PFODA (1000 mg/kg bw per day, Hirata-Koizumi et al., 2012). Hepatocellular necrosis was
5433 induced by PFBA (175 mg/kg bw per day, Foreman et al., 2009), PFDoDA (2.5 mg/kg bw per day, Kato
5434 et al., 2015b), PFHxS (3 mg/kg bw per day, Chang et al., 2018), or 8:2 FTOH (100 mg/kg bw per day,
5435 Iwase et al., 2006).

The reasons for the increased serum transaminases, which indicate membrane damage of hepatocytes, and the occurrence of necrotic hepatocytes in the histopathological evaluations, are not completely understood. It has been assumed repeatedly that this damage may be related to the profound alterations in the hepatocellular lipid metabolism of PFAS-treated rodents. Via activation of PPAR α in rodents, PFASs induce the β -oxidation cycle of fatty acids in the hepatic peroxisomes, which degrades the long-chain fatty acids to acyl-CoA-moieties for use in other metabolic pathways. Despite an increased peroxisomal degradation of long-chain fatty acids, the hepatic lipid content appears to be elevated by PFASs (EFSA CONTAM Panel, 2018). This is due to accumulation of triglycerides, as described for PFOA (5 mg/kg bw per day, Hui et al., 2017; Wu et al., 2018), PFOS (0.09 mg/kg bw per day, Huck et al., 2018), PFNA (0.2 mg/kg bw per day, Wang et al., 2015), PFDA (1.5 mg/g bw per day, Kawashima et al., 1995), and PFDoDA (0.2 mg/kg bw per day, Zhang et al., 2013d). Also enhanced storage of cholesterol may account for this effect, as observed after application of PFNA (0.2 mg/kg bw per day, Wang et al., 2015) or PFDoDA (10 mg/kg bw per day, Zhang et al., 2013). Steatosis does not occur in livers of rodents treated with typical PPs and appears to be very specific for PFASs. Mechanistic studies indicate a disbalance between (i) the reduced export of lipids from hepatocytes and expression/activity of lipid catabolism genes and (ii) increased expression/activity of genes involved in lipid uptake by hepatocytes and in fatty acid, triglyceride and cholesterol biosynthesis. The development of steatosis is independent of a PPAR α -mediated mechanism and is even very pronounced in KO mice, as described in the subchapter above.

Intrahepatic lipid overload is a symptom for severe metabolic disturbances and predisposes the hepatocytes to undergo necrosis. Necrotic cells alarm the immune system and may elicit a pro-inflammatory response in the liver. Indeed, elevated intrahepatic levels of pro-inflammatory cytokines, like IL1 β , IL10 or TNF α , were observed after treatment with PFNA (Fang et al., 2012a). It has also been hypothesized that the increased peroxisomal β -oxidation of long-chain fatty acids leads to enhanced production of hydrogen peroxide and subsequent lipid peroxidation. Eriksen et al. (2010) did not find evidence that PFNA (0–2000 μ M) induces ROS production or oxidative stress in HepG2 cells. However, elevated hepatic levels of malondialdehyde (MDA)/ thiobarbituric acid reactive substances (TBARS) were seen after *in vivo* treatment of rodents with PFOS (1 mg/kg bw per day; Han et al., 2018a), PFOA (5 mg/kg bw per day; Wang et al., 2017c), or PFNA (5 mg/kg bw per day; Fang et al., 2012b). Also, PFDoDA was found to cause intrahepatic lipid peroxidation when applied at 0.5 mg/kg bw per day (Liu et al., 2016) and to induce SOD activity at 1 mg/kg bw per day (Zhang et al., 2008).

To conclude, like PPs, PFASs seem to cause liver hyperplasia via murine PPAR α , considering no clear hyperplastic liver growth in the KO and humanized mouse models. PFASs induce steatosis in rodent hepatocytes in a PPAR α -independent way. The accumulated lipids increase the hepatocellular volume and contribute to the elevated weight of the liver due to hypertrophy. The hepatocellular steatosis seems to be causally related to the occurrence of necrotic hepatocytes and the increased serum transaminase levels. These various effects in the rodent liver appear to be common for most of the PFASs, investigated so far. Thorough knowledge of the MoA underlying the development of hepatocellular steatosis in PFAS-treated rodents is missing, as outlined also in EFSA (2018).

3.3.5.4 Immunotoxicity

A clear mode of action of immunotoxicity by PFOS and PFOA has not been established. Just anecdotal data are available that may offer some leads for further investigation, but do as yet not offer a clear mechanism along which PFASs influence the immune system. Information on immunotoxicity by PFASs other than PFOS and PFOA and whether such effects on the immune system might be brought about by similar mechanisms as PFOS and PFOA is even more limited.

In some studies on PFOS and PFOA, effects on the immune system were shown at doses where also effects on body weight were observed and it might be argued that effects on the immune system may have been secondary to general toxicity. However, other studies showed effects at concentrations where

no effects on body weight were observed (see section 3.3.3.5). The pivotal studies showing effects on the antibody response to immunization with sheep red blood cells (Peden-Adams et al., 2008; Dong et al., 2009), showed effects at dose levels below those that induced effects on body weights. As PFASs have been shown to affect thyroid hormone levels (see section 3.3.5.6), Fair et al. (2011) investigated whether this could explain the effects observed by Peden-Adams et al. (2008) and found effects on the immune system at doses lower than where effects on thyroid hormone levels occurred. Dong et al. (2009) also measured corticosterone levels in serum and did not find changes that might have been responsible for the suppressed antibody response.

As reviewed in the previous opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018), one component of a mechanism along which immunotoxic effects by these chemicals could be brought about, is by impacting NF- κ B regulated gene transactivation and affecting gene expression of apoptotic regulators, resulting in apoptosis of lymphoid cells, and consequently suppressed proliferation and activation of T-cell receptor signalling and depressed immune functionality.

An association between immune parameters, PPAR δ mRNA expression and PFASs has been observed in cord blood, in addition to mRNA expression of several immunomodulatory genes (Pennings et al., 2016). Qazi et al. (2009b), comparing WT and PPAR α KO mice, reported effects of PFOS on the thymus and the spleen that were partly dependent on PPAR α . These findings would be in line with the notion that different PPARs have been described to interact with both the innate, as well as the acquired immune system (Daynes and Jones, 2002), and that PPAR agonists in fact induce anti-inflammatory activity (Le Menne et al., 2018; Wang et al., 2018). This is in keeping with suppressed LPS-dependent release of TNF α in human peripheral blood leukocytes by PFOS (Brieger et al. (2011) and IL-18 by PFOA in THO-1 cells (Corsini et al., 2011). In addition, also immune effects independent of PPAR α have been shown, *in vitro* by PPAR silencing (Corsini et al., 2011) and *in vivo* by using PPAR α KO animals (DeWitt et al., 2009, 2016). Hence a clear picture on the involvement of PPARs is lacking, and further research on this topic is necessary.

There may be a common MoA behind the immunotoxic effects of PFASs. However, there may also be differences in the mechanisms behind some of the immunotoxic effects observed with different PFASs. For instance, cytokine profiles in lymphoid cells have shown to be differentially affected by PFOS and PFOA (Corsini et al., 2011; Midgett et al., 2015). It should be noted, however, that individual interleukin values, influenced by PFASs, cannot easily be interpreted in terms of how they play a role in the suppressive effect on antibody production by PFOS and PFOA.

At least for PFOS, other mechanisms, that may or may not be related to those mentioned above, have been put forward as well:

- Zarei et al. (2017) investigated the cytotoxic activity of PFOS on human lymphocytes after *in vitro* incubation. The authors noted increased ROS production, lipid peroxidation, glutathione depletion, activation of caspase-3, as well as damage to cell organelles such as mitochondria and lysosomes.
- Suo et al. (2017) showed that PFOS increased IL-22 excretion by lamina propria lymphocytes isolated from the large intestines of wild-type mice. This effect was not observed using F1 mice from an AhRf/f and a RORc-cre cross to specifically delete the Ah receptor and it was concluded that this response must be dependent on the Ah receptor.
- Lee et al. (2018), using rat basophilic leukemia cells *in vitro*, showed that PFOS significantly increased histamine release by IgE stimulated cells, which coincided with enhanced gene expression of proinflammatory cytokines and activation of nuclear NF- κ B. However, even if IgE dependent allergic responses are immune-related, it is not clear if there is a relation to immune suppression such as evidenced by depressed antibody responses.

5530 As indicated above, information on immunotoxicity by other PFASs than PFOS and PFOA is even more
5531 limited.

5532 Fang et al. (2008, 2009) studied effects of oral exposure to PFNA in both mice and rats, and RT-PCR of
5533 thymus cells revealed increased gene expression of PPAR α and PPAR γ . However, PFNA did not seem to
5534 influence genes encoding for the NF- κ B signalling pathway.

5535 In an *in vitro* study performed by Zhou et al. (2017), the human gastric cell line AGS was exposed to
5536 PFDA, and increased IL-13 and IL-18 expression, both at the protein level and the mRNA level, were
5537 observed. Also NLRP3 and NF- κ B were increased, indicating enhanced inflammasome activity. Increased
5538 IL-13 and IL-18 production were also observed in the stomach of mice, exposed to PFDA in drinking
5539 water, accompanied by disorganized alignment of cells and increased inflammatory cell infiltration.

5540 Both these studies by Fang et al. (2008, 2009) and by Zhou et al. (2017) do therefore indicate that
5541 components of the immune system are affected by PFNA and PFDA. Whether such effects of PDFA
5542 would have an impact on the functionality of the immune system, including antibody production, has
5543 not been studied.

5544 In a study on PFHpA, PFNA, PFDA, and PFUnDA, Lee and Kim (2018) compared effects of the chain
5545 length of PFASs in an *in vivo* system of anaphylaxis and an *in vitro* assay using IgE stimulated mast
5546 cells. The longer chain compounds PFDA and PFUnDA increased the release of histamine and
5547 proinflammatory cytokines and intracellular calcium concentrations. RBL cells, transfected with a NF-
5548 κ B-luciferase reporter construct, showed enhanced luciferase activity when treated with PFDA and
5549 PFUnDA, but not the other compounds. *In vivo*, PFNA, PFDA and PFUnDA aggravated allergic reactions
5550 in an ovalbumin model, such as an aggravation in the decrease of rectum temperature after injection
5551 of ovalbumin. These results indicate that the chain length is a determinant in effects on allergic
5552 processes. Again, these data support the notion that components of the immune system are influenced
5553 by these compounds. However, even if allergic responses are immune-related, it is not clear whether
5554 these effects may lead to suppressed antibody responses to antigens other than the allergen ovalbumin,
5555 and if so would follow similar mechanisms as such potential immunosuppressive effects, if they would
5556 be present.

5557 In summary, the studies that were performed to shed light on possible mechanisms, support the notion
5558 that PFASs influence the immune system. Immune effects of PFOS and PFOA have been mostly studied,
5559 but their mode of action has not been fully identified. NF- κ B regulated gene transactivation seems
5560 involved. There are interactions of PPARs with the immune system, but it is not clear in how far these
5561 receptors may be involved in immune effects by PFASs.

5562 3.3.5.5 Impaired mammary gland development

5563 The mammary gland develops through three major stages into a fully lactating mammary gland. In the
5564 embryonic stage, distinct placodes are formed from the ectoderm-derived mammary line that descend
5565 into the mesenchyme and form the rudimentary ductal structure present at birth. This occurs under the
5566 control of epithelial/mesenchymal interactions. During later major developmental stages during puberty
5567 (ductal elongation), and in reproduction (alveolar growth in pregnancy and lactation, and tissue
5568 remodelling during involution at weaning), the development is regulated by both hormones and
5569 epithelial/mesenchymal interactions (Macias and Hinck, 2012). The transition stages of the mammary
5570 gland development are regarded as highly sensitive windows of susceptibility in both humans and
5571 rodents, with the terminal end buds and ductal growth as the most fragile structures in the rodent
5572 (Russo and Russo, 1996; Martinson et al., 2013).

5573 The *in vivo* studies on mammary gland development in mice (described in section 3.3.3.3.2) indicate
5574 that all the developmental stages can be affected by PFOA. However, the cross-fostering study with
5575 PFOA implies that postnatal exposure is sufficient to decrease mammary gland development lasting

beyond sexual maturity in mice (White et al., 2009). Of note, the MoA is not necessarily similar at the different developmental stages. There are no studies on other PFASs than PFOA for this effect.

After PFOA dosing of C57Bl/6 mice, starting in peri-puberty, both inhibitory but also stimulatory effects on mammary gland development have been reported (see 3.3.3.3.2), whereas in other mouse strains only inhibitory effects have been reported. Prenatal and/or early postnatal exposure affected mammary gland development at lower levels than exposure later in life.

Only few studies on effects of PFASs on mammary cells *in vitro* were identified. When grown on reconstituted extracellular matrix, the cell line MCF-10A can be used as a model for studying ER-independent breast tissue development, as the cells form acini, which are highly-organized epithelial structures that share some aspects of glandular breast tissue architecture. Acini development was dose-relatedly disturbed, starting at 0.6 μ M (lowest concentration tested) of PFOS, PFNA (both from day 5) and PFDA (from day 3), and completely absent at 60 μ M on day 10 (Halsne et al., 2016). Acini development was disturbed to a much lesser extent with PFOA and PFUnDA, and started to occur at least at a 10-fold higher concentration and at later time points. When grown in monolayer, only at much higher concentrations (100 to 500 μ M for 72 h) than those causing effects on acini, some effects on cell cycle were observed with PFOS, PFNA, or PFDA, whereas PFOA and PFUnDA had no effect. It is however doubtful that this model, which according to Qu et al. (2015) "exhibits a unique differentiated phenotype in 3D culture which may not exist or be rare in normal human breast tissue" can mimic early-life breast gland differentiation. Similar doubt applies to results from studies on PFOS or PFOA in MCF-10A cells in monolayer (Pierozan et al., 2018 a,b).

The involvement of PPAR α in mammary gland development is of interest since PFOA can activate this receptor. PPAR α mRNA is expressed in luminal epithelial cells and stromal adipocytes in the mouse mammary gland, and the level of PPAR α mRNA decreases during pregnancy and lactation (Gimble et al., 1998). The mammary gland develops normally in PPAR α KO mice. However, PPAR α activation influences mammary lobuloalveolar development in pregnancy. Transgenic mice expressing constitutively active PPAR α in basal epithelial cells (and not, as is normal, in luminal epithelial cells) in the mammary gland during pregnancy had defects in lactation, resulting in mortality of new-borns (Yang et al., 2006a). This was due to impaired development of lobuloalveoli, and reduced proliferation together with increased apoptosis in mammary epithelia, which were accompanied by reduction in β -catenin expression and the target gene Cyclin D1. Treatment of pregnant WT mice with synthetic PPAR α ligand (WY-14,643) also led to impaired development of lobuloalveoli, an effect not observed in similarly treated PPAR α KO mice (Yang et al., 2006b).

The stimulating effect (increased growth) in the mammary gland, observed in C57BL/6 mice after 5 mg/kg bw PFOA exposure five days per week during puberty, as reported by Yang et al. (2009), occurred also in PPAR α KO mice on a C57BL/6 background, and was thus independent of PPAR α (Zhao et al., 2010). In contrast, the decrease in mammary gland development at higher PFOA dose (7.5 mg/kg bw per day, 5 days per week) during puberty was not seen in PPAR α KO mice (Zhao et al., 2012). However, since the serum PFOA concentration in PPAR α C57BL/6 KO mice was approximately only 50% of that in WT at both 5 and 7.5 mg/kg bw per day, the dependency on PPAR α expression for the observed effects could not be fully elucidated in the study.

An important mechanistic issue to understand is whether effects induced by PFOA on mammary gland development occur without general effects on pubertal transition. In CD-1 mice treated during gestation, it appears that puberty is not affected, since Tucker et al. (2015) reported no impact of PFOA treatment on vaginal opening. However, when mice were treated during puberty at higher levels that resulted in decreased mammary gland development, Zhao et al. (2012) reported later vaginal opening in Balb/c and absent vaginal opening in C57Bl/6 mice, and there was absence of oestrus cycle in both strains.

Zhao et al. (2012) observed in two mice strains treated with PFOA during puberty (Balb/c with 2.5 mg/kg bw per day and C57Bl/6 with 7.5 mg/kg bw per day, 5 days per week), a decrease of the number

of terminal end buds, a decrease in number of stimulated terminal ducts and a reduced ductal length (see 3.3.3.3.2). In the ovaries, reduced levels of proteins important for steroidogenesis (StAR, CYP11A1, HSD3 β 1 and HSD17 β 1) were reported, whereas levels of aromatase were not affected. In the mammary gland, the important growth factors HGF and AREG were down-regulated, whereas IGF-1 levels were unaffected. In line with this, levels of the proliferation marker PCNA were significantly reduced in mammary glands. The effects of PFOA could partially, but not entirely, be prevented by co-treatment with estradiol or progesterone, or combinations thereof. The effects of PFOA on mammary gland development in animals dosed during puberty thus seem to involve interference with ovarian steroid production, and expression of growth factors in the mammary gland.

In summary, the MoA behind the most sensitive PFOA effect, which is a decrease in mammary gland development in animals dosed during gestation and neonatally, is unknown. Normal mammary gland development does not require PPAR α expression, but PPAR α activation in pregnancy can reduce mammary gland development in the dam.

3.4. Critical Effects, dose-response assessment and derivation of a health-based guidance value

3.4.1 Critical effects

The potential effects of PFASs have been studied extensively, both in laboratory animals and in humans. In animals the effects that were most often reported were increased liver weight and effects on thyroid hormones. In addition, effects on the immune system and mammary gland development were observed in animals at lower levels than those showing effects on liver and thyroid hormone levels.

For several PFASs, exposure of particularly mice resulted in a decrease in the T-cell dependent antibody response. In the two most informative studies on PFOS (Peden-Adams et al., 2008; Dong et al., 2009), applying multiple dose levels, the lowest NOAEC serum level of 17.8 ng/mL was noted for Peden-Adams et al 2008 (see section 3.3.3.5)). Effects were also observed with other PFASs and also in rats, but the studies currently available do not allow a formal comparison of potential differences in potencies (see section 3.3.3.5, Table 20).

In addition, various effects have been observed in developmental studies with rodents. The effect shown at the lowest exposure is that of PFOA on the development of the mammary glands in mice, observed in several studies with different strains. These effects concerned an impaired development of the mammary glands of animals exposed in utero and/or during lactation, and effect on the further development when exposed during peri-puberty (Macon et al., 2011; White et al., 2011; Tucker et al., 2015), but also a decreased involution of the glands in the dams after the lactation period (White et al., 2007). In particular the impaired development was associated with relatively low serum levels in three different studies (LOAECs around 20 ng/mL on PND 22 corresponding to a maternal LOAEC of around 66 ng/mL, no NOAECs). Effects on mammary gland development have thus far not been studied for other PFASs.

In human studies, various associations between serum levels and a number of outcomes have been reported. In the previous opinion (EFSA CONTAM Panel, 2018), four endpoints were selected as potential critical effects for PFOS and/or PFOA. These were (i) increased serum total and LDL cholesterol (risk factor for cardiovascular disease), (ii) increased ALT levels (indicating effects on liver cells), (iii) reduced birth weight, and (iv) effects on the immune system as shown by decreased antibody response to vaccines.

In the previous opinion (EFSA CONTAM Panel, 2018), the CONTAM Panel used the effects on serum cholesterol levels to derive TWIs for both PFOS and PFOA. Those TWIs were also protecting towards the other potential critical endpoints. Although the association with increased cholesterol was observed in a large number of studies, the CONTAM Panel now considers the uncertainty regarding causality

larger. This is primarily due to a postulated biological process around the enterohepatic cycling of both PFASs and bile acids, the latter affecting serum cholesterol levels.

As already explained in the previous opinion on PFOA and PFOS (EFSA CONTAM Panel, 2018), the association with reduced birth weight might at least partly be explained by changes in the physiology during pregnancy, although a recent study seemed to strengthen the causality of the effect (Meng et al., 2018; see also section 3.3.4.1.1). The remaining decrease in birth weight after adjusting for confounders was not large and the adversity of such a decrease might be questioned. Thus far, there is little evidence for an increase in the proportion of children with low birth weight (<2500 g).

The consistent increase in ALT levels in general population studies seems supported by observed effects in animal studies, but was not observed in most of the occupational studies with much higher exposure (EFSA CONTAM Panel, 2018). In the critical study (Gallo et al., 2012), the increase in subjects with high ALT levelled off at relatively low serum concentrations (about 30 ng/mL of PFOS and PFOA) and above that, it did not increase further. This is in contrast to rodent studies showing increase in ALT only at the high end of the dose-response curve. This inconsistency creates some uncertainty that should be addressed in future studies. For these reasons, this endpoint was not considered as the critical effect.

Reduction in thyroid hormone levels is often observed in animal studies. Epidemiological studies provide insufficient support for associations between exposure to PFASs and changes in thyroid hormone levels or thyroid function.

Considering the selection of the most critical effect, those on the immune system were observed at the lowest serum levels in both animals and humans. The findings were considered robust since they were consistently observed for several PFASs and for several species. This is not the case for effects on mammary gland development, which are observed at similar low serum levels in mice but have not been studied in other animal models or humans. Therefore, the CONTAM Panel decided to base the present assessment on PFASs on effects on the immune system.

A decrease in vaccination response is seen as adverse by the scientific community, as summarised by WHO/IPCS (2012) in the Guidance for immunotoxicity risk assessment for chemicals. This may in particular apply to vulnerable population groups, i.e. infants and the elderly, considering their higher infection risk.

For compounds that accumulate in the body, the preference of the CONTAM Panel is to identify serum or tissue levels associated with adverse effects. Based on observations on animals and humans, the CONTAM Panel decided to combine its assessment on the serum levels for the sum of four PFASs, i.e. PFOA, PFNA, PFHxS and PFOS. At present, these are the PFASs that contribute most to the levels observed in human serum (see section 3.3.2 on biomonitoring). Although some other PFASs like PFBA and PFHxA also contribute significantly to the exposure (see section on current exposure), these compounds show much shorter half-lives in humans and thus much higher exposure is required to obtain similar serum levels as the 4 PFASs selected. As will be discussed below, the available data are insufficient to derive potency factors for the different PFASs, although such differences in potency are likely to exist.

A study on children in the Faroe Islands (Grandjean et al., 2012) showed several inverse associations between serum levels of PFOA, PFNA, PFHxS and PFOS, as well as the sum of PFOA, PFHxS and PFOS at five years of age, before booster vaccination, and antibody titres against diphtheria and tetanus at both the age of 5, shortly after booster vaccination, and at 7.5 years. For this study, additional data on the sum of PFOA, PFNA, PFHxS and PFOS were obtained (see Appendix L). The CONTAM Panel identified a NOAEC serum level at the age of 5 years for the sum of PFOA, PFNA, PFHxS and PFOS of 27.0 ng/mL, based on decreased antibody titres for diphtheria at the age of 7 years.

This study was supported by a more recent study from Germany. In this latter study, an inverse association was observed between serum levels of PFOA (Abraham et al., 2020), but also the sum of PFOA, PFNA, PFOS and PFHxS (Appendix K), and antibody titres against haemophilus influenzae type b (Hib), diphtheria and tetanus in serum sampled from 1-year-old children predominantly breastfed for a median duration of 7.4 months (see section 3.3.4.4.2). A NOAEC of 31.9 ng/mL at the age of 1 year was derived for the sum of PFOA, PFNA, PFOS, and PFHxS based on association with reduction in antibody titres against Hib (See Appendix K). For PFOS, PFHxS and PFNA alone, no significant associations were observed in this study. The association with reduced tetanus antibody titres was also significant, whereas the association between the sum of 4 PFASs and diphtheria was only borderline significant.

The possible confounding by a number of contaminants like PCBs, dioxins, organochlorine pesticides, lead and mercury was examined in these studies but these had no effect on the observed associations.

A substantial decrease in antibody response after vaccination was reported, with inverse association for several antigens in several independent cohorts, but not all (see Section 3.3.4.4.2). In the German study, at the LOAEC the antibody titres for Hib had already decreased by more than 50%. In the study from the Faroe Islands, at the LOAEC antibody titres for diphtheria were lower by around 50%. In that study, the proportion of children having vaccination titres below the protective limit after vaccination was also increased at higher PFAS levels. Such decreases in antibody responses are clearly adverse on a population level, not only in terms of protection against the pathogen to which the vaccine is directed, but also in terms of general immunologic defence against other pathogens. Indeed, there are some data suggesting that PFAS exposure is associated with increased infection risk, and also with decreased specific antibody formation after virus exposure in infants.

The study by Abraham et al. indicates that children are sensitive in their first year, and this was also suggested by Grandjean et al. (2017), comparing measured and predicted serum levels of PFASs during the first 18 months with measured antibody titres against diphtheria and tetanus at 5 years of age before applying a booster. However, the observed associations between serum levels at 5 years of age and antibody titres at 7 years (Grandjean et al., 2012) imply that the decreased antibody response also occurs in older children. Although performed with a small group and thus not suitable for deriving a critical serum level, the study by Kielsen et al. (2016) with 12 adults shows that the decrease in vaccination response may also occur at much older age and low serum levels of PFASs (median levels for PFOA, PFHxS and PFOS of, respectively, 1.7, 0.7, 0.4 and 9.5 ng/mL). This is supported by the results from mice studies that were performed with adult animals (see section 3.3.3.5). One study performed with treatment of pregnant dams did not show higher sensitivity of the offspring

3.4.2 Mixture Approach

In the previous opinion (EFSA CONTAM Panel, 2018) it was decided to derive separate TWIs for PFOS and PFOA, "since both toxicity as well as underlying modes of toxic action for PFOS and PFOA are not sufficiently understood and might differ, but also overlap." Since that Opinion, EFSA launched a new Guidance document on how to evaluate the effects of mixtures (EFSA Scientific Committee, 2019) and it was considered that similarities in chemical properties and effects warrant a mixture approach for a number of PFASs. The CONTAM Panel decided to focus on four PFASs (PFOA, PFNA, PFHxS and PFOS) but acknowledges that more data are needed on other PFASs in the future. In humans, these four PFASs share toxicokinetic properties and show similar accumulation and long half-lives. Furthermore, these four PFASs show the highest concentrations in blood plasma and serum (See section 3.3.2). Also in terms of effects, these compounds in general show the same effects when studied in animals. This is exemplified by the NTP studies (NTP, 2019a,b), which studied a number of effects on organs and tissues, but e.g. also serum levels of hormones, bile acids, cholesterol, triglycerides and the expression of a several genes in the liver, like Cyp4a1, Cyp2b1, Cyp2b2, and Acox1. Overall, most of the seven PFASs showed an effect on these parameters, confirming that in general these compounds cause similar effects

(see Table 21). Differences in potencies were also observed and these could partly be explained by differences in kinetics. There are also sex-related differences in kinetics, in particular in rats, but these are not observed in humans.

An important question is whether these four PFASs show similar potencies for the critical effects. As shown in section 3.3.3.5, effects of PFOS and PFOA on vaccination response have been studied in animals as well as more general effects of the immune system for some other PFASs (in addition to PFOS and PFOA, also PFNA and PFDA). However, there are no comparative studies that provide reliable insight in the relative potencies.

The study by Abraham et al. (2020) showed that only for PFOA there was a significant association with antibody titres against three different vaccines. For PFOS, PFHxS and PFNA this was not observed. The association was, however, also significant for the sum of the four PFASs, based on additional information from the authors (see Appendix K). Grandjean et al. (2012) showed significant associations for PFOS, PFOA and the sum of PFOS, PFOA and PFHxS. Furthermore, the decrease in titres was also significant for the sum of four PFASs (PFOA, PFNA, PFHxS and PFOS), based on additional data provided by the authors (see Appendix L). Although in a later study by Grandjean et al. (2017), focussing on serum levels at young age and antibody titres at 5 years before the booster in two cohorts, PFOA showed stonger associations than PFOS, these were also observed for PFOS. Granum et al. (2013) reported an inverse association between maternal PFASs and rubella titres at age 3 years for all four PFASs, and similar conclusions were reached when each compound was mutually adjusted for the other. In summary all three studies showed positive associations for PFOA. Since PFOA and PFOS serum concentrations are higher as compared to serum concentrations of PFNA and PFHxS, and PFOA highly correlates with the serum levels of the other PFASs, it is uncertain whether PFOA has a higher potency for this critical endpoint as compared to the other PFASs and thus drives the association. Furthermore, the CONTAM Panel noted that a potential higher potency of PFOA seems to contradict the outcome of various studies with animals, which thus far showed effects at much lower serum levels for PFOS than for PFOA, keeping in mind that a thorough comparison was never made. The latter also applies to effects on mammary glands in mice, which were only studied for PFOA.

Another issue is whether to base relative potencies on applied dose or e.g. serum levels (internal dose). The NTP studies (NTP 2019a,b) reported effects on absolute liver weight, and also serum levels at the last day of treatment. This allows comparison of dose-response curves based on external dose but also on internal dose, i.e. the serum level. This comparison reveals large differences between the two approaches, due to kinetic differences. This adds additional uncertainty to deriving potency factors for different PFASs.

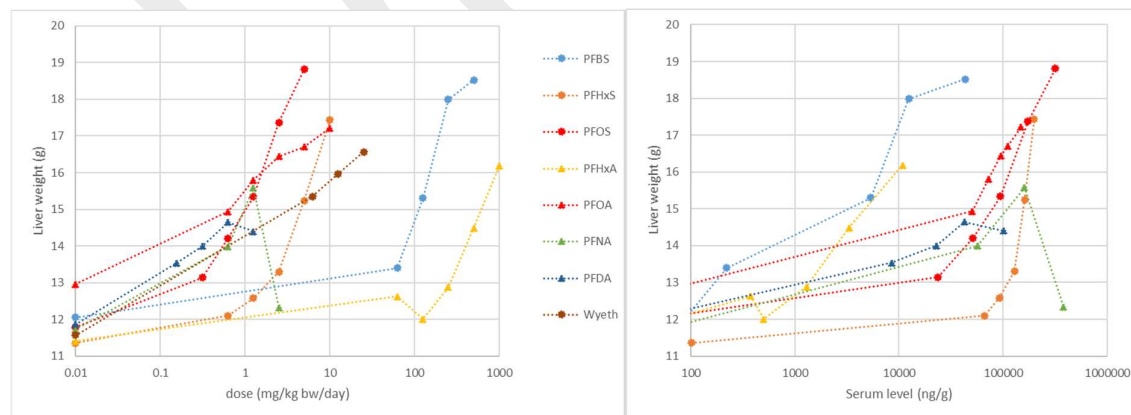


Figure 11: Absolute liver weights in male rats exposed to various PFASs, based on applied dose (left) or serum levels (right). For WY-14,643 serum levels were not determined. There is a clear shift in the order of the different PFASs; compare e.g. PFBS (blue dots) and PFOS (red dots).

Despite many studies, the mode of action behind various adverse effects is still unclear. This applies to effects on the liver but certainly on the immune system and mammary gland development. It has been shown that some PFASs act on the PPAR α receptor and it was hypothesized that activation of this receptor is an important event behind some of the observed adverse effects. However, PFASs show partly different effects than classical agonists, also in PPAR α knock-out mice. Therefore, the MoA is still unclear, even for effects on the liver, like steatosis. Due to the lack of a clear MoA, results of *in vitro* studies can help to improve our understanding but cannot be used to derive potency factors at this stage. Also the differences in kinetics between species, sex and PFASs makes this rather complicated. The absence of a clear MoA is not a reason for not applying a mixture approach.

As a pragmatic approach, the CONTAM Panel decided to restrict the mixture approach to the four most abundant PFASs in human serum and in the absence of more specific information, to assume equal potencies by default for these four PFASs on immune outcomes. Although scientifically less correct, this was done on a weight base rather than a molar base, to allow easier comparison with the exposure assessment.

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5816 Table 21: Effects of PFASs in male and female rats (NTP, 2019a, b). Figures express the lowest/highest ratio compared to the controls observed
 5817 for either one of the dose levels. In some cases most animals died and these dose groups were not included.

PFAS	Liver weight		Serum total T4		Serum free T4		Serum T3		Serum triglycerides		Serum cholesterol		Serum bile salts/acids		Gene expression liver					
	(g)		(µg/dL)		(ng/dL)		(ng/dL)		(mg/dL)		(mg/dL)		(µmol/mL)		Acox1		Cyp4a1		Cyp2b1	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
PFBS	1.5	1.3	0.03	0.3	0.1	0.2	0.4	0.6	0.3	---	0.2	0.8	1.9	5.3	4.8	3.1	24	4.5	63	510
PFHxS	1.5	1.2	0.3	0.7	0.2	0.6	0.6	---	0.6	---	0.7	---	---	---	2.8	---	16	1.3	27	38
PFOS	1.6	1.5	0.1	0.1	0.1	0.3	0.6	0.6	0.2	0.7	0.2	0.7	3.4	3.7	5.3	3.0	30	2.4	349	802
PFHxA	1.4	1.4	0.4	---	0.3	---	0.7	---	---	---	0.8	---	2.2	2.6	2.0	1.6	12	2.5	6	68
PFOA	1.3	1.6	0.03	0.5	0.1	0.7	0.6	---	0.6	1.6	0.6	1.2	4.5	---	5.7	3.9	30	7.6	18	71
PFNA	1.3	1.3	0.1	0.6	0.1	0.5	---	---	0.5	---	0.7	---	16.9	2.8	5.9	5.2	22	13	6.8	14
PFDA	1.2	1.3	0.7	---	0.1	0.3	---	2.1	0.5	---	0.6	0.7	13.1	7.6	9.1	5.4	60	17	10	89
Wyeth	1.4	1.6	0.8	---	0.6	1.5	---	1.3	---	---	0.8	0.9	7.1	---	6.5	5.6	43	7.1	3.7	33

5818 Dose levels (mg/kg bw per day; males/females): PFBS (62.5-1000/62.6-1000), PFHxS (0.625-10/3.12-50), PFOS (0.312-5/0.312-5), PFHxA (62.6-1000/62.6-1000), PFOA (0.625-10/6.25-100), PFNA
 5819 (0.625-10/1.56-25), PFDA (0.156-2.5/0.156-2.5), Wyeth 14,346 (6.25-25/6.25-25). Blue colour indicates significantly higher than controls, brown colour significantly lower.

5820 Serum TSH levels decreased in males treated with PFOA, PFNA, and Wyeth 14,643, but increased in females treated with PFOA and Wyeth 14,643.

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3.4.3 Dose-response assessment

3.4.3.1 Effects on the immune system

In the previous opinion (EFSA CONTAM Panel, 2018), BMD analysis was performed on the PFOS data in 5-year-old children from the Faroe Islands, resulting in a BMD₀₅ and BMDL₀₅ of, respectively, 11.6 and 10.5 ng/mL. However, the modelling approach was criticized during the expert meeting (EFSA/CONTAM/3503), including the use of the antibody titre in the lowest decile as the reference value rather than extrapolate and evaluate the BMR for a serum PFOS concentration of zero. Data for PFOA were not modelled, since the levels were much lower than those for PFOS, and there were no indications that PFOA was more potent than PFOS. Modelling of the data from both the Faroe Islands and Germany with the BMD modelling programmes normally applied by EFSA (PROAST and BMDs) resulted in wide BMDL-BMDU intervals, as a consequence of extrapolating to zero exposure (well below the lowest observed serum levels). Therefore, NOAECs were derived in the present opinion, based on the distribution of the participants into quintiles. The issue with the BMD-modelling also applied to the two animal studies (Peden-Adams et al., 2008; Dong et al., 2009) showing effects for PFOS at rather low doses and serum levels, but also detectable serum levels of PFOS in the control groups.

For the study from the Faroe Islands, a NOAEC was derived for the sum of the four PFASs (PFOA, PFNA, PFHxS and PFOS) of 27.0 ng/mL in serum of 5-year-old children (serum level in 4th quintile, see Appendix L). For the study from Germany, a NOAEC of 31.9 ng/mL was obtained for 1-year-old children (3rd quintile, see Appendix K.). Since PFAS serum levels in breastfed children are in general higher at 1 years of age than at 5 years (see simulations below), this NOAEC of 31.9 ng/mL corresponds to a lower intake by the child and thus the mother in her life up to pregnancy. Therefore, this NOAEC was used to estimate the daily intake by mothers that would result in this critical serum concentration at 1 year of age in breastfed children. This daily intake was subsequently used to derive a maternal reference point for the sum of PFOA, PFNA, PFHxS and PFOS.

In the previous Opinion (EFSA CONTAM Panel, 2018), a PBPK model was used to translate the critical serum levels into a daily intake. This model was originally developed for adults, but was adjusted to estimate the serum levels also in growing children and also including the lactation period. Data from human biomonitoring studies on milk versus plasma levels, were used to estimate the levels in human milk corresponding to a certain serum level in the mother. In addition, the prenatal exposure and resulting body burden of the new-born was estimated based on levels in cord blood and mother's blood. This modelling was previously performed for PFOS only, but in the current Opinion this model was extended to PFOA, based on available data (see Appendix M for a description of the PBPK modelling). It has been shown that during breastfeeding, a substantial part of the PFASs in the mother is transferred to the infant, and as a result, serum levels in the mother but also milk levels decrease over the lactation period. This decline was also included in the model. Data available for PFNA and PFHxS were insufficient, but it was assumed that these compounds behave like PFOA and PFOS, respectively.

In the case of the German study, the serum level of 31.9 ng/mL was the sum of levels of PFOA, PFNA, PFHxS and PFOS of 15.1, 0.5, 2.1 and 14.2 ng/mL, respectively, or 15.6 ng/mL for the PFCAs and 16.3 ng/mL for the PFASs. Using the model, and assuming 12 months of breastfeeding, it was estimated that for PFOA/PFNA this corresponds to an intake by the mother of 0.33 ng/kg bw per day and for PFHxS/PFOS of 0.83 ng/kg bw per day, or combined 1.16 ng/kg bw per day for the sum of the four PFASs. Such intakes would result in serum levels in the mother at 35 years of age of 3.5 ng/mL for PFOA/PFNA and 9.1 ng/mL PFHxS/PFOS, or combined 12.6 ng/mL. This serum level would result in initial milk levels of 0.11 and 0.14 ng/mL for PFOA/PFNA and PFHxS/PFOS, respectively, based on the applied milk to serum ratios of 0.03 and 0.015. The CONTAM Panel decided to apply a breastfeeding duration of 12 months, also based on the WHO recommendations to breastfeed exclusively for 6 months with continued breastfeeding along with appropriate complementary foods up to two years of age or beyond.

Figures 12 and 13 show the serum levels in women exposed in utero, via breastfeeding for 12 months and subsequently regular food consumption, the latter resulting in an intake of 0.33 ng/kg bw per day of PFOA or 0.83 ng/kg bw per day of PFOS. The model is developed for PFOA and PFOS, but these are assumed to be representing also PFNA and PFHxS, respectively. The major purpose is to show the serum level around child-bearing age. The graphs do not include the effect of having a child and of subsequent breastfeeding, which results in a (temporary) decline in the serum levels of the mothers.

Figures 14 and 15 show the serum levels in a child during the first 10 years of age, born to the mothers described above, and being exposed in utero, then breastfed for 12 months, followed by 9 years exposure via food, for either PFOA or PFOS. Exposure during the age 1 to 10 years was assumed to be double that of the mothers, to compensate for the higher food per kg bw intake and resulting higher exposure (see section 3.2.1). This resulted in serum levels at the age of 5 years of 4.2 and 7.8 ng/mL for PFOA and PFOS, respectively, or 12.0 ng/mL for the sum. This is much lower than the NOAEC of 27.0 ng/mL observed in the study from the Faroe Islands, showing that the NOAEC from the German study corresponds to a lower intake by the mothers.

The CONTAM Panel decided to use the daily intake of 1.16 ng/kg bw per day as the starting point for the derivation of a HBGV for the sum of PFOA, PFNA, PFHxS and PFOS.

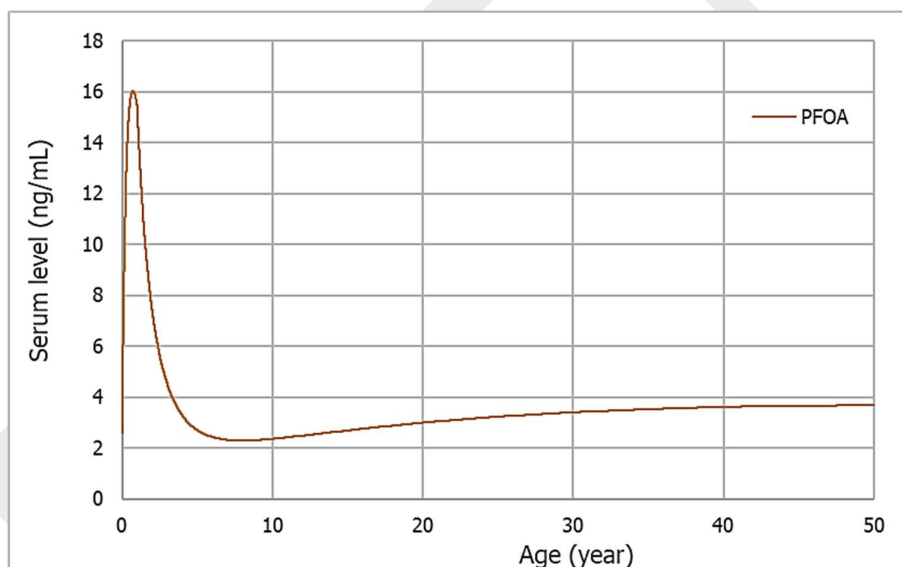


Figure 12: Serum levels in a woman exposed in utero, via breastfeeding for 12 months and subsequently via food intake for 49 years to 0.33 ng/kg bw per day of PFOA. The level peaks at 9 months of breastfeeding around 16 ng/mL, decreases and then increases to a steady state level around 3.7 ng/mL.

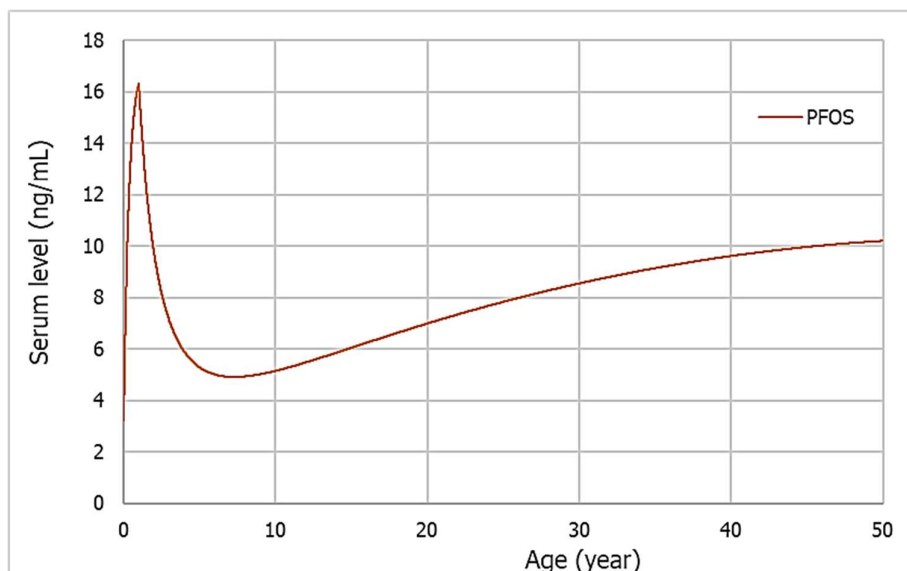


Figure 13: Serum levels in a woman exposed in utero, via breastfeeding for 12 months and subsequently via food intake for 49 years to 0.83 ng/kg bw per day of PFOS. The level peaks at the end of breastfeeding around 16.2 ng/mL, decreases and then increases to a steady state level around 10.2 ng/mL.

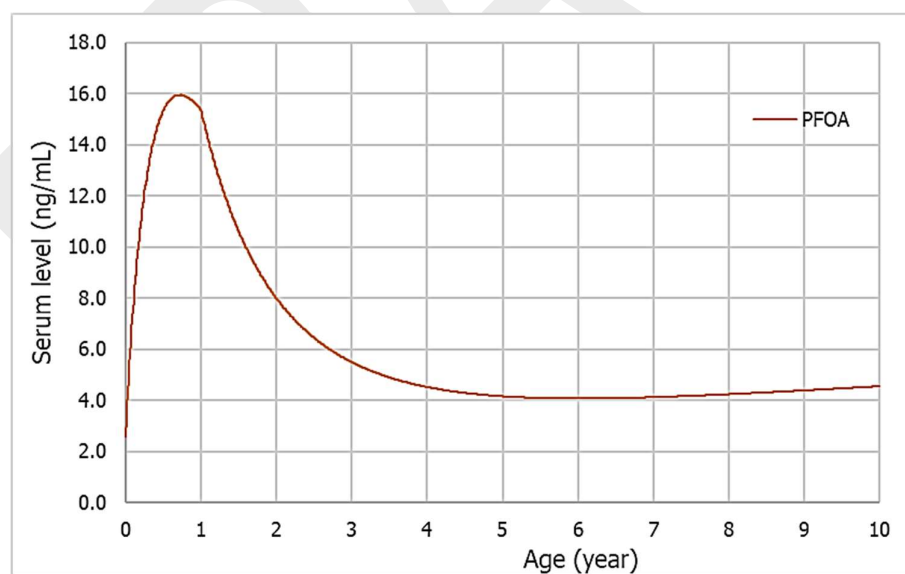
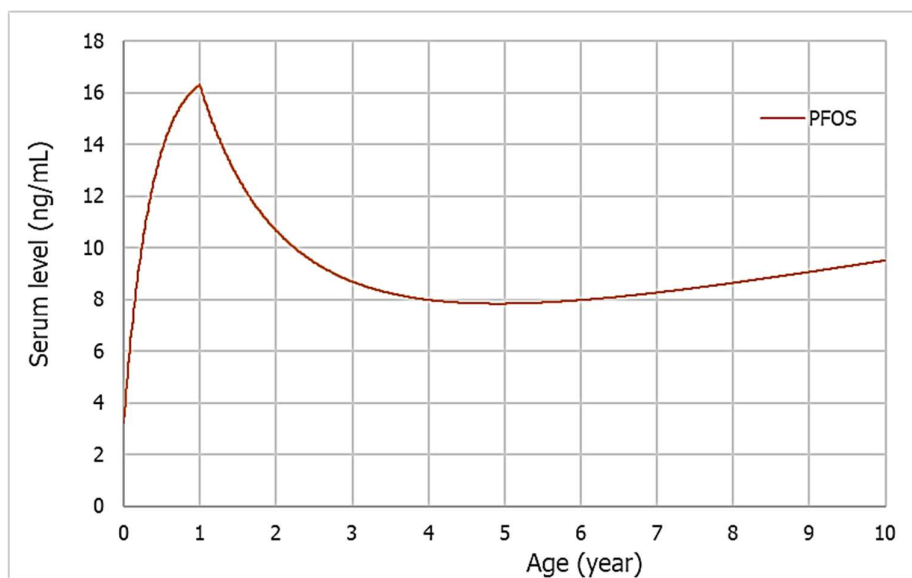


Figure 14: Serum levels in a child exposed in utero, via breastfeeding for 12 months and subsequently via food intake for 9 years to 0.66 ng/kg bw per day of PFOA. The level peaks at 9 months before the end of breastfeeding around 16 ng/mL, decreases and then reaches levels of 4.2 and 4.6 ng/mL at, respectively, 5 and 10 years of age.

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5904 **Figure 15:** Serum levels in a child exposed in utero, via breastfeeding for 12 months and
 5905 subsequently via food intake for 9 years to 1.66 ng/kg bw per day of PFOS. The level peaks at
 5906 the end of breastfeeding around 16.2 ng/mL, decreases and then reaches levels of 7.8 and 9.5
 5907 ng/mL at, respectively, 5 and 10 years of age.

5908 In animal studies, the lowest NOAEC observed for immune effects was 18 ng/mL for mice treated with
 5909 PFOS (see Section 3.3.3.5.1). Using this critical serum level means that the UF of 4 for interspecies
 5910 toxicokinetic differences should not be applied and only a total UF of 25 is required to consider possible
 5911 inter- and intraspecies differences. That would result in a critical serum level of 0.72 ng/mL (18/25).
 5912 This level is 44-fold lower than the NOAEC of 31.9 ng/mL observed in the German study. This critical
 5913 serum level would correspond to a much lower daily intake than the one estimated from the human
 5914 studies. It should be noted that the age of the mice in these studies corresponds more to an adult
 5915 situation in humans and at this age serum levels will be 3-fold lower at the TWI intake (see Figures 12
 5916 and 13). This comparison of the results from animal and human studies suggests that the application
 5917 of the various uncertainty factors is too conservative and supports the use of the human data to derive
 5918 a HBGV.

5919 3.4.3.2 Mammary gland effects

5920 The CONTAM Panel considered also the mammary gland effects as potentially adverse for humans.
 5921 Three different studies imply that such effects are associated with serum levels in pups around 20
 5922 ng/mL, as measured at PND 21. It should be stressed that the critical window for this effect is unclear
 5923 and that serum levels in pups vary during lactation. Therefore, it may be more appropriate to use the
 5924 critical serum level in the dam, which in the three-generation study was around 66 ng/mL (see section
 5925 3.3.3.3.2 on mammary gland development). This is a LOAEC and a UF of 3 was applied to determine a
 5926 NOAEC of 20 ng/mL in the dam. Using the critical serum level means that the UF of 4 for interspecies
 5927 differences in toxicokinetic differences should not be applied and only a UF of 25 is required to consider
 5928 possible inter- and intraspecies differences. This would result in a critical serum level in the mother of
 5929 $20/25 = 0.8$ ng/mL. This level is 16-fold lower than the serum level of 12.9 ng/mL in mothers around
 5930 the age of 35 years, corresponding to the NOAEC of 31.9 ng/mL in 1-year-old children (see above). This

5931 shows that basing the assessment on the effects on mammary gland development would result in a
5932 much lower HBGV.

5933 Based on the uncertainties on whether these effects on mammary gland development may occur in
5934 humans and the high uncertainties in extrapolation between species, the CONTAM Panel decided to use
5935 the effects on vaccination response in humans as the critical endpoint. Nevertheless, this developmental
5936 effect is of concern and more studies should be performed to study this effect in humans.

5937 **3.4.4 Derivation of a Health Based Guidance Value**

5938 The effects on the immune system were consistently observed in both animals and humans and support
5939 that the immune effects from PFAS exposure in humans are causally related. The critical serum level
5940 derived from animal studies would correspond to a lower HBGV than if is based on human studies, when
5941 taking standard UFs into consideration. The CONTAM Panel decided to derive a Health Based Guidance
5942 Value (HBGV) based on immune effects in humans. Two studies showed a dose-response and from the
5943 most sensitive one the NOAEC was used to derive a reference point, being 1.16 ng/kg bw per day for
5944 the sum of the 4 PFASs (PFHxS, PFOS, PFOA and PFNA).

5945 The CONTAM Panel decided that no additional uncertainty factors need to be applied, because the
5946 NOAEC is based on infants which are expected to be a sensitive population group, as is true for many
5947 immunotoxic chemicals. The CONTAM Panel also took into account that the NOAEC is based on risk
5948 factors for disease rather than disease.

5949 The CONTAM Panel established a group tolerable weekly intake (TWI) of $7 \times 1.16 = 8$ ng/kg bw per
5950 week, in order to take into account the long half-lives of these PFASs.

5951 This TWI should prevent that mothers reach a body burden that results in levels in milk that would lead
5952 to serum levels in the infant that are associated with effects on the immune system. In doing this, the
5953 higher exposure of breastfed infants is already taken into account in the derivation of the TWI, and this
5954 TWI is not applicable for infants.

5955 The CONTAM Panel noticed that this TWI is protective for the other potential critical endpoints (increase
5956 in serum cholesterol, reduced birth weight and high serum levels of ALT) considered in the previous
5957 opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018). This is based on the fact that the serum levels
5958 in adults exposed at the TWI, stay below the combined BMDLs that were derived in the previous Opinion
5959 on PFOS and PFOA.

5960 **3.5. Risk characterisation**

5961 Based on the occurrence data obtained from the Member States, the exposure to individual PFASs was
5962 estimated for different age groups across different food consumption surveys. Since it was decided to
5963 limit the risk assessment to PFOA, PFNA, PFHxS and PFOS, also the combined exposure to these four
5964 PFASs was estimated. Table 22 shows the exposure, but on a weekly basis to allow a better comparison
5965 with the TWI of 8 ng/kg bw per week.

5966 For infants, mean LB exposure varied between 17 and 85 ng/kg bw per week, as compared to P95
5967 exposure between 32 and 195 ng/kg bw per week. UB exposure for these age groups was much higher,
5968 for the mean varying between 299 and 802 ng/kg bw per week, with P95 exposure between 649 and
5969 1574 ng/kg bw per week. As mentioned above, the exposure of infants during lactation was taken into
5970 account in the derivation of the TWI. For this reason, the risk for the breastfed infant should be
5971 evaluated by assessing the long-term exceedance of the TWI by the mothers. To exemplify this,
5972 according to the PBPK model, the TWI corresponds to a milk level of 0.24 ng/mL for the sum of four
5973 PFASs. With an intake of 800 mL human milk and an average body weight of 6.1 kg during the first
5974 year, this results in an exposure of 31 ng/kg bw per day or 220 ng per kg bw per week (this intake will
5975 actually start at a higher point and decrease over time due to increasing body weight and decreasing

milk levels). Although the estimated intake for infants in Table 22 is from food, a direct comparison with the TWI is therefore not appropriate. This exposure of breastfed infants corresponding to the TWI could be a better starting point. In addition, the CONTAM Panel noted that the high exposure from food is primarily due to a high intake of PFNA and associated with high uncertainty, since, based on only one measured level in an important food category (see section 3.2.1).

For toddlers and other children, mean LB exposure varied between 6 and 46 ng/kg bw per week, as compared to P95 exposure between 19 and 96 ng/kg bw per week. UB exposure was much higher, for the mean varying between 270 and 785 ng/kg bw per week, with P95 UB exposure between 553 and 1603 ng/kg bw per week. The issue of the high PFNA contribution also applied for toddlers, but not for "Other children" (3-10 years). It is difficult to evaluate the impact of the exceedance of the TWI by toddlers and other children. As shown in section 3.4.3, a two-fold higher intake than the TWI by children did not result in serum levels higher than the NOAEC derived from the Faroe Islands. It seems possible that some of the children have an exposure that could result in adverse effects on the immune response.

The group of adolescents, adults, elderly and very elderly showed in general lower exposure, due to the lower intake of food per kg bw. Mean LB exposure varied between 3 and 22 ng/kg bw per week, as compared to P95 exposure between 9 and 70 ng/kg bw per week. UB exposure for these age groups was again higher, for the mean varying between 81 and 290 ng/kg bw per week, with P95 exposure between 153 and 294 ng/kg bw per week. The highest mean LB exposure for adolescents and adults, exceeds the TWI by a factor of 1.5. At the highest LB P95 exposure this is a factor 4.5. At the UB exposure the exceedance is much larger.

Table 22: Summary statistics of the mean and 95th percentile for the combined LB and UB chronic dietary exposure to the sum of PFOA, PFOS, PFHxS and PFNA extended to a week for each age group across surveys.

Age group	Range of mean dietary exposure (LB-UB) (ng/kg bw per week)					
	Mean LB dietary exposure			Mean UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
Infants	17	34	85	299	500	802
Toddlers	10	21	46	428	519	785
Other children	6	11	21	270	373	572
Adolescents	3	6	11	144	185	290
Adults	4	6	9	95	112	154
Elderly	5	6	15	81	105	131
Very elderly	3	6	22	88	108	139
Age group	Range of 95th percentile dietary exposure (LB-UB) (ng/kg bw per week)					
	95th percentile LB dietary exposure			95th percentile UB dietary exposure		
	Minimum	Median	Maximum	Minimum	Median	Maximum
Infants	32	96	195	649	856	1574
Toddlers	23	53	96	705	938	1603
Children	19	29	68	553	763	1157
Adolescents	9	15	37	309	399	626
Adults	9	16	35	184	229	439
Elderly	12	17	39	161	201	327
Very elderly	9	16	70	153	198	294

Values in shaded grey cells are higher than the TWI

The modelled serum level in the mothers at 35 years, corresponding to the TWI is 12.6 ng/mL (see dose-response modelling). As shown in Tables 16 and 17 (see section 3.3.2), biomonitoring data showed median serum levels of PFOA, PFNA, PFHxS and PFOS of, respectively, 1.9, 0.6, 0.7 and 7.7 ng/mL or combined 10.9 ng/mL. This level is close to the level corresponding to the TWI and suggests that a considerable part of the adult population exceeds this level. The same conclusion could be derived from the estimated dietary intake. On the other hand, it also indicates that the LB exposure is a more accurate

6006 prediction of the exposure than the UB estimates which would lead to a much higher exceedance of the
6007 critical serum levels.

6008 The CONTAM Panel decided to focus on 4 PFASs (PFOA, PFNA, PFHxS and PFOS). Based on the exposure
6009 assessment, these four PFASs contribute about half to the sum of the 17 PFASs that were included. This
6010 seems to contradict the biomonitoring data that show a much higher contribution of these four.
6011 However, a major part of the additional exposure came from PFBA and PFHxA, which have a shorter
6012 half-life and accumulate much less in humans.

6013 **3.6. Uncertainty analysis**

6014 **3.6.1 Uncertainty in exposure estimates**

6015 The number of occurrence data submitted to EFSA on PFASs differed in terms of both food products
6016 and compounds, with most of the samples relating to fish meat (33% of the results), and to PFOS and
6017 PFOA. Most of the food samples (~84%) were submitted by only three European countries (Germany,
6018 Norway and France), thus levels of PFASs in food commodities might not be representative for all of
6019 Europe. This may introduce uncertainty concerning the occurrence values used in the exposure
6020 assessment and lead to both an under- and overestimation of exposure.

6021 Whilst PFASs released from materials in contact with food may not be a significant contributor to
6022 exposure on a population basis, this may have a disproportionately large impact on some individuals. It
6023 is not known to what extent such release is covered by the occurrence database. This might lead to an
6024 underestimation of the exposure.

6025 The LODs/LOQs of the PFAS data reported to EFSA varied between laboratories, food matrices and
6026 substances. Overall, 95% of the results were reported below LOD/LOQ, increasing the disparity between
6027 LB and UB exposure values and thus contributing to the uncertainty associated with the dietary exposure
6028 estimations (an underestimation linked to the LB value and an overestimation linked to the UB value).
6029 This varied between substances.

6030 High LODs/LOQs did also result in a very limited set of detected data for specific compounds in some
6031 food groups, especially in cases where the number of samples available were low. This has for example
6032 resulted in "Food for infants and small children" being a major contributor to the total exposure to the
6033 sum of PFOA, PFNA, PFHxS and PFOS for infants and children, based on one sample with concentrations
6034 above the LOQ for PFNA.

6035 There are also food groups where the measured levels were below the LOQs/LODs of most other
6036 reported data sets, meaning that the mean levels used in the assessment were underestimated. This is
6037 especially relevant for widely consumed products that still contribute to the exposure despite the low
6038 levels (e.g. fruits and vegetables). As a result the exposure is underestimated.

6039 For several food groups, it was assumed that measured levels in a subgroup are representative for the
6040 whole food group, despite the absence of data for other products (e.g. data in apples used for all fruit).
6041 This could lead to both an overestimation (other subgroups not liable to contamination) or
6042 underestimation (other subgroups more contaminated) of the exposure.

6043 During the last fifty years different time trends have been observed for various PFASs in the environment
6044 and thus in food. This is among others due to restrictions on the use of PFOS and PFOA and at the
6045 same time increased use of short chain PFASs. This means that estimates of exposure that are based
6046 on data collected over a period of time will not necessarily reflect the current situation.

6047 Uncertainties and limitations related to the use of the EFSA Comprehensive Food Consumption
6048 Database, as described by EFSA (EFSA, 2011a), are applicable in the present assessment and are not
6049 further described in this Scientific Opinion.

For the most prevalent PFASs in human serum, i.e. PFOA, PFNA, PFHxS and PFOS, diet has been recognized as the main source of exposure in the general population. For other PFASs, almost nothing is known on the relative contribution of various exposure routes. Also, human metabolism of precursors may contribute to internal exposure. In the current exposure assessment, neither non-dietary exposure nor exposure to precursors have been considered, but both of these are included when biomonitoring data are used as a measure of exposure. This contributes to an underestimation of the exposure.

3.6.2 Uncertainty in biomonitoring data

Blood serum and blood plasma have been the most commonly used matrices for biomonitoring of PFASs, even though for some specific PFASs (not PFHxS, PFOS, PFOA and PFNA, but PFHxA and FOSA), whole blood may be more appropriate.

The representativeness of the biomonitoring data is affected by several factors, such as a limited amount of data for many PFASs and a non-equal distribution of studies between the European countries. Also, the studies included samples collected over ten years. As significant time trends have been observed in human samples from several studies, the collection time points may have had an influence on the aggregated data such as mean and median concentrations.

3.6.3 Uncertainties in hazard identification and characterisation

3.6.3.1 Experimental animal data

For PFASs other than PFOS and PFOA, no or only a limited number of chronic exposure studies are available.

There is a lack of data on effects on the immune system and in particular the T-cell directed antibody response for PFASs other than PFOS and PFOA. Moreover, the underlying immunotoxic mode of action of PFASs is unknown.

Concerning mammary gland development, there are no studies on other PFASs than PFOA. Information on the possible effects on milk production in animals affected by impaired mammary gland development after perinatal exposure is limited. This adds to the uncertainty related to this developmental endpoint.

3.6.3.2 Epidemiological studies in humans

The previous Opinion (EFSA CONTAM Panel, 2018) described in detail the uncertainties related to epidemiological studies. In general, studies performed adjustment for potential confounding factors (factors associated with both exposure to PFASs and the outcome considered) in the statistical models. Examples are age, sex, occupation and diet. There is, however, always a possibility of residual confounding not taken into account, for example certain life-style factors associated with socioeconomic status. The possibility of confounding is discussed in the respective section on the various outcomes, especially for the potential critical effects considered, i.e. effects on the immune response.

For rare outcomes, the number of cases in the study base is usually small and therefore the statistical power to demonstrate or refute an association with PFAS exposure is usually limited. In most cases this will result in risk estimates that are non-informative, or at least not statistically significant.

For the selected critical effect in this risk assessment, the conclusion on adversity is based on reduced antibody response, which is an effect marker of impaired immune function, while the evidence for increased risk of infections is more limited.

3.6.4 Uncertainties in dose-response assessment and HBGV derivation

3.6.4.1 Estimation of the reference point

BMD modelling is preferred over deriving a NOAEL or NOAEC, giving a better estimate of the critical levels. Existing guidelines on BMD modelling have been derived for its application in controlled studies in experimental animals. Although the same methodology has, in selected human cases, been applied for few data rich contaminants (Cd, Pb), the existing guidance on BMD modelling is not directly applicable for human studies. Extrapolation well beyond the lowest observed exposure level (no zero dose) and high variability between humans are sources of uncertainty that have not been reflected on or addressed in existing EFSA guidance or experimental work. In the case of PFASs, the serum concentrations were well above zero in both critical studies, the means for the sum of the 4 PFASs in the lowest quintile being 10 and 16 ng/mL for, respectively, Abraham et al. (2020) and Grandjean et al. (2012). Furthermore, booster vaccination in children results in an increase in antibody titers that has high between-person variability. This combined with a rather steep decrease (reduction in antibody titres >50% for levels of the sum of 4 PFASs at the LOAECs) was considered a logical explanation for the wide BMDL/BMDU confidence intervals observed when attempting to apply existing guidance on these studies. Instead, NOAECs were identified to provide an estimate for the Reference Point.

Also in two independent studies on effects of PFOS on the antibody response after immunisation of mice with sheep red blood cells, the BMD modelling resulted in wide BMDL/BMDU confidence intervals and extrapolation outside the observable range, since also in these experiments there were background PFOS levels measured in the untreated controls. Also for the most sensitive study, a NOAEC was derived.

The CONTAM Panel therefore decided to use the NOAEC approach for the selection of the Reference Point. It was noted that NOAECs are affected by dose spacing and study design in the case of experimental animals and by the number of quantiles in the case of human studies. It was also noted that the decrease in antibody titres around 50% or more at the LOAEC in the two studies on the Faroe Islands and Germany, is quite large.

3.6.4.2 Mixture effects and potency factors

Most PFASs cause similar effects in experimental animals, although there are differences in the effective doses, partly due to kinetic differences. For potential critical effects identified in this opinion including vaccination response and mammary gland development, no studies are available that compare the dose-response curves for different PFASs and thus would allow to derive potencies for these PFASs. Furthermore, there are clear interspecies and even sex-related differences that further complicate the derivation of potency factors. Therefore, it was decided to assume equal potencies for the four selected PFASs, which in humans share similar long half-lives. To allow easier comparison with estimated exposure, this was based on a weight basis rather than molar basis, although the latter is likely to be scientifically more correct. Given the quite similar molecular weight of the four compounds (PFHxS = 400, PFOS = 538, PFOA = 414 and PFNA = 464) this would contribute little to the overall uncertainty in the assessment. The fact that the NOAECs are based on the sum of 4 PFASs adds to the uncertainty, since the observed associations may be more related to one of the PFASs in the mixture.

3.6.4.3 PBPK modelling

Human PBPK models for PFOA and PFOS were used to estimate exposure to PFOA/PFNA and PFOS/PFHxS for women of 35 years (child-bearing age) leading to a defined serum level in mothers, and to simulate PFAS concentrations in the infant at 1 year of age after 12-months breastfeeding. This was done to calculate the intake by the mothers that would eventually result in serum levels in the 1-year-old infant equal to the NOAEC. Due to the lack of PBPK models for PFHxS and PFNA, the PBPK modelling was based on summing PFOA and PFNA, as well as PFHxS and PFOS and applying the models

for PFOA and PFOS, respectively. Since in practice PFNA and PFHxS contribute little to the overall exposure, this will have a minor effect on the estimations.

The variation in reported half-lives for PFOS and PFOA is a source of uncertainty, since in the respective PBPK models defined values of 5.4 and 2.3 years for PFOS and PFOA, respectively, were taken into account for developing the models. Tissue/plasma partition coefficients for PFASs for all tissues in the model were calculated from tissue concentration data derived from experimental animals rather than humans. Although validated against human studies, uncertainty remains for extrapolation from animal to human.

The models do not take into account possible physiological changes during pregnancy (bodyweight, volume of distribution), which may alter the toxicokinetics of PFASs during this period and could influence the transfer into the fetus. This also applies for the decrease in BW from the delivery and during the lactation period. Body weight is a sensitive parameter and all tissue volumes and flows are scaled to this parameter.

There is uncertainty in the modelling of plasma levels of infants, in terms of the contribution from breast milk. Data on PFOS/PFOA transfer to breast milk are available, and the average intake in mL per day is known. The milk consumption was kept constant over 1 year, i.e. the known decline in the milk volumes after 6 months was not taken into account, which could result in an overestimation of the exposure. If taken into account, it would have slowed down the decline in milk PFAS levels, so the PFAS exposure from a lower milk volume might not have been that different. Furthermore, additional exposure from other food in the breastfeeding period was not taken into account. The parameters used for the transfer to milk, based on breast milk to serum ratio in women, and decline in the milk levels are based on limited information. It was decided to apply a breastfeeding duration of 12 months, because of the current breastfeeding practice in Europe (Theurich et al., 2019) and based on the WHO recommendations to breastfeed exclusively for 6 months with continued breastfeeding along with appropriate complementary foods up to two years of age or beyond. This is supported by information on breastfeeding 6 to 12 months and beyond in a number of European countries. Using a duration of breastfeeding of less than 12 months would imply that the TWI would not be protective for women breastfeeding for periods in line with the WHO recommendations. However, applying a longer period than 12 months might be too conservative and not in line with common practice to breastfeed up to 12 months. When assuming a 6-month breastfeeding period, corresponding daily intakes by the mothers for PFOA/PFNA, PFHxS/PFOS or the sum would be, respectively, 0.53, 1.35 and 1.88 ng/kg bw per day. This would result in serum levels in the mothers at 35 years of 5.7, 14.8 and 20.5 ng/mL, and initial milk levels of 0.17 and 0.16 ng/mL for PFOA/PFNA and PFHxS/PFOS, respectively, based on ratios of 0.03 and 0.015. These intakes and critical serum levels in mothers are higher than those estimated for 12 months of breastfeeding. With 6 months of breastfeeding the peak serum levels in the children are achieved at 6 months of age, being 24.8 and 22.2 ng/mL for PFOA/PFNA and PFHxS/PFOS, respectively, and then decrease during the next 6 months to the NOAEC serum levels.

Further, there is neither information on possible differences between infants and adults regarding absorption and/or excretion of PFOS/PFOA, nor on specific kinetic parameters, such as plasma protein binding and renal clearance in infants.

When comparing the modelled data with those from the study by Abraham et al. (2020), the CONTAM Panel observed that the modelled levels in serum from mothers before pregnancy (3.4 ng/mL for PFOA/PFNA and 9.2 ng/mL PFHxS/PFOS, or combined 12.6 ng/mL) are lower than the observed levels (4.9, 0.4, 1.8 and 17.2 ng/mL for respectively PFOA, PFNA, PFHxS and PFOS, combined 24 ng/mL). However, in that study, the levels before pregnancy or birth were not determined, and it was thought that these starting levels are best represented the serum levels in the 21 mothers that did not breastfeed, since their levels would not have been affected by the decline due to the breastfeeding. The mean serum levels in the 1-year-old breastfed children were 17 ng/mL for PFOA/PFNA and 17 ng/mL for PFHxS/PFOS, or 34 ng/mL for the sum, so being close to the NOAEC of 31.9 ng/mL. It thus appears

that the PBPK model underestimates the levels (12.6 vs 24 ng/mL) in the mothers that correspond to the NOAEC in the 1-year-old children. This seems especially true for PFOS (9.2 vs 19 ng/mL) and less for PFOA (3.4 vs 5 ng/mL). This difference might be explained by a lower transfer to milk, and/or a higher decline rate of PFAS levels in breast milk during the lactation. These parameters are in principle linked but in the model this was not the case and published data were used instead for both parameters. Furthermore, a high exposure to PFOS and PFOA by mothers during the year after birth could have resulted in a strong increase in serum levels. Time trend studies show that serum levels were not at steady state and peaking around the year 2000, when the study was performed. Furthermore, 49 of the 101 mothers gave birth before and 40 of these breastfed their infants, resulting in lower serum levels when giving birth to the infants included in the study. This applied more for mothers in the new study that breastfed (36/80) than those that did not (4/21 and much shorter duration of breastfeeding) (Abrahams, 2020, personal communication) Therefore, their mean serum level before pregnancy would be lower than the 24 ng/mL observed in non-breastfeeding mothers, and thus be closer to the modelled level of 12.6 ng/mL. All these factors could have contributed to the apparent discrepancies between the modelled serum concentrations and the levels in the non-breastfeeding mothers 1 year after birth.

In a second comparison, the PBPK models were also used to estimate the serum levels in a 35 year old consumer resulting from the mean LB exposure of adults to PFOA and PFOS, as presented in table 10. For PFOA, the exposure of 0.13, 0.18 and 0.28 ng/kg bw per day (min, median, max across surveys), would result in serum levels of 1.4, 1.9 and 3.0 ng/mL. These levels are in the range observed in biomonitoring data with a median of 1.9 (min-max 0.76-4.9) ng/mL (Table 17). For PFOS, the mean exposure of 0.29, 0.58 and 0.93 ng/kg bw per day (min, median, max), would result in serum levels of 3.2, 6.4 and 10.2 ng/mL, as compared to the median of biomonitoring data being 7.7 (min-max 1.7-27.4) ng/mL (Table 16). This implies that both for PFOA and PFOS, the PBPK models correspond quite well with the serum levels in the adults.

3.6.5 Summary of Uncertainties

In Table 23, a summary of the uncertainty evaluation is presented, highlighting the main sources of uncertainty and indicating an estimate of whether the respective source of uncertainty might have led to an over- or underestimation of the exposure or the resulting risk.

Table 23: Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of PFASs in food

Sources of uncertainty	Direction
Extrapolation of occurrence data few Member States to whole of Europe	+/- ^(a)
Limited occurrence data for some PFASs and several food groups	+/-
Large proportion of left-censored data in the final dataset	+/-
Using the substitution method at the lower bound (LB) and upper bound (UB) scenario	+/-
Including data over the last 10 years while levels in food and humans are declining	+
Non-dietary exposure and contribution from precursors	-
Residual confounding, e.g. from life-style factors in human epi studies	+/-
Limited data on PFASs other than PFOS and PFOA	+/-
Evidence for an association between PFASs exposure and increased risk of infections is limited	+
The derivation of reference point	+/-
PBPK modelling	+/-
Lack of information on the relative potencies of PFASs/ mixture approach	+/-

Variability in vaccination response

+/-

(a): + = uncertainty with potential to cause over-estimation of exposure/risk; - = uncertainty with potential to cause under-estimation of exposure/risk

The CONTAM Panel has used LB exposures for the risk characterisation as it considers that the UB exposure assessment results in a large overestimation, also based on comparison of the critical serum level in mothers with the data from biomonitoring (see section 3.5 risk characterisation). Therefore, the risk characterisation is better based on the LB exposure, although there are also issues with levels based on too few quantified results, so that not all contributing food groups may be covered in the exposure assessment.

Overall, the CONTAM Panel considered that the impact of the uncertainties on the risk assessment for the sum of PFOA, PFNA, PFHxS and PFOS is high.

4. Conclusions

4.1 Occurrence/Exposure

- An initial number of 97,434 results for food samples analysed for PFASs from 16 European countries were available for the assessment. The final cleaned data set contained 69,433 analytical results for 26 PFASs with 92% of left-censored (LC) data (results below LOD/LOQ).
- The exposure assessment was limited to 17 PFASs (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTTrDA, PFTeDA, PFBS, PFHxS, PFHpS, PFOS, PFDS and FOSA). For this 67,839 analytical data were available. Most data were on PFOS (n = 8,498), PFOA (n = 8,197), PFDA (n = 5,770), PFNA (n = 5,594), PFHxA (n = 5,448) and PFHxS (n = 4,745).
- For most food categories and PFASs, calculated mean UB levels were much higher than LB levels. Due to high proportions of results below LOD/LOQ and/or only limited availability of data for all PFASs, the exposure calculations should be considered only as a rough indication of the range of chronic dietary exposure, and should thus be interpreted with caution. The CONTAM Panel concluded that the calculated LB exposure is likely to be more realistic than UB exposure.
- The CONTAM Panel decided to limit the risk assessment to the sum of PFOA, PFNA, PFHxS and PFOS. For this sum, the mean exposure in adolescents, adults, elderly and very elderly ranged from 0.42 to 3.1 ng/kg bw per day at the LB. Toddlers and other children had approximately two-fold higher mean intake than older age groups (adolescents, adults, elderly, very elderly), ranging from 0.84 to 6.5 ng/kg bw per day at the LB. In infants the mean exposure ranges were at the LB 2.4 to 12.2 ng/kg bw per day. The 95th percentile exposures ranged across surveys and age groups at the LB from 1.3 (adults) to 27.9 (infants) ng/kg bw per day.
- The four PFASs contributed in adults approximately 46% to the sum of all PFASs for which the exposure was calculated. Other PFASs that contributed more than 5% to this sum were PFBA (16%) and PFHxA (15%), which have short half-lives in humans.

- For the combined exposure to PFOA, PFNA, PFHxS and PFOS, the main contributing categories were "Fish meat" and "Fruit and fruit products" and "Eggs and egg products" for all population groups.
- Diet is the major source of PFAS exposure for most of the population, but other routes such as dust ingestion and indoor air inhalation may also contribute substantially.

4.2 Hazard Identification and characterisation

4.2.1 Toxicokinetics

- Many of the 27 PFASs considered in this opinion are shown to be readily absorbed through the gastrointestinal tract in mammals including humans; then they distribute predominantly to the plasma and depending on the PFAS to liver. They are excreted in both urine and faeces.
- Neither PFCAs nor PFSAs are metabolised by animals or humans, whereas precursors such as fluorotelomer alcohols (FTOHs) and polyfluoroalkyl phosphate esters (PAPs) are biotransformed to several metabolites, including PFCAs.
- Routes and rates of elimination of PFASs vary according to the chemical end-group, the chain length and the species. In rats, but not in humans, sex differences were observed in toxicokinetics.
- In rodents, half-lives vary from a few hours to several weeks and are in general much shorter than in humans.
- In humans, the estimated half-lives for short-chain PFASs (such as PFBA, PFBS and PFHxA) were found to range from a few days to approximately one month whereas for compounds having a long perfluorinated carbon chain length (such as PFOA, PFNA, PFDA, PFHxS or PFOS), it can be several years.
- The long elimination half-lives of PFASs mainly originate from their interactions with various transporters involved in the reabsorption processes occurring at the hepatic, intestinal and renal level.
- The maternal transfer of PFASs to offspring occurs both prenatally (in utero) and postnatally (via breastfeeding).

4.2.2 Transfer to plants and animals

- PFASs are transferred from soil to plants. In general, transfer rates are higher for the short chain PFASs and decrease from roots to leaves to fruits.
- In food producing animals, PFASs transfer from feed to animal derived food like milk, eggs and meat, with clear differences between species and the type of PFAS. This is also relevant for soil ingestion by foraging farm animals.

4.2.3 Biomonitoring

- 6283 • Serum and plasma are suitable matrices for monitoring the exposure to PFASs.
- 6284 • Generally, after the year 2000, the concentrations in serum/plasma of PFOS, PFOA and in some
- 6285 studies PFHxS have decreased, while the concentrations of PFNA, PFDA and PFUnDA have
- 6286 increased. No clear trends have been reported for the remaining PFASs.
- 6287 • Summary statistics was performed on median levels from the various studies published from
- 6288 2007-2018. Median values for the study medians were determined, referred to as median
- 6289 concentrations.
- 6290 • The most prominent PFAS for adults was PFOS (64%), followed by PFOA (16%), PFHxS (5.6%)
- 6291 and PFNA (5.1%). For children, PFOS and PFOA contributed almost the same with 35.0% and
- 6292 36.6% of the total, followed by PFNA (8.8%) and PFHxS (6.7%).
- 6293 • For adults the median concentrations in serum or plasma were 7.7, 1.9, 0.67, 0.61, 0.30 and
- 6294 0.28 ng/mL for PFOS, PFOA, PFHxS, PFNA, PFDA and PFUnDA, respectively, while the
- 6295 concentrations of the remaining PFASs were below 0.25 ng/mL.
- 6296 • For children the median concentrations in plasma were 3.2, 3.3, 0.79, 0.60 and 0.30 ng/mL for
- 6297 PFOS, PFOA, PFNA, PFHxS and PFDA, respectively, while the concentrations of the remaining
- 6298 PFASs were below 0.25 ng/mL.
- 6299 • Considerably higher concentrations have been observed for some individuals, including both
- 6300 occupationally exposed adults, and children and adults, which have experienced elevated
- 6301 exposure from, e.g. contaminated drinking water. In these cases, the relative abundance of the
- 6302 various PFASs may deviate considerably from what is observed in general populations.

6303 4.2.4 Toxicity in experimental animals

6304 *Repeated dose exposure*

- 6305 • For most PFASs, repeated dose toxicity studies were identified, the most consistent endpoint
- 6306 was increased liver weight. This was seen for all PFASs studied, but with clear differences in
- 6307 relative potencies. Disturbances in lipid metabolism, including hepatocellular steatosis as well
- 6308 as hepatotoxic effects were evident mostly at higher dose levels.
- 6309 • Many PFASs decreased the levels of thyroid hormones (both T4 and T3).
- 6310 • For some PFASs, increased relative kidney weight, alterations of the mucosa in the nasal cavity
- 6311 and olfactory epithelium were observed.

6312 *Developmental toxicity*

- 6313 • The most sensitive developmental effect observed was impaired development of mammary
- 6314 gland in mice, after exposure late in gestation or via lactation. This effect persisted beyond
- 6315 sexual maturity. Only PFOA has been investigated in relation to this outcome, with a maternal
- 6316 LOAEC around 66 ng/mL serum.

- 6317 • Developmental toxicity studies in rodents were identified for most of the PFASs. The effects
 6318 most often observed were increased fetal and/or neonatal mortality and reduction in fetal
 6319 weight and/or postnatal growth. In general, developmental toxicity occurred at similar or slightly
 6320 lower doses than those inducing maternal toxicity. LOAELs and NOAELs were orders of
 6321 magnitude higher than those for developmental effects on mammary glands seen with PFOA.

6322 *Reproductive toxicity*

- 6323 • Effects on male reproductive parameters, including atrophy of the testicular interstitium,
 6324 accompanied by reduced serum testosterone levels and epididymal and testicular weights have
 6325 been reported for PFNA and PFDA. Degenerative changes and spermatid retention in
 6326 seminiferous tubules were also observed.

6327 *Neurotoxicity*

- 6328 • PFOS and PFOA exert developmental neurotoxic effects in rodents at doses of 0.1–0.3 mg/kg
 6329 bw per day or higher. Studies with PFDA, PFHxS and PFDoDA indicate developmental neurotoxic
 6330 effects.

6331 *Immunotoxicity*

- 6332 • PFOS and PFOA have been shown to cause a reduced response to vaccination (T-cell dependent
 6333 antibody response) and PFOS also caused a reduced resistance to infection. Effects were noted
 6334 at doses where no overt toxicity was evident.
- 6335 • Whereas effects on the immune system have also been observed for other PFASs, i.e. PFNA
 6336 and PFDA, the available data base for these compounds is more limited and does not include
 6337 vaccination response.
- 6338 • The CONTAM Panel concluded that the immune system is a prime target of PFASs.

6339 *Genotoxicity*

- 6340 • For PFOS and PFOA no evidence for a direct genotoxic mode of action was identified.
- 6341 • For PFASs other than PFOS and PFOA, the number of studies and data are limited. However,
 6342 structural similarity for PFHxS and PFOS, as well as for PFNA and PFOA, indicates that also for
 6343 these PFASs a direct genotoxic mode of action is unlikely.

6344 *Long-term toxicity and carcinogenicity*

- 6345 • Available studies indicate that PFOS and PFOA are tumour promoters in rodent liver and that
 6346 PFOA also induces Leydig cell tumours in rats.
- 6347 • The only long-term study available for PFHxA provides no evidence for carcinogenicity. PFNA
 6348 and PFDA promoted liver tumor formation in a trout two-stage model of hepatocarcinogenesis,
 6349 while 8:2 FTOH failed to do so.

4.2.5 Human Observations

Immune outcomes

- Epidemiological studies published since the publication of the previous EFSA CONTAM Panel opinion on PFOS and PFOA, provide further support for the conclusion that PFOS and PFOA are associated with reduced antibody response to vaccination, observed in several studies.
- The evidence for other PFASs is weaker, related to the mixture of PFASs in the blood, the lower concentrations compared to PFOS and PFOA, and the small number of studies.
- Studies with children on the Faroe Islands showed various associations between the serum levels of individual PFASs but also the sum of PFOA, PFNA, PFHxS and PFOS and antibody titres against diphtheria and tetanus. A NOAEC of 27.0 ng/mL was identified for the association between the sum of these four PFASs at 5 years of age and the antibody titres against diphtheria at 7 years.
- The CONTAM Panel identified a new study with children from Germany showing an inverse association between serum levels of PFOA, but also the sum of PFOA, PFNA, PFHxS and PFOS, and antibody titres against haemophilus influenzae type b (HiB), diphtheria and tetanus in serum sampled from 1-year-old children predominantly breastfed. A NOAEC of 31.9 ng/mL at the age of 1 year was derived for the sum of PFOA, PFNA, PFHxS and PFOS. This study supports the results from the study on the Faroe Islands.
- Some of the studies suggest that serum levels of PFOS and PFOA are associated with increased propensity for infection.
- Epidemiological studies provide insufficient evidence to conclude on associations between exposure to PFASs and asthma and allergies.

Metabolic outcomes

- Epidemiological studies provide clear evidence for an association between exposure to PFOS, PFOA and PFNA and increased serum levels of cholesterol.
- Epidemiological studies provide evidence for an association between exposure to PFASs and increased serum levels of the liver enzyme alanine transferase (ALT). The magnitude of the associations was small, however, and few studies found associations with ALT outside the reference range. There were no associations with liver disease.
- There is insufficient evidence for associations with diabetes, obesity and metabolic syndrome.

Fertility and Pregnancy outcomes

- 6384 • Studies on PFOS and PFOA published since 2018 confirm previous conclusions from the PFOS
6385 and PFOA opinion (EFSA CONTAM Panel, 2018) that “there may well be a causal association
6386 between PFOS and PFOA and birth weight”.
- 6387 • Maternal serum levels in studies reporting results on other PFASs were generally much lower
6388 and those studies provide no evidence for an adverse association for other PFASs and birth
6389 weight.
- 6390 • Epidemiological studies show no associations between other PFASs and fertility and reproductive
6391 outcomes in both males and females.

6392 *Other potential effects in humans*

- 6393 • As for PFOS and PFOA, epidemiological studies provide no evidence for associations between
6394 other PFASs and fertility and reproductive outcomes in both males and females.
- 6395 • Epidemiological studies provide no evidence for associations between exposure to PFASs and
6396 neurodevelopmental outcomes, growth in infancy or childhood, neurobehavioural,
6397 neuropsychiatric, cognitive outcomes or thyroid function.
- 6398 • Epidemiological studies show no evidence for carcinogenicity of PFOS and PFOA in humans, as
6399 concluded in the 2018 opinion (EFSA CONTAM Panel, 2018). Limited information was identified
6400 for other PFASs.
- 6401 • Epidemiological studies provide insufficient evidence to conclude on associations between
6402 exposure to PFASs and increased risk of cardiovascular disease.
- 6403 • Epidemiological studies provide insufficient evidence for associations between exposure to PFASs
6404 and changes in kidney function or serum levels of uric acid, as well as low bone mineral density
6405 or osteoporosis.

6406 **4.2.6 Mode of Action**

- 6407 • Like peroxisome proliferators, PFASs seem to cause liver hyperplasia in rodents via activation
6408 of PPAR α .
- 6409 • PFASs induce steatosis in rodent hepatocytes in a PPAR α -independent way. Hepatocellular
6410 steatosis appears to be causally related to the occurrence of necrotic hepatocytes and the
6411 increased serum transaminase levels. Thorough knowledge of the MoA causing steatosis is
6412 missing.
- 6413 • There is no clear mode of action behind the strong decrease observed in thyroid hormone levels
6414 (T₄, T₃) in rodents exposed to PFASs, which is normally not accompanied by increased TSH
6415 levels. PFASs compete with T₄ for binding to transthyretin and can induce
6416 glucuronosyltransferase activity in the liver, but it is unclear if this is behind the decrease in
6417 hormone levels observed in experimental animals.

6418 • A mode of action of immunotoxicity by PFASs has not been established. Data from *in vivo* and
 6419 *in vitro* studies on PFOS and PFOA suggests that immunotoxic effects may originate from
 6420 modulation of PPARs, NF-κB regulated gene transactivation and/or regulation of apoptosis.

6421 • The MOA behind the impaired mammary gland development in mice dosed with PFOA is
 6422 unknown.

6423 **4.3 Critical effects, dose response assessment, derivation of HBGV**

6424 • In human studies, various associations between serum levels and a number of outcomes have
 6425 been reported. In the previous Opinion (EFSA CONTAM Panel, 2018), four endpoints were
 6426 selected as potential critical effects for PFOS and/or PFOA. These were (i) increased serum total
 6427 and LDL cholesterol (risk factor for cardiovascular disease), (ii) increased ALT levels (indicating
 6428 effects on liver cells), (iii) reduced birth weight, and (iv) effects on the immune system, as
 6429 shown by decreased antibody response to vaccines.

6430 • The CONTAM Panel concluded that effects on the immune system, which were observed at the
 6431 lowest serum PFAS levels in both animals and humans, is the critical effect. The findings were
 6432 considered robust since in animals they were consistently observed for the two studied PFASs
 6433 (PFOA, PFOS) and also in humans.

6434 • The CONTAM Panel noted that this is not the case for effects on mammary gland development,
 6435 which are observed at similar low serum levels in mice but have not been studied in other
 6436 animal models or humans.

6437 • Therefore, the CONTAM Panel decided to base the present assessment on PFASs on effects on
 6438 the immune system.

6439 • Based on observations on animals and humans, the CONTAM Panel decided to combine its
 6440 assessment on the sum of four PFASs, i.e. PFOA, PFNA, PFHxS and PFOS. At present, these are
 6441 the PFASs that contribute most to the levels observed in human serum.

6442 • In humans, these four PFASs share toxicokinetic properties and show similar accumulation and
 6443 long half-lives. Also in terms of effects, these compounds in general show the same effects
 6444 when studied in animals.

6445 • Current data do not allow the derivation of potency factors. As a pragmatic approach, the
 6446 CONTAM Panel assumed by default equal potencies for effects of these four PFASs on immune
 6447 outcomes.

6448 • The CONTAM Panel identified a NOAEC serum level at the age of 5 years for the sum of PFOA,
 6449 PFNA, PFHxS and PFOS of 27.0 ng/mL, based on decreased diphtheria antibody titres at the
 6450 age of 7 years, from the Faroe Islands study.

- 6451 • From the study from Germany, a NOAEC of 31.9 ng/mL at the age of 1 year was derived for
6452 the sum of PFOA, PFNA, PFHxS, PFOS, based on the inverse association between serum levels
6453 of the sum of these 4 PFASs and antibody titres against haemophilus influenzae type b (HiB).
- 6454 • This NOAEC of 31.9 ng/mL corresponds to a lower intake by the child and thus the mother in
6455 her life up to pregnancy, since PFAS serum levels in breastfed children are in general higher at
6456 1 years of age than at 5 years. Therefore, this NOAEC was used to estimate the daily intake by
6457 mothers that would result in this critical serum concentration at 1 year of age in breastfed
6458 children. This daily intake was subsequently used to derive a maternal reference point for the
6459 sum of PFOA, PFNA, PFHxS and PFOS.
- 6460 • Using a PBPK model, and assuming 12 months of breastfeeding, it was estimated that the
6461 NOAEC in infants corresponds to an intake by the mother of 1.16 ng/kg bw per day for the sum
6462 of the four PFASs. Such intake would result in a serum level in the mother at 35 years of age
6463 of 12.6 ng/mL.
- 6464 • The CONTAM Panel decided to use the daily intake of 1.16 ng/kg bw per day as the starting
6465 point and established a group tolerable weekly intake (TWI) of $7 \times 1.16 = 8$ ng/kg bw per week
6466 for the sum of PFOA, PFNA, PFHxS and PFOS.
- 6467 • The CONTAM Panel decided not to use additional uncertainty factors, because the NOAEC is
6468 based on effects in infants which are expected to be a sensitive population group, as is true for
6469 many immunotoxic chemicals. The CONTAM Panel also took into account that the NOAEC is
6470 based on risk factors for disease rather than disease.
- 6471 • This TWI should prevent that mothers reach a body burden that results in levels in milk that
6472 would lead to serum levels in the infant, associated with decreases in vaccination response. As
6473 a result, the higher exposure of breastfed infants is taken into account in the derivation of the
6474 TWI, and this TWI should not be applied for infants.
- 6475 • The CONTAM Panel noted that this TWI is protective for the other potential critical endpoints
6476 (increase in serum cholesterol, reduced birth weight and high serum levels of ALT) considered
6477 in the previous opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018).

6478 4.4 Risk Characterisation

- 6479 • Based on the occurrence data obtained from the Member States, the exposure to individual
6480 PFASs was estimated for different age groups across different food consumption surveys. Since
6481 it was decided to limit the risk assessment to PFOA, PFNA, PFHxS and PFOS, also the combined
6482 exposure to these four PFASs was estimated.
- 6483 • The CONTAM Panel noted that several factors contribute to the high uncertainty of the present
6484 exposure assessment. Large differences between LB and UB concentrations were observed in
6485 foods, as a result of analytical methods being used that are not sufficiently sensitive. This results

6486 in a large difference between maximum UB and minimum LB chronic dietary exposure estimates
6487 for PFASs.

6488 • The CONTAM Panel considers that the exposure levels for PFOA, PFNA, PFHxS and PFOS are
6489 closer to the LB than the UB values.

6490 • Regarding the combined exposure of the four PFASs, for toddlers and other children, mean LB
6491 exposure varied between 6 and 46 ng/kg bw per week. The high (P95) LB exposure ranged
6492 from 19 and 96 ng/kg bw per week. For toddlers, the high exposure from food is influenced by
6493 a high contribution from PFNA and associated with high uncertainty.

6494 • The group of adolescents, adults, elderly and very elderly showed in general lower exposure.
6495 Mean LB exposure varied between 3 and 22 ng/kg bw per week, as compared to high (P95) LB
6496 exposure between 9 and 70 ng/kg bw per week. The highest mean LB exposure for adolescents
6497 and adults exceeds the TWI by a factor of 1.5. At the highest LB P95 exposure this is a factor
6498 of 4.5.

6499 • These exceedances of the TWI at LB exposure estimates indicate a health concern.

6500 **5. Recommendations**

6501 • For individual PFASs more sensitive analytical methods with high levels of quality control (to
6502 avoid matrix effects or impact of background contamination) are needed in order to reduce
6503 uncertainty in the dietary exposure assessment.

6504 • Occurrence data are needed for all PFASs found in the environment and in a broad range of
6505 widely consumed food products.

6506 • For the determination of the total amount of PFASs, sensitive and accurate methods, which
6507 facilitate determination in samples of food and drinks are needed.

6508 • Additional studies on the relative contribution of sources other than food are needed, especially
6509 for PFASs which are present in the highest concentrations in indoor air and house dust, such as
6510 n:2 FTOHs and PAPs.

6511 • More studies on the effect of cooking and food processing, in particular in relation to transfer
6512 to food from food contact materials that contain PFASs, are needed.

6513 • More information is needed on the transfer of PFASs along the food chain.

6514 • Studies on effects of other PFASs, and in particular PFOS, PFNA and PFHxS, on mammary gland
6515 development should be conducted.

6516 • Studies on effects of other PFASs, and in particular PFNA and PFHxS on the immune system
6517 should be conducted.

- 6518 • Studies for the critical effects that allow for a derivation of potency factors for PFASs should be
6519 conducted.
- 6520 • Studies to characterise the mode of action of immunotoxicity and mammary gland development
6521 of PFASs should be performed.
- 6522 • More longitudinal epidemiological studies are needed on human endpoints, in particular
6523 prospective vaccination studies covering more varied types of vaccines, different populations,
6524 as well as more studies on other immune outcomes in humans, including risk of infections.
- 6525 • Epidemiological studies should include the sum of several PFASs.
- 6526 • Experimental evidence is needed to understand and quantify the association between PFASs
6527 and blood lipids, for example the importance of enterohepatic circulation.
- 6528 • PBPK models for PFASs should be further optimised.
- 6529
- 6530

References

- 6532 Abbott BD, Wolf CJ, Schmid JE, Das KP, Zehr RD, Helfant L, Nakayama S, Lindstrom AB, Strynar MJ and
6533 Lau C, 2007. Perfluorooctanoic acid induced developmental toxicity in the mouse is dependent on
6534 expression of peroxisome proliferator activated receptor- α . *Toxicological Sciences*, 98, 571–581.
- 6535 Abbott BD, Wolf CJ, Das KP, Zehr RD, Schmid JE, Lindstrom AB, Strynar MJ and Lau C, 2009.
6536 Developmental toxicity of perfluorooctane sulfonate (PFOS) is not dependent on expression of
6537 peroxisome proliferator activated receptor- α (PPAR α) in the mouse. *Reproductive Toxicology*,
6538 27, 258–265.
- 6539 Abbott BD, Wood CR, Watkins AM, Tatum-Gibbs K, Das KP and Lau C, 2012. Effects of perfluorooctanoic
6540 acid (PFOA) on expression of peroxisome proliferator-activated receptors (PPAR) and nuclear
6541 receptor-regulated genes in fetal and postnatal CD-1 mouse tissues. *Reproductive Toxicology*, 33,
6542 491–505. <https://doi.org/10.1016/j.reprotox.2011.11.005>
- 6543 Abe T, Takahashi M, Kano M, Amaike Y, Ishii C, Maeda K, Kudoh Y, Morishita T, Hosaka T, Sasaki T,
6544 Kodama S, Matsuzawa A, Kojima H and Yoshinari K, 2017. Activation of nuclear receptor CAR by an
6545 environmental pollutant perfluorooctanoic acid. *Archives of Toxicology*, 91, 2365–2374.
6546 doi:10.1007/s00204-016-1888-3
- 6547 Abraham K, Mielke H, Fromme H, Volkel W, Menzel J, Peiser M, Zepp F, Willich SN and Weikert C, 2020.
6548 (Internal exposure to perfluoroalkyl substances (PFASs) and biological marker in 101 healthy one-
6549 year-old children: Associations between levels of perfluorooctanoic acid (PFOA) and vaccine
6550 response. *Archives of Toxicology*, SUBMITTED.
- 6551 Adinehzadeh M, Reo NV, Jarnot BM, Taylor CA and Mattie DR, 1999. Dose-response hepatotoxicity of
6552 the peroxisome proliferator, perfluorodecanoic acid and the relationship to phospholipid metabolism
6553 in rats. *Toxicology*, 134, 179–195.
- 6554 Adler G, Beglinger C, Braun U, Reinshagen M, Koop I, Schafmayer A, Rovati L and Arnold R, 1991.
6555 Interaction of the cholinergic system and cholecystokinin in the regulation of endogenous and
6556 exogenous stimulation of pancreatic secretion in humans. *Gastroenterology*, 100, 537–543.

- 6557 Ahrens L, Siebert U and Ebinghaus R, 2009. Temporal trends of polyfluoroalkyl compounds in harbor
6558 seals (*Phoca vitulina*) from the German Bight, 1999–2008. *Chemosphere*, 76, 151–158.
6559 <https://doi.org/10.1016/j.chemosphere.2009.03.053>
- 6560 Ahrens L, Yeung LWY, Taniyasu S, Lam PKS and Yamashita N, 2011a. Partitioning of perfluorooctanoate
6561 (PFOA), perfluorooctane sulfonate (PFOS) and perfluorooctane sulfonamide (PFOSA) between water
6562 and sediment. *Chemosphere*, 85, 731–737.
- 6563 Ahrens L, Herzke D, Huber S, Bustnes JO, Bangjord G and Ebinghaus R, 2011b. Temporal trends and
6564 pattern of polyfluoroalkyl compounds in Tawny Owl (*Strix aluco*) eggs from Norway, 1986–2009.
6565 *Environmental Science & Technology*, 45, 8090–8097. doi:10.1021/es103473v
- 6566 Ahrens L, Shoeib M, Harner T, Lee SC, Guo R and Reiner EJ, 2011c. Wastewater Treatment Plant and
6567 Landfills as Sources of Polyfluoroalkyl Compounds to the Atmosphere. *Environmental Science &*
6568 *Technology*, 45, 8098–8105. doi:10.1021/es1036173
- 6569 Ahrens L and Bundschuh M, 2014. Fate and effects of poly- and perfluoroalkyl substances in the aquatic
6570 environment: a review. *Environmental Toxicology and Chemistry*, 33, 1921–1929.
- 6571 Albrecht PP, Torsell NE, Krishnan P, Ehresman DJ, Frame SR, Chang SC, Butenhoff JL, Kennedy GL,
6572 Gonzalez FJ and Peters JM, 2013. A species difference in the peroxisome proliferator-activated
6573 receptor α -dependent response to the developmental effects of perfluorooctanoic acid. *Toxicological*
6574 *Sciences*, 131, 568–582. <https://doi.org/10.1093/toxsci/kfs318>
- 6575 Alves A, Kucharska A, Erratico C, Xu F, Den Hond E, Koppen G, Vanermen G, Covaci A and Voorspoels
6576 S, 2014. Human biomonitoring of emerging pollutants through non-invasive matrices: state of the
6577 art and future potential. *Analytical and Bioanalytical Chemistry*, 406, 4063–4088.
6578 <https://doi.org/10.1007/s00216-014-7748-1>
- 6579 Alves A, Jacobs G, Vanermen G, Covaci A and Voorspoels S, 2015. New approach for assessing human
6580 perfluoroalkyl exposure via hair. *Talanta*, 144, 574–583.
6581 <https://doi.org/10.1016/j.talanta.2015.07.009>
- 6582 Alves RN, Maulvault AL, Barbosa VL, Cunha S, Kwadijk CJAF, Álvarez-Muñoz D, Rodríguez-Mozaz S,
6583 Aznar-Alemany Ò, Eljarrat E, Barceló D, Fernandez-Tejedor M, Tediosi A and Marques A, 2017.
6584 Preliminary assessment on the bioaccessibility of contaminants of emerging concern in raw and
6585 cooked seafood. *Food and Chemical Toxicology*, 104, 69–78.
6586 <https://doi.org/10.1016/j.fct.2017.01.029>
- 6587 Andersen CS, Fei C, Gamborg M, Nohr EA, Sørensen TI and Olsen J, 2010. Prenatal exposures to
6588 perfluorinated chemicals and anthropometric measures in infancy. *American Journal of Epidemiology*,
6589 172, 1230–1237.
- 6590 Andersen ME, Clewell III HJ, Tan YM, Butenhoff JL and Olsen GW. 2006. Pharmacokinetic modeling of
6591 saturable, renal resorption of perfluoroalkylacids in monkeys-Probing the determinants of long
6592 plasma half-lives. *Toxicology*, 227, 156–164. <https://doi.org/10.1016/j.tox.2006.08.004>
- 6593 Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL and Goldman LR, 2007a.
6594 Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in
6595 relation to weight and size at birth. *Environmental Health Perspectives*, 115, 1670–1676.
6596 <https://doi.org/10.1289/ehp.10334>
- 6597 Apelberg BJ, Goldman LR, Calafat AM, Herbstman JB, Kuklanyik Z, Heidler J, Needham LL, Halden RU
6598 and Witter FR, 2007b. Determinants of fetal exposure to polyfluoroalkyl compounds in Baltimore,
6599 Maryland. *Environmental Science & Technology*, 41, 3891–3897. <https://doi.org/10.1021/es0700911>

- 6600 Arbuckle TE, Kubwabo C, Walker M, Davis K, Lalonde K, Kosarac I, Wen SW and Arnold DL, 2013.
 6601 Umbilical cord blood levels of perfluoroalkyl acids and polybrominated flame retardants. *International*
 6602 *Journal of Hygiene and Environmental Health*, 216, 184-194. doi:10.1016/j.ijheh.2012.03.004
- 6603 Armstrong DL, Lozano N, Rice CP, Ramirez M and Torrents A, 2016. Temporal trends of perfluoroalkyl
 6604 substances in limed biosolids from a large municipal water resource recovery facility. *Journal of*
 6605 *Environmental Management*, 165, 88-95. https://doi.org/10.1016/j.jenvman.2015.09.023
- 6606 Arrebola JP, Castaño A, Esteban M, Bartolomé M, Pérez-Gómez B, Ramos JJ and BIOAMBIENT.ES, 2018.
 6607 Differential contribution of animal and vegetable food items on persistent organic pollutant
 6608 serum concentrations in Spanish adults. Data from BIOAMBIENT.ES project., *Science of the Total*
 6609 *Environment*, 634, 235-242. https://doi.org/10.1016/j.scitotenv.2018.03.283
- 6610 Arvaniti OS and Stasinakis AS, 2015. Review on the occurrence, fate and removal of perfluorinated
 6611 compounds during wastewater treatment. *Science of the Total Environment*, 524-525, 81-92.
 6612 https://doi.org/10.1016/j.scitotenv.2015.04.023
- 6613 Asakawa A, Toyoshima M, Harada KH, Fujimiya M, Inoue K and Koizumi A, 2008. The ubiquitous
 6614 environmental pollutant perfluorooctanoic acid inhibits feeding behavior via peroxisome proliferator-
 6615 activated receptor- α . *International Journal of Molecular Medicine*, 21, 439-445. DOI:
 6616 10.3892/ijmm.21.4.439
- 6617 Ashley-Martin J, Dodds L, Levy AR, Platt RW, Marshall JS and Arbuckle TE, 2015. Prenatal exposure to
 6618 phthalates, bisphenol A and perfluoroalkyl substances and cord blood levels of IgE, TSLP and IL-33.
 6619 *Environmental Research*, 140, 360-368. https://doi.org/10.1016/j.envres.2015.04.010
- 6620 ATSDR (Agency for Toxic Substances and Disease Registry), 2013. Health consultation. Exposure
 6621 investigation report. Perfluorochemical serum sampling in the vicinity of Decatur, Alabama, Morgan,
 6622 Lawrence, and Limestone counties. U.S. Department of Health and Human Services, Atlanta.
 6623 Available online:
 6624 http://www.atsdr.cdc.gov/HAC/pha/Decatur/Perfluorochemical_Serum%20Sampling.pdf
- 6625 ATSDR (Agency for Toxic Substances and Disease Registry), 2018. Draft Toxicological Profile for
 6626 Perfluoroalkyls. Available online: https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf
- 6627 Axmon A, Axelsson J, Jakobsson K, Lindh CH and Jönsson BA, 2014. Time trends between 1987 and
 6628 2007 for perfluoroalkyl acids in plasma from Swedish women. *Chemosphere*, 102, 61-67.
 6629 https://doi.org/10.1016/j.chemosphere.2013.12.021
- 6630 Averina M, Brox J, Huber S and Furberg AS, 2018. Perfluoroalkyl substances in adolescents in northern
 6631 Norway: Lifestyle and dietary predictors. The Tromsø study, Fit Futures 1. *Environment International*,
 6632 114, 123-130.
- 6633 Averina M, Brox J, Huber S, Furberg AS and Sørensen M, 2019. Serum perfluoroalkyl substances (PFAS)
 6634 and risk of asthma and various allergies in adolescents. The Tromsø study Fit Futures in Northern
 6635 Norway. *Environmental Research*, 169, 114-121.
- 6636 Ayanda I, Min Y, Yu Z and Zha J, 2018. Cytotoxic and genotoxic effects of perfluorododecanoic acid
 6637 (PFDoA) in Japanese medaka. *Knowledge & Management of Aquatic Ecosystems*, 419, 9, 7 pp.
 6638 https://doi.org/10.1051/kmae/2017058
- 6639 Bach CC, Bech BH, Nohr EA, Olsen J, Matthiesen NB, Bossi R, Uldbjerg N, Bonefeld-Jørgensen EC and
 6640 Henriksen TB, 2015a. Serum perfluoroalkyl acids and time to pregnancy in nulliparous women.
 6641 *Environmental Research*, 142, 535-541. https://doi.org/10.1016/j.envres.2015.08.007
- 6642 Bach CC, Bech BH, Brix N, Nohr EA, Bonde JP and Henriksen TB, 2015b. Perfluoroalkyl and
 6643 polyfluoroalkyl substances and human fetal growth: a systematic review. *Critical Reviews in*
 6644 *Toxicology*, 45, 53-67. https://doi.org/10.3109/10408444.2014.952400

- 6645 Bach CC, Bech BH, Nohr EA, Olsen J, Matthiesen NB, Bonefeld-Jørgensen EC, Bossi R and Henriksen
6646 TB, 2016. Perfluoroalkyl acids in maternal serum and indices of fetal growth: the Aarhus birth cohort.
6647 *Environmental Health Perspectives*, 124, 848–854. <https://doi.org/10.1289/ehp.1510046>
- 6648 Bach CC, Matthiesen NB, Olsen J and Henriksen TB, 2018. Conditioning on Parity in Studies of
6649 Perfluoroalkyl Acids and Time to Pregnancy: An Example from the Danish National Birth Cohort.
6650 *Environmental Health Perspectives*, 126, 117003. <https://doi.org/10.1289/EHP1493>
- 6651 Ballesteros-Gomez A, Rubio S and van Leeuwen S, 2010. Tetrahydrofuran-water extraction, in-line
6652 clean-up and selective liquid chromatography/tandem mass spectrometry for the quantitation of
6653 perfluorinated compounds in food at the low picogram per gram level. *Journal of Chromatography*
6654 *A*, 1217, 5931-5921.
- 6655 Bao J, Karrman A, Bavel B and Jin Y, 2014. Perfluoroalkyl substances in the blood samples from a male
6656 population of Sweden. *Chinese Science Bulletin*, 59, 388–395.
- 6657 Bao WW, Qian ZM, Geiger SD, Liu E, Liu Y, Wang SQ, Lawrence WR, Yang BY, Hu LW, Zeng XW and
6658 Dong GH, 2017. Gender-specific associations between serum isomers of perfluoroalkyl substances
6659 and blood pressure among Chinese: Isomers of C8 Health Project in China. *Science of the Total*
6660 *Environment*, 607-608:1304-1312. doi: 10.1016/j.scitotenv.2017.07.124
- 6661 Barbosa V, Maulvault AL, Alves RN, Kwadijk C, Kotterman M, Tediosi A, Fernández-Tejedor M, Sloth JJ,
6662 Granby K, Rasmussen RR, Robbens J, De Witte B, Trabalón L, Fernandes JO, Cunha SC and Marques
6663 A, 2018. Effects of steaming on contaminants of emerging concern levels in seafood. *Food and*
6664 *Chemical Toxicology*, 118, 490-504. <https://doi.org/10.1016/j.fct.2018.05.047>
- 6665 Barry V, Winqvist A and Steenland K, 2013. Perfluorooctanoic acid (PFOA) exposures and incident
6666 cancers among adults living near a chemical plant. *Environmental Health Perspectives*, 121, 1313-
6667 1318.
- 6668 Bartell SM, Calafat AM, Lyu C, Kato K, Ryan PB and Steenland K. 2009. Rate of decline in serum PFOA
6669 concentrations after granular activated carbon filtration at two public water systems in Ohio and
6670 West Virginia. *Environmental Health Perspectives*, 118, 222-228.
- 6671 Bartolome M, Gallego-Pico A, Cutanda F, Huetos O, Esteban M, Perez-Gomez B and Castano A, 2017.
6672 Perfluorinated alkyl substances in Spanish adults: Geographical distribution and determinants of
6673 exposure. *Science of the Total Environment*, 603-604, 352-360. doi:10.1016/j.scitotenv.2017.06.031
- 6674 Baduel C, Lai FY, Townsend K and Mueller JF, 2014. Size and age-concentration relationships for
6675 perfluoroalkyl substances in stingray livers from eastern Australia. *Science of the Total Environment*,
6676 496, 523-530. doi:10.1016/j.scitotenv.2014.07.010
- 6677 Bassler J, Ducatman A, Elliott M, Wen S, Wahlang B, Barnett J and Cave MC, 2019. Environmental
6678 perfluoroalkyl acid exposures are associated with liver disease characterized by apoptosis and altered
6679 serum adipocytokines. *Environmental Pollution*, 247, 1055-1063.
- 6680 Beesoon S, Webster GM, Shoeib M, Harner T, Benskin JP and Martin JW, 2011. Isomer profiles of
6681 perfluorochemicals in matched maternal, cord, and house dust samples: manufacturing sources and
6682 transplacental transfer. *Environmental Health Perspectives*, 119, 1659-1664.
- 6683 Begley TH, White K, Honigfort P, Twaroski ML, Neches R and Walker RA, 2005. Perfluorochemicals:
6684 Potential sources of and migration from food packaging. *Food Additives and Contaminants*, 22, 1023-
6685 1031
- 6686 Bell EM, Yeung E, Ma W, Kannan K, Sundaram R, Smarr MM and Buck Louis GM, 2018. Concentrations
6687 of endocrine disrupting chemicals in newborn blood spots and infant outcomes in the upstate KIDS
6688 study. *Environment International*, 121, 232-239.

- Benninghoff AD, Orner GA, Buchner CH, Hendricks JD, Duffy AM and Williams DE, 2012. Promotion of Hepatocarcinogenesis by Perfluoroalkyl Acids in Rainbow Trout. *Toxicological Sciences*, 125, 69–78.
- Benskin JP, De Silva AO, Martin LJ, Arsenault G, McCrindle R, Riddell N, Mabury SA and Martin JW, 2009. Disposition of perfluorinated acid isomers in Sprague-Dawley rats; part 1: single dose. *Environmental Toxicology and Chemistry*, 28, 542–554. <https://doi.org/10.1897/08-239.1>
- Benskin JP, De Silva AO and Martin JW, 2010. Isomer profiling of perfluorinated substances as a tool for source tracking: a review of early findings and future applications. *Reviews of Environmental Contamination and Toxicology*, 208, 111–160.
- Benskin JP, Phillips V, St Louis VL and Martin JW, 2011. Source Elucidation of Perfluorinated Carboxylic Acids in Remote Alpine Lake Sediment Cores. *Environmental Science & Technology*, 45, 7188–7194.
- Berk M, Williams LJ, Andreazza AC, Pasco JA, Dodd S, Jacka FN, Moylan S, Reiner EJ and Magalhaes PV, 2014. Pop, heavy metal and the blues: secondary analysis of persistent organic pollutants (POP), heavy metals and depressive symptoms in the NHANES National Epidemiological Survey. *BMJ Open*, 4, e005142. doi:10.1136/bmjopen-2014-005142
- Berg V, Nøst TH, Huber S, Rylander C, Hansen S, Veyhe AS, Fuskevåg OM, Odland JØ and Sandanger TM, 2014. Maternal serum concentrations of per- and polyfluoroalkyl substances and their predictors in years with reduced production and use. *Environment International*, 69, 58–66.
- Berg V, Nost TH, Hansen S, Elverland A, Veyhe AS, Jorde R, Odland JO and Sandanger TM, 2015. Assessing the relationship between perfluoroalkyl substances, thyroid hormones and binding proteins in pregnant women; a longitudinal mixed effects approach. *Environment International*, 77, 63–69. doi: 10.1016/j.envint.2015.01.007
- Berg V, Nost TH, Pettersen RD, Hansen S, Veyhe AS, Jorde R, Odland JO and Sandanger TM, 2017. Persistent Organic Pollutants and the Association with Maternal and Infant Thyroid Homeostasis: A Multipollutant Assessment. *Environmental Health Perspectives*, 125, 127–133. doi: 10.1289/ehp152
- Berger U, Glynn A, Homström KE, Berglund M, Halldin Ankarberg E and Törnkqvist, 2009. Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden – Analysis of edible fish from Lake Vättern and the Baltic Sea. *Chemosphere*, 76, 799–804.
- Berntsen HF, Bjorklund CG, Audinot JN, Hofer T, Verhaegen S, Lentzen E, Gutleb AC and Ropstad E, 2017. Time-dependent effects of perfluorinated compounds on viability in cerebellar granule neurons: Dependence on carbon chain length and functional group attached. *Neurotoxicology*, 63, 70–83. doi:10.1016/j.neuro.2017.09.005
- Berntsen HF, Bjorklund CG, Strandabo R, Haug TM, Moldes-Anaya A, Fuentes-Lazaro J, Verhaegen S, Paulsen RE, Tasker RA and Ropstad E, 2018. PFOS-induced excitotoxicity is dependent on Ca²⁺ influx via NMDA receptors in rat cerebellar granule neurons. *Toxicology and Applied Pharmacology*, 15, 19–32. doi: 10.1016/j.taap.2018.08.015
- Berthiaume J and Wallace KB, 2002. Perfluorooctanoate, perfluorooctanesulfonate, and N-ethyl perfluorooctanesulfonamido ethanol; peroxisome proliferation and mitochondrial biogenesis. *Toxicological Letters*, 129, 23–32.
- Binnington MJ, Lei YD, Pokiak L, Pokiak J, Ostertag SK, Loseto LL, Chan HM, Yeung LWY, Huang H and Wania F, 2017. Effects of preparation on nutrient and environmental contaminant levels in Arctic beluga whale (*Delphinapterus leucas*) traditional foods. *Environmental Science: Processes & Impacts*, 19, 1000–1015. doi:10.1039/c7em00167c
- Bijland S, Rensen PC, Pieterman EJ, Maas AC, van der Hoorn JW, van Erk MJ, Havekes LM, Willems van Dijk K, Chang SC, Ehresman DJ, Butenhoff JL and Princen HM, 2011. Perfluoroalkyl sulfonates cause

- alkyl chain length-dependent hepatic steatosis and hypolipidemia mainly by impairing lipoprotein production in APOE*3-Leiden CETP mice. *Toxicological Sciences*, 123, 290-303.
- Bjermo H, Darnerud PO, Pearson M, Barbieri HE, Lindroos AK, Nälsén C, Lindh CH, Jönsson BA and Glynn A, 2013. Serum concentrations of perfluorinated alkyl acids and their associations with diet and personal characteristics among Swedish adults. *Molecular Nutrition Food Research*, 57, 2206-2215.
- Bjerregaard-Olesen C, Bach CC, Long M, Ghisari M, Bossi R, Bech BH, Nohr EA, Henriksen TB, Olsen J and Bonefeld-Jørgensen EC, 2016. Time trends of perfluorinated alkyl acids in serum from Danish pregnant women 2008-2013. *Environment International*, 1, 14-21.
- Bjork JA and, Wallace KB, 2009. Structure-activity relationships and human relevance for perfluoroalkyl acid-induced transcriptional activation of peroxisome proliferation in liver cell cultures. *Toxicological Sciences*, 111, 89-99.
- Bjork JA, Butenhoff JL and Wallace KB, 2011. Multiplicity of nuclear receptor activation by PFOA and PFOS in primary human and rodent hepatocytes. *Toxicology*, 288, 8-17.
- Blaine AC, Rich CD, Sedlako EM, Hundal LS, Kuma K, Lau C, Mills MA, Harris KM and Higgins CP, 2014. Perfluoroalkyl Acid Distribution in Various Plant Compartments of Edible Crops Grown in Biosolids-Amended soils. *Environmental Science & Technology*, 48, 7858-7865.
- Bloom MS, Kannan K, Spliethoff HM, Tao L, Aldous KM and Vena JE, 2010. Exploratory assessment of perfluorinated compounds and human thyroid function. *Physiology & Behavior*, 99, 240-245. doi: 10.1016/j.physbeh.2009.02.005
- Blum A, Balan SA, Scherlinger M, Trier X, Goldenman G, Cousins IT, Diamond M, Fletcher T, Higgins C, Lindeman AE, Peaslee G, Voogt Pd, Wang Z and Weber R, 2015. The Madrid Statement on Poly- and Perfluoroalkyl Substances (PFASs). *Environmental Health Perspectives*, 123, A107-A111. doi: 10.1289/ehp.1509934
- Bodin J, Groeng EC, Andreassen M, Dirven H and Nygaard UC, 2016. Exposure to perfluoroundecanoic acid (PFUnDA) accelerates insulinitis development in a mouse model of type 1 diabetes. *Toxicology Reports*, 3, 664-672. doi:10.1016/j.toxrep.2016.08.009
- Bogdanska J, Sundstrom M, Bergstrom U, Borg D, Abedi-Valugerdi M, Bergman A, DePierre J and Nobel S, 2014. Tissue distribution of 35S-labelled perfluorobutanesulfonic acid in adult mice following dietary exposure for 1-5 days. *Chemosphere*, 98, 28-36. doi:10.1016/j.chemosphere.2013.09.062
- Borg D, Lund BO, Lindquist NG and Håkansson H, 2013. Cumulative health risk assessment of 17 perfluoroalkylated and polyfluoroalkylated substances (PFASs) in the Swedish population. *Environment International*, 59, 112-123.
- Boujrad N, Vidic B, Gazouli M, Culty M and Papadopoulos V, 2000. The peroxisome proliferator perfluorodecanoic acid inhibits the peripheral-type benzodiazepine receptor (PBR) expression and hormone-stimulated mitochondrial cholesterol transport and steroid formation in Leydig cells. *Endocrinology*, 141, 3137-3148.
- Brantsæter AL, Whitworth KW, Ydersbond TA, Haug LS, Haugen M, Knutsen HK, Thomsen C, Meltzer HM, Becher G, Sabaredzovic A, Hoppin JA, Eggesbø M and Longnecker MP, 2013. Determinants of plasma concentrations of perfluoroalkyl substances in pregnant Norwegian women. *Environment International*, 54, 74-84.
- Braun JM, Kalkbrenner AE, Just AC, Yolton K, Calafat AM, Sjödin A, Hauser R, Webster GM, Chen A and Lanphear BP, 2014. Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4- and 5-year-old children: the HOME study. *Environmental Health Perspectives*, 122, 513-520.

- 6778 Brede E, Wilhelm M, Göen T, Müller J, Rauchfuss K, Kraft M and Hölzer J, 2010. Two-year follow-up
6779 biomonitoring pilot study of residents' and controls' PFC plasma levels after PFOA reduction in public
6780 water system in Arnsberg, Germany. *International Journal of Hygiene and Environmental Health*,
6781 213, 217–223.
- 6782 Brieger A, Bienefeld N, Hasan R, Goerlich R and Haase H, 2011. Impact of perfluorooctane sulfonate
6783 and perfluorooctanoic acid on human peripheral leukocytes. *Toxicology In Vitro*, 25, 960–968.
- 6784 Buck RC, Franklin J, Berger U, Conder JM, Cousins IT, de Voogt P, Jensen AA, Kannan K, Mabury SA
6785 and van Leeuwen SPJ, 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment:
6786 terminology, classification, and origins. *Integrated Environmental Assessment and Management*, 7,
6787 513–541. <https://doi.org/10.1002/ieam.258>
- 6788 Buck Louis GM, Sundaram R, Schisterman EF, Sweeney AM, Lynch CD, Gore-Langton RE, Maisog J, Kim
6789 S, Chen Z and Barr DB, 2013. Persistent environmental pollutants and couple fecundity: the LIFE
6790 study. *Environmental Health Perspectives*, 121, 231–236. <https://doi.org/10.1289/ehp.1205301>
- 6791 Buck Louis GM, Sapra KJ, Barr DB, Lu Z and Sundaram R, 2016. Preconception perfluoroalkyl and
6792 polyfluoroalkyl substances and incident pregnancy loss, LIFE Study. *Reproductive Toxicology*, 65,
6793 11–17. <https://doi.org/10.1016/j.reprotox.2016.06.011>
- 6794 Buck Louis GM, Zhai S, Smarr MM, Grewal J, Zhang C, Grantz KL, Hinkle SN, Sundaram R, Lee S, Honda
6795 M, Oh J and Kannan K, 2018. Endocrine disruptors and neonatal anthropometry, NICHD Fetal Growth
6796 Studies – Singletons. *Environmental international*, 119, 515–526. doi: 10.1016/j.envint.2018.07.024
- 6797 Budtz-Jorgensen E and Grandjean P, 2018. Application of benchmark analysis for mixed contaminant
6798 exposures: Mutual adjustment of perfluoroalkylate substances associated with immunotoxicity. *PLoS*
6799 *ONE* 13(10): e0205388. <https://doi.org/10.1371/journal.pone.0205388>
- 6800 Buhrke T, Kibellus A and Lampen A, 2013. *In vitro* toxicological characterization of perfluorinated
6801 carboxylic acids with different carbon chain lengths. *Toxicology Letters*, 218, 97–104.
- 6802 Buhrke T, Krüger E, Pevny S, Rößler M, Bitter K and Lampen A, 2015. Perfluorooctanoic acid (PFOA)
6803 affects distinct molecular signalling pathways in human primary hepatocytes. *Toxicology*, 333, 53–
6804 62.
- 6805 Bull S, Burnett K, Vassaux K, Ashdown L, Brown T and Rushton L, 2014. Extensive literature search and
6806 provision of summaries of studies related to the oral toxicity of perfluoroalkylated substances
6807 (PFASs), their precursors and potential replacements in experimental animals and humans. Area 1:
6808 Data on toxicokinetics (absorption, distribution, metabolism, excretion) in *in vitro* studies,
6809 experimental animals and humans. Area 2: Data on toxicity in experimental animals. Area 3: Data
6810 on observations in humans. EFSA supporting publication 2014;11(4):EN-572, 345 pp.
6811 doi:10.2903/sp.efsa.2014.EN-572
- 6812 Butenhoff JL, Kennedy GL Jr, Hinderliter PM, Lieder PH, Jung R, Hansen KJ, Gorman GS, Noker PE and
6813 Thomford PJ, 2004a. Pharmacokinetics of perfluorooctanoate in cynomolgus monkeys. *Toxicological*
6814 *Sciences*, 82, 394–406. <https://doi.org/10.1093/toxsci/kfh>
- 6815 Butenhoff JL, Olsen GW and Pfahles-Hutchens A, 2006. The applicability of biomonitoring data for
6816 perfluorooctanesulfonate to the environmental public health continuum. *Environmental Health*
6817 *Perspectives*, 114, 1776–1782.
- 6818 Butenhoff JL, Chang SC, Ehresman DJ and York RG, 2009. Evaluation of potential reproductive and
6819 developmental toxicity of potassium perfluorohexanesulfonate in Sprague Dawley rats. *Reproductive*
6820 *Toxicology* 27, 331–41.

- 6821 Butenhoff JL, Bjork JA, Chang SC, Ehresman DJ, Parker GA, Das K, Lau C, Lieder PH, van Otterdijk FM
6822 and Wallace KB, 2012. Toxicological evaluation of ammonium perfluorobutyrate in rats: twenty-eight-
6823 day and ninety-day oral gavage studies. *Reproductive Toxicology*, 33, 513–530.
- 6824 Bojes HK, Germolec DR, Simeonova P, Bruccolrei A, Schoonhoven R, Luster MI and Thurman RG, 1997.
6825 Antibodies to tumor necrosis factor alpha prevent increases in cell replication in liver due to the
6826 potent peroxisome proliferator WY14,643. *Carcinogenesis*, 18, 669–674.
- 6827 Bursch W, Lauer B, Timmermann-Trosiener I, Barthel G, Schuppler J and Schulte-Hermann R, 1984.
6828 Controlled death (apoptosis) of normal and putative preneoplastic cells in rat liver following
6829 withdrawal of tumor promoters. *Carcinogenesis*, 5, 453–458.
- 6830 Buser MC and Scinicariello F, 2016. Perfluoroalkyl substances and food allergies in adolescents.
6831 *Environment International*, 88, 74–79. <https://doi.org/10.1016/j.envint.2015.12.020>
- 6832 Butt CM, Mabury SA, Kwan M, Wang X and Muir DC, 2008. Spatial trends of perfluoroalkyl compounds
6833 in ringed seals (*Phoca hispida*) from the Canadian Arctic. *Environmental Technology and Chemistry*,
6834 27, 542–553.
- 6835 Bytingsvik J, Simon E, Leonards PE, Lamoree M, Lie E, Aars J, Derocher AE, Wiig O, Jenssen BM and
6836 Hamers T, 2013. Transthyretin-binding activity of contaminants in blood from polar bear (*Ursus*
6837 *maritimus*) cubs. *Environmental Science & Technology*, 47, 4778–4786. doi:10.1021/es305160v
- 6838 Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Tully JS and Needham LL, 2007. Serum concentrations of
6839 11 polyfluoroalkyl compounds in the U.S. population: data from the national health and nutrition
6840 examination survey (NHANES). *Environmental Science & Technology*, 41, 2237–2242.
- 6841 Calfee-Mason KG, Spear BT and Glauert HP, 2004. Effects of vitamin E on the NF-kappaB pathway in
6842 rats treated with the peroxisome proliferator, ciprofibrate. *Toxicology and Applied Pharmacology*,
6843 199, 1–9.
- 6844 Callan AC, Rotander A, Thompson K, Heyworth J, Mueller JF, Odland JØ and Hinwood AL, 2016. Maternal
6845 exposure to perfluoroalkyl acids measured in whole blood and birth outcomes in offspring. *Science*
6846 *of the Total Environment*, 569–570, 1107–1113. <https://doi.org/10.1016/j.scitotenv.2016.06.177>
- 6847 Campo J, Masiá A, Picó Y, Farré M and Barceló D, 2014. Distribution and fate of perfluoroalkyl substances
6848 in Mediterranean Spanish sewage treatment plants. *Science of The Total Environment* 472, 912–922.
- 6849 Campo J, Pérez F, Masiá A, Picó Y, Farré M and Barceló D, 2015. Perfluoroalkyl substance contamination
6850 of the Llobregat River ecosystem (Mediterranean area, NE Spain). *Science of the Total Environment*
6851 503–504, 48–57.
- 6852 Cardenas A, Gold DR, Hauser R, Kleinman KP, Hivert MF, Calafat AM, Ye X, Webster TF, Horton ES and
6853 Oken E, 2017. Plasma Concentrations of Per- and Polyfluoroalkyl Substances at Baseline and
6854 Associations with Glycemic Indicators and Diabetes Incidence among High-Risk Adults in the
6855 Diabetes Prevention Program Trial. *Environmental Health Perspectives*, 125, 107001. doi:
6856 10.1289/ehp1612
- 6857 Cariello NF, Romach EF, Colton HM, Ni H, Yoon L, Falls GJ, Casey W, Creech D, Anderson SP, Benavides
6858 GR, Hoivik DJ, Brown R and Miller RT, 2005. Gene Expression Profiling of the PPAR-alpha Agonist
6859 Ciprofibrate in the Cynomolgus Monkey Liver. *Toxicological Sciences*, 88, 1, 250–264.
6860 <https://doi.org/10.1093/toxsci/kfi273>
- 6861 Case MT, York RG and Christian MS, 2001. Rat and rabbit oral developmental toxicology studies with
6862 two perfluorinated compounds. *International Journal of Toxicology*, 20, 101–109.

- 6863 Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Tully JS and Needham LL, 2007a. Serum concentrations
6864 of 11 polyfluoroalkyl compounds in the U.S. population: data from the National Health and Nutrition
6865 Examination Survey (NHANES) 1999–2000. *Environmental Science & Technology*, 41, 2237–2242.
- 6866 Cariou R, Veyrand B, Yamada A, Berrebi A, Zalko D, Durand S, Pollono C, Marchand P, Leblanc JC,
6867 Antignac JP and Le Bizec B, 2015. Perfluoroalkyl acid (PFAA) levels and profiles in breast milk,
6868 maternal and cord serum of French women and their newborns. *Environment International*, 84, 71-
6869 81.
- 6870 Cassone CG, Vongphachan V, Chiu S, Williams KL, Letcher RJ, Pelletier E, Crump D and Kennedy SW,
6871 2012. *In ovo* effects of perfluorohexane sulfonate and perfluorohexanoate on pipping success,
6872 development, mRNA expression, and thyroid hormone levels in chicken embryos. *Toxicological*
6873 *Sciences*, 127, 216–224. doi: 10.1093/toxsci/kfs072.
- 6874 Cervený D, Grabic R, Fedorova G, Grabicova K, Turek J, Kodes V, Golovko O, Zlábek V and Randak T,
6875 2016. Perfluoroalkyl substances in aquatic environment-comparison of fish and passive sampling
6876 approaches. *Environmental Research*, 144, 92-98.
- 6877 Chan E, Burstyn I, Cherry N, Bamforth F and Martin JW, 2011. Perfluorinated acids and
6878 hypothyroxinemia in pregnant women. *Environmental Research*, 111, 559-564. doi:
6879 10.1016/j.envres.2011.01.011
- 6880 Chang SC, Thibodeaux JR, Eastvold ML, Ehresman DJ, Bjork JA, Froehlich JW, Lau C, Singh RJ, Wallace
6881 KB and Butenhoff JL, 2008. Thyroid hormone status and pituitary function in adult rats given oral
6882 doses of perfluorooctanesulfonate (PFOS). *Toxicology*, 20, 243, 330-339.
- 6883 Chang S, Allen BC, Andres KL, Ehresman DJ, Falvo R, Provencher A, Olsen GW and Butenhoff JL, 2017.
6884 Evaluation of Serum Lipid, Thyroid, and Hepatic Clinical Chemistries in Association with Serum
6885 Perfluorooctanesulfonate (PFOS) in Cynomolgus Monkeys After Oral Dosing with Potassium PFOS.
6886 *Toxicological Sciences*, 156, 387-401. doi:10.1093/toxsci/kfw267
- 6887 Chang S, Butenhoff JL, Parker GA, Coder PS, Zitzow JD, Krisko RM, Bjork JA, Wallace KB and Seed JG,
6888 2018. Reproductive and developmental toxicity of potassium perfluorohexanesulfonate in CD-1 mice.
6889 *Reproductive Toxicology*, 78, 150-168. doi:10.1016/j.reprotox.2018.04.007
- 6890 Chen MH, Ha EH, Liao HF, Jeng SF, Su YN, Wen TW, Lien GW, Chen CY, Hsieh WS and Chen PC, 2013.
6891 Perfluorinated compound levels in cord blood and neurodevelopment at 2 years of age.
6892 *Epidemiology*, 24, 800-808. doi: 10.1097/EDE.0b013e3182a6dd46
- 6893 Chen MH, Ha EH, Wen TW, Su YN, Lien GW, Chen CY, Chen PC and Hsieh WS, 2012. Perfluorinated
6894 compounds in umbilical cord blood and adverse birth outcomes. *PLoS ONE*, 7, e42474.
6895 <https://doi.org/10.1371/journal.pone.0042474>
- 6896 Chen Y, Hu W, Huang C, Hua S, Wei Q, Bai C, Chen J, Norris MB, Winn R, Yang D and Dong Q, 2016.
6897 Subchronic perfluorooctanesulfonate (PFOS) exposure induces elevated mutant frequency in an *in*
6898 *vivo* λ transgenic medaka mutation assay. *Science Reports*, 6, 38466. doi:10.1038/srep38466
- 6899 Chen W-L, Bai F-Y, Chang Y-C, Chen P-C and Chen C-Y, 2018. Concentrations of perfluoroalkyl
6900 substances in foods and the dietary exposure among Taiwan general population and pregnant
6901 women. *Journal of Food and Drug Analysis*, 26, 994-1004.
6902 <https://doi.org/10.1016/j.jfda.2017.12.011>
- 6903 Chen Y, Li H, Mo J, Chen X, Wu K, Ge F, Ma L, Li X, Guo X, Zhao J and Ge RS, 2019. Perfluorododecanoic
6904 Acid Blocks Rat Leydig Cell Development during Prepuberty. *Chemical Research in Toxicology*, 32,
6905 146-155.

- 6906 Cheng X and Klaassen CD, 2008b. Perfluorocarboxylic acids induce cytochrome P450 enzymes in mouse
6907 liver through activation of PPAR-alpha and CAR transcription factors. *Toxicological Sciences*, 106, 29-
6908 36.
- 6909 Cheng X and Klaassen CD, 2008a. Critical role of PPAR-alpha in perfluorooctanoic acid- and
6910 perfluorodecanoic acid-induced downregulation of Oatp uptake transporters in mouse livers.
6911 *Toxicological Sciences*, 106, 37-45.
- 6912 Chengelis CP, Kirkpatrick JB, Radovsky A and Shinohara M, 2009. A 90-day repeated dose oral (gavage)
6913 toxicity study of perfluorohexanoic acid (PFHxA) in rats (with functional observational battery and
6914 motor activity determinations). *Reproductive Toxicology*, 27, 342-351.
- 6915 Cheung C, Akiyama TE, Ward JM, Nicol CJ, Feigenbaum L, Vinson C and Gonzalez FJ, 2004. Diminished
6916 hepatocellular proliferation in mice humanized for the nuclear receptor peroxisome proliferator-
6917 activated receptor alpha. *Cancer Research*, 64, 3849-3854.
- 6918 Christensen KY, Maisonet M, Rubin C, Holmes A, Calafat AM, Kato K, Flanders WD, Heron J, McGeehin
6919 MA and Marcus M, 2011. Exposure to polyfluoroalkyl chemicals during pregnancy is not associated
6920 with offspring age at menarche in a contemporary British cohort. *Environment International*, 37,
6921 129-135. doi:10.1016/j.envint.2010.08.007
- 6922 Choi H, Bae IA, Choi JC, Park SJ and Kim M, 2018. Perfluorinated compounds in food simulants after
6923 migration from fluorocarbon resin-coated frying pans, baking utensils, and non-stick baking papers
6924 on the Korean market. *Food Additives and Contaminants B*, 11, 264-272.
6925 doi:10.1080/19393210.2018.1499677
- 6926 Chou WC and Lin Z, 2019. Bayesian evaluation of a physiologically based pharmacokinetic (PBPK) model
6927 for perfluorooctane sulfonate (PFOS) to characterize the interspecies uncertainty between mice, rats,
6928 monkeys, and humans: Development and performance verification. *Environment International*, 129,
6929 408-422. doi: 10.1016/j.envint.2019.03.058
- 6930 Chu S, Wang J, Leong G, Woodward LA, Letcher RJ and Li QX, 2015. Perfluoroalkyl sulfonates and
6931 carboxylic acids in liver, muscle and adipose tissues of black-footed albatross (*Phoebastria nigripes*)
6932 from Midway Island, North Pacific Ocean. *Chemosphere*, 138, 60-66.
6933 <https://doi.org/10.1016/j.chemosphere.2015.05.043>
- 6934 CLH report (Proposal for Harmonised Classification and Labelling). 8:2 Fluorotelomer alcohol (8:2
6935 FTOH). Version number: 1 Date: 20. March 2012
- 6936 Cohn BA, La Merrill MA, Krigbaum NY, Wang M, Park JS, Petreas M, Yeh G, Hovey RC, Zimmermann L
6937 and Cirillo PM, 2019. *In Utero* Exposure to Poly and Perfluoroalkyl Substances (PFASs) and
6938 Subsequent Breast Cancer. *Reproductive Toxicology*, pii: S0890-6238(18)30486-6. doi:
6939 10.1016/j.reprotox.2019.06.012
- 6940 de Cock M, de Boer MR, Lamoree M, Legler J and van de Bor M, 2014. First year growth in relation to
6941 prenatal exposure to endocrine disruptors - a Dutch prospective cohort study. *International Journal*
6942 *of Environmental Research and Public Health*, 11, 7001-7021.
- 6943 Convertino M, Church TR, Olsen GW, Liu Y, Doyle E, Elcombe CR, Barnett AL, Samuel LM, MacPherson
6944 IR and Evans TRJ, 2018. Modeling for Assessing the Systemic Health Risk of Perfluorooctanoate
6945 (PFOA). *Toxicological Sciences*, 163, 293-306.
- 6946 Coperchini F, Pignatti P, Lacerenza S, Negri S, Sideri R, Testoni C, de Martinis L, Cottica D, Magri F,
6947 Imbriani M, Rotondi M and Chiovato L, 2015. Exposure to perfluorinated compounds: *in vitro* study
6948 on thyroid cells. *Environmental Science and Pollution Research*, 22, 2287-2294.

- 6949 Corsini E, Avogadro A, Galbiati V, dell'Agli M, Marinovich M, Galli CL and Germolec DR, 2011. In vitro
6950 evaluation of the immunotoxic potential of perfluorinated compounds (PFCs). *Toxicology and Applied*
6951 *Pharmacology*, 250, 108–116.
- 6952 Corton JC, Peters JM and Klaunig JE, 2017. The PPAR α -dependent rodent liver tumor response is not
6953 relevant to humans: addressing misconceptions. *Archives of Toxicology*, 92, 83-119. doi:
6954 10.1007/s00204-017-2094-7
- 6955 Corton C, Cunningham ML, Hummer BT, Lau C, Meek B, Peters JM, Popp JA, Rhomberg L, Seed J and
6956 Klaunig JE, 2014. Mode of action framework analysis for receptor-mediated toxicity: The peroxisome
6957 proliferator-activated receptor alpha (PPAR α) as a case study. *Critical Reviews in Toxicology*, 44, 1-
6958 49, DOI: 10.3109/10408444.2013.835784
- 6959 Crawford NM, Fenton SE, Strynar M, Hines EP, Pritchard DA and Steiner AZ, 2017. Effects of
6960 perfluorinated chemicals on thyroid function, markers of ovarian reserve, and natural fertility.
6961 *Reproductive Toxicology*, 69, 53-59. doi: 10.1016/j.reprotox.2017.01.006
- 6962 Cui L, Zhou QF, Liao CY, Fu JJ and Jiang GB, 2009. Studies on the toxicological effects of PFOA and
6963 PFOS on rats using histological observation and chemical analysis. *Archives of Environmental*
6964 *Contamination and Toxicology*, 56, 338-349. doi: 10.1007/s00244-008-9194-6
- 6965 Cui Q, Pan Y, Zhang H, Sheng N and Dai J, 2018. Elevated concentrations of perfluorohexanesulfonate
6966 and other per and polyfluoroalkyl substances in Baiyangdian Lake (China): Source characterization
6967 and exposure assessment. *Environmental Pollution*, 241, 684-691.
6968 <https://doi.org/10.1016/j.envpol.2018.05.099>
- 6969 Curran I, Hierlihy SL, Liston V, Pantazopoulos P, Nunnikhoven A, Tittlemier S, Barker M, Trick K and
6970 Bondy G, 2008. Altered fatty acid homeostasis and related toxicologic sequelae in rats exposed to
6971 dietary potassium perfluorooctanesulfonate (PFOS). *Journal of Toxicology and Environmental Health*,
6972 71, 1526-1541.
- 6973 Dagnino S, Strynar MJ, McMahan RL, Lau CS, Ball C, Garantziotis S, Webster TF, McClean MD and
6974 Lindstrom AB, 2016. Identification of Biomarkers of Exposure to FTOHs and PAPs in Humans Using
6975 a Targeted and Nontargeted Analysis Approach. *Environmental Science & Technology*, 50, 10216-
6976 10225. doi:10.1021/acs.est.6b01170
- 6977 Dalsager L, Christensen N, Husby S, Kyhl H, Nielsen F, Host A, Grandjean P and Jensen TK, 2016.
6978 Association between prenatal exposure to perfluorinated compounds and symptoms of infections at
6979 age 1-4 years among 359 children in the Odense Child Cohort. *Environment International*, 96, 58-
6980 64. doi: 10.1016/j.envint.2016.08.026
- 6981 Das KP, Grey BE, Zehr RD, Wood CR, Butenhoff JL, Chang SC, Ehresman DJ, Tan YM and Lau C, 2008.
6982 Effects of perfluorobutyrate exposure during pregnancy in the mouse. *Toxicological Sciences*, 105,
6983 173-181. doi:10.1093/toxsci/kfn099
- 6984 Das KP, Grey BE, Rosen MB, Wood CR, Tatum-Gibbs KR, Zehr RD, Strynar MJ, Lindstrom AB and Lau C,
6985 2015. Developmental toxicity of perfluorononanoic acid in mice. *Reproductive Toxicology*, 51, 133-
6986 144. doi:10.1016/j.reprotox.2014.12.012
- 6987 Das KP, Wood CR, Lin MT, Starkov AA, Lau C, Wallace KB, Corton JC and Abbott BD, 2017. Perfluoroalkyl
6988 acids-induced liver steatosis: Effects on genes controlling lipid homeostasis. *Toxicology*, 378, 37-52.
6989 doi:10.1016/j.tox.2016.12.007
- 6990 Dassuncao C, Hu XC, Nielsen F, Weihe P, Grandjean P and Sunderland EM, 2018. Shifting Global
6991 Exposures to Poly- and Perfluoroalkyl Substances (PFASs) Evident in Longitudinal Birth Cohorts from
6992 a Seafood-Consuming Population. *Environmental Science & Technology*, 52, 3738-3747.

- 6993 Dassuncao C, Hu XC, Zhang X, Bossi R, Dam M, Mikkelsen B and Sunderland EM, 2017. Temporal Shifts
6994 in Poly- and Perfluoroalkyl Substances (PFASs) in North Atlantic Pilot Whales Indicate Large
6995 Contribution of Atmospheric Precursors. *Environmental Science & Technology*, 51, 4512-4521.
6996 doi:10.1021/acs.est.7b00293
- 6997 Ding L, Hao F, Shi Z, Wang Y, Zhang H, Tang H and Dai J, 2009. Systems biological responses to chronic
6998 perfluorododecanoic acid exposure by integrated metabolomic and transcriptomic studies. *Journal*
6999 *of Proteome Research*, 8, 2882-2891. doi:10.1021/pr9000256
- 7000 Dixon D1, Reed CE, Moore AB, Gibbs-Flournoy EA, Hines EP, Wallace EA, Stanko JP, Lu Y, Jefferson
7001 WN, Newbold RR and Fenton SE, 2012. Histopathologic changes in the uterus, cervix and vagina of
7002 immature CD-1 mice exposed to low doses of perfluorooctanoic acid (PFOA) in a uterotrophic assay.
7003 *Reproductive Toxicology*, 33, 506-512.
- 7004 De Felip E, Abballe A, Albano FL, Battista T, Carraro V, Conversano M, Franchini S, Giambanco L,
7005 Iacovella N, Ingelido AM, Maiorana A, Maneschi F, Marra V, Mercurio A, Nale R, Nucci B, Panella V,
7006 Pirola F, Porpora MG, Procopio E, Suma N, Valentini S, Valsenti L and Vecchiè V, 2015. Current
7007 exposure of Italian women of reproductive age to PFOS and PFOA: A human biomonitoring study.
7008 *Chemosphere*, 137, 1-8.
- 7009 Del Vento S, Halsall C, Gioia R, Jones K and Dachs J, 2012. Volatile per- and polyfluoroalkyl compounds
7010 in the remote atmosphere of the western Antarctic Peninsula: an indirect source of perfluoroalkyl
7011 acids to Antarctic waters? *Atmospheric Pollution Research*, 3, 450-455.
7012 <https://doi.org/10.5094/APR.2012.051>
- 7013 Denys S, Fraize-Frontier S, Moussa O, Le Bizec B, Veyrand B and Volatier JL, 2014. Is the fresh water
7014 fish consumption a significant determinant of the internal exposure to perfluoroalkylated substances
7015 (PFAS)? *Toxicology Letters*, 231, 233-238.
- 7016 De Silva AO and Mabury SA, 2006. Isomer distribution of perfluorocarboxylates in human blood:
7017 Potential correlation to source. *Environmental Science & Technology*, 40, 2903-2909.
- 7018 De Silva AO, Benskin JP, Martin LJ, Arsenault G, McCrindle R, Riddell N, Martin JW and Mabury SA, 2009.
7019 Disposition of perfluorinated acid isomers in Sprague-Dawley rats; part 2: subchronic dose.
7020 *Environmental Toxicology and Chemistry*, 28, 555-567. doi:10.1897/08-254.1
- 7021 DeWitt JC, Copeland CB, Strynar MJ and Luebke RW, 2008. Perfluorooctanoic acid-induced
7022 immunomodulation in adult C57BL/6J or C57BL/6N female mice. *Environmental Health Perspectives*,
7023 116, 644-650.
- 7024 Dewitt JC, Copeland CB and Luebke RW, 2009. Suppression of humoral immunity by perfluorooctanoic
7025 acid is independent of elevated serum corticosterone concentration in mice. *Toxicological Sciences*,
7026 109, 106-112.
- 7027 DeWitt JC, Williams WC, Creech NJ and Luebke RW, 2016. Suppression of antigen-specific antibody
7028 responses in mice exposed to perfluorooctanoic acid: Role of PPAR α and T- and B-cell targeting.
7029 *Journal of Immunotoxicology*, 13, 38-45. doi: 10.3109/1547691X.2014.996682.
- 7030 D'Eon JC and Mabury SA, 2007. Production of perfluorinated carboxylic acids (PFCAs) from the
7031 biotransformation of polyfluoroalkyl phosphate surfactants (PAPS): exploring routes of human
7032 contamination. *Environmental Science & Technology*, 41, 4799-4805.
- 7033 D'Eon JC and Mabury SA, 2011. Exploring indirect sources of human exposure to perfluoroalkyl
7034 carboxylates (PFCAs): evaluating uptake, elimination, and biotransformation of polyfluoroalkyl
7035 phosphate esters (PAPs) in the rat. *Environmental Health Perspectives*, 119, 344-350.
- 7036 Di Nisio A, Sabovic I, Valente U, Tescari S, Rocca, MS, Guidolin D, Dall'Acqua S, Acquasaliente L, Pozzi
7037 N, Plebani M, Garolla A and Foresta C, 2019. Endocrine Disruption of Androgenic Activity by

- 7038 Perfluoroalkyl Substances: Clinical and Experimental Evidence. *Clin Endocrinol Metab*, 104, 1259–
7039 1271. doi: 10.1210/jc.2018-01855
- 7040 Dobraca D, Israel L, McNeel S, Voss R, Wang M, Gajek R, Park JS, Harwani S, Barley F, She J and Das
7041 R, 2015. Biomonitoring in California firefighters: metals and perfluorinated chemicals. *Occupational*
7042 *and Environmental Medicine*, 57(1), 88-97.
- 7043 Domingo JL, 2011. Influence of cooking processes on the concentrations of environmental pollutants in
7044 food: a review of the published literature. *Critical Reviews in Food Science and Nutrition*, 51, 29–37.
- 7045 Domingo JL, Jogsten IE, Eriksson U, Martorella I, Perello G, Nadala M and van Bavel B, 2012. Human
7046 dietary exposure to perfluoroalkyl substances in Catalonia, Spain. *Temporal Trend. Food Chemistry*,
7047 135, 1575–1582.
- 7048 Domingo JL and Nadal M, 2017. Per- and Polyfluoroalkyl Substances (PFASs) in Food and Human Dietary
7049 Intake: A Review of the Recent Scientific Literature. *Journal of Agricultural and Food Chemistry*, 65,
7050 533-543. doi:10.1021/acs.jafc.6b04683
- 7051 Donat-Vargas C, Bergdahl IA, Tornevi A, Wennberg M, Sommar J, Koponen J, Kiviranta H and Akesson
7052 A, 2019. Associations between repeated measure of plasma perfluoroalkyl substances and
7053 cardiometabolic risk factors. *Environment International*, 124, 58-65.
- 7054 Dong GH, Zhang YH, Zheng L, Liu W, Jin YH and He QC, 2009. Chronic effects of
7055 perfluorooctanesulfonate exposure on immunotoxicity in adult male C57BL/6 mice. *Archives of*
7056 *Toxicology*, 83, 805–815.
- 7057 Dong GH, Liu MM, Wang D, Zheng L, Liang ZF and Jin YH, 2011. Sub-chronic effect of
7058 perfluorooctanesulfonate (PFOS) on the balance of type 1 and type 2 cytokine in adult C57BL6 mice.
7059 *Archives of Toxicology*, 85, 1235–1244.
- 7060 Dong GH, Wang J, Zhang YH, Liu MM, Wang D, Zheng L and Jin YH, 2012. Induction of p53-mediated
7061 apoptosis in splenocytes and thymocytes of C57BL/6 mice exposed to perfluorooctane sulfonate
7062 (PFOS). *Toxicology and Applied Pharmacology*, 264, 292–299.
- 7063 Dong GH, Tung KY, Tsai CH, Liu MM, Wang D, Liu W, Jin YH, Hsieh WS, Lee YL and Chen PC, 2013.
7064 Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-
7065 control study of Taiwanese children. *Environmental Health Perspectives*, 121, 507-513. doi:
7066 10.1289/ehp.1205351
- 7067 Dong Z, Wang H, Yu YYB, Naidu R and Liu Y, 2019. Using 2003-2014 U.S. NHANES data to determine
7068 the associations between per- and polyfluoroalkyl substances and cholesterol: Trend and
7069 implications. *Ecotoxicology and Environmental Safety*, 173, 461-468.
- 7070 Dreyer A, Weinberg I, Temme C and Ebinghaus R, 2009. Polyfluorinated Compounds in the Atmosphere
7071 of the Atlantic and Southern Oceans: Evidence for a Global Distribution. *Environmental Science &*
7072 *Technology*, 43, 6507-6514. doi:10.1021/es9010465
- 7073 Doull J, Cattley R, Elcombe C, Lake BG, Swenberg J, Wilkinson C, Williams G and van Gemert M.A, 1999.
7074 Cancer risk assessment of di(2-ethylhexyl)phthalate: application of the new U.S. EPA Risk
7075 Assessment Guidelines. *Regulatory Toxicology and Pharmacology*, 29, 327–357.
- 7076 Ducatman A, Zhang J and Fan H, 2015. Prostate-specific antigen and perfluoroalkyl acids in the C8
7077 health study population. *Journal of Occupational and Environmental Medicine*, 57, 111-114. doi:
7078 10.1097/JOM.0000000000000319.
- 7079 Dzierlenga AL, Robinson VG, Waidyanatha S, DeVito MJ, Eifrid MA, Gibbs ST, Granville CA and Blystone
7080 CR, 2019. Toxicokinetics of perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA) and

- 7081 perfluorodecanoic acid (PFDA) in male and female Hsd:Sprague dawley SD rats following intravenous
- 7082 or gavage administration. *Xenobiotica*. DOI: 10.1080/00498254.2019.1683776
- 7083 ECHA (European Chemicals Agency), 2013. Committee for Risk Assessment RAC Annex 1 Background
- 7084 document to the Opinion proposing harmonised classification and labelling at EU level of 8:2
- 7085 Fluorotelomer alcohol (8:2 FTOH). Available online:
- 7086 <https://echa.europa.eu/documents/10162/983ac315-260c-12a8-661a-3f5302e4941b>
- 7087 EFSA (European Food Safety Authority), 2008. Opinion of the Scientific Panel on Contaminants in the
- 7088 Food chain on Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. *EFSA*
- 7089 *Journal* 2008;6(7):653, 131 pp. <https://doi.org/10.2903/j.efsa.2008.653>
- 7090 EFSA (European Food Safety Authority), 2010a. Standard sample description for food and feed. *EFSA*
- 7091 *Journal* 2010;8(1):1457, 54 pp. <https://doi.org/10.2903/j.efsa.2010.1457>
- 7092 EFSA (European Food Safety Authority), 2010b. Management of left-censored data in dietary exposure
- 7093 assessment of chemical substances. *EFSA Journal* 2010;8(3):1557, 96 pp.
- 7094 <https://doi.org/10.2903/j.efsa.2010.1557>
- 7095 EFSA (European Food Safety Authority), 2011a. Use of the EFSA Comprehensive European Food
- 7096 Consumption Database in Exposure Assessment. *EFSA Journal* 2011;9(3):2097, 34 pp.
- 7097 <https://doi.org/10.2903/j.efsa.2011.2097>
- 7098 EFSA (European Food Safety Authority), 2011b. Evaluation of the FoodEx, the food classification system
- 7099 applied to the development of the EFSA Comprehensive European Food Consumption Database.
- 7100 *EFSA Journal* 2011;9(3):1970, 27 pp. <https://doi.org/10.2903/j.efsa.2011.1970>
- 7101 EFSA (European Food Safety Authority), 2011c. Report on the development of a Food Classification and
- 7102 Description System for exposure assessment and guidance on its implementation and use. *EFSA*
- 7103 *Journal* 2011;9(12):2489, 84 pp. <https://doi.org/10.2903/j.efsa.2011.2489>
- 7104 EFSA (European Food Safety Authority), 2012. Perfluoroalkylated substances in food: occurrence and
- 7105 dietary exposure. *EFSA Journal* 2012;10(6):2743, 55 pp. <https://doi.org/10.2903/j.efsa.2012.2743>
- 7106 EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific
- 7107 Committee, Scientific Panels and Units in the absence of actual measured data. *EFSA (European Food*
- 7108 *Safety Authority)*, 2015. The food classification and description system FoodEx2 (revision 2). EFSA
- 7109 supporting publication 2015:EN-804, 90 pp. <https://doi.org/10.2903/sp.efsa.2015.EN-804>
- 7110 EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2013. Scientific Opinion on
- 7111 nutrient requirements and dietary intakes of infants and young children in the European Union. *EFSA*
- 7112 *Journal* 2013;11(10):3408, 103 pp. <https://doi.org/10.2903/j.efsa.2013.3408>
- 7113 EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2010. Scientific Opinion on Lead
- 7114 in Food. *EFSA Journal* 2010;8(4):1570, 151 pp. <https://doi.org/10.2903/j.efsa.2010.1570>
- 7115 EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK, Alexander J,
- 7116 Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B,
- 7117 Hogstrand C, Hoogenboom LR, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot A-C, Vleminckx
- 7118 C, Vollmer G, Wallace H, Bodin L, Cravedi J-P, Halldorsson TI, Haug LS, Johansson N, van Loveren
- 7119 H, Gergelova P, Mackay K, Levorato S, van Manen M and Schwerdtle T, 2018. Scientific Opinion on
- 7120 the risk to human health related to the presence of perfluorooctane sulfonic acid and
- 7121 perfluorooctanoic acid in food. *EFSA Journal* 2018;16(12):5194, 284 pp. <https://doi.org/10.2903/j.efsa.2018.5194>
- 7122
- 7123 EFSA Scientific Committee, More SJ, Bampidis V, Benford D, Bennekou SH, Bragard C, Halldorsson TI,
- 7124 Hernandez-Jerez AF, Koutsoumanis K, Naegeli H, Schlatter JR, Silano V, Nielsen SS, Schrenk D, Turck
- 7125 D, Younes M, Benfenati E, Castle L, Cedergreen N, Hardy A, Laskowski R, Leblanc JC, Kortenkamp A,

- 7126 Ragas A, Posthuma L, Svendsen C, Solecki R, Testai E, Dujardin B, Kass GEN, Manini P, Jeddi MZ,
7127 Dorne J-LCM and Hogstrand C, 2019. Guidance on harmonised methodologies for human health,
7128 animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA
7129 Journal 2019;17(3):5634, 77 pp. <https://doi.org/10.2903/j.efsa.2019.5634>
- 7130 Ehresman DJ, Froehlich JW, Olsen GW, Chang SC and Butenhoff JL, 2007. Comparison of human whole
7131 blood, plasma, and serum matrices for the determination of perfluorooctanesulfonate (PFOS),
7132 perfluorooctanoate (PFOA), and other fluorochemicals. *Environmental Research*, 103, 176-184.
- 7133 Eke D, Celik A, Yilmaz MB, Aras N, Kocaturk Sel S and Alptekin D, 2017. Apoptotic gene expression
7134 profiles and DNA damage levels in rat liver treated with perfluorooctane sulfonate and protective
7135 role of curcumin. *International Journal of Biological Macromolecules*, 104, 515-520.
7136 doi:10.1016/j.ijbiomac.2017.06.075
- 7137 Eggen T, Moeder M and Arukwe A, 2010. Municipal landfill leachates: a significant source for new and
7138 emerging pollutants. *Science of the Total Environment*, 408, 5147-557.
- 7139 Elcombe CR, Elcombe BM, Foster JR, Chang SC, Ehresman DJ and Butenhoff JL, 2012. Hepatocellular
7140 hypertrophy and cell proliferation in Sprague-Dawley rats from dietary exposure to potassium
7141 perfluorooctanesulfonate results from increased expression of xenosensor nuclear receptors PPAR α
7142 and CAR/PXR. *Toxicology*, 293, 16-29.
- 7143 Ellis DA, Martin JW, De Silva AO, Mabury SA, Hurley MD, Sulbaek Andersen MP and Wallington TJ, 2004.
7144 Degradation of fluorotelomer alcohols: A likely atmospheric source of perfluorinated carboxylic acids.
7145 *Environmental Science & Technology*, 38, 3316–3321.
- 7146 Emmett EA, Shofer FS, Zhang H, Freeman D, Desai C and Shaw LM. 2006. Community exposure to
7147 perfluorooctanoate: Relationships between serum concentrations and exposure sources. *Journal of*
7148 *Occupational and Environmental Medicine*, 48, 759-770.
- 7149 Eryasa B, Grandjean P, Nielsen F, Valvi D, Zmirou-Navier D, Sunderland E, Weihe P and Oulhote Y,
7150 2019. Physico-chemical properties and gestational diabete predict transplacental transfer and
7151 partitioning of perfluoroalkyl substances. *Environment International*, 130, 104874.
- 7152 Health Effects Support Document for Perfluorooctanoic Acid (PFOA), EPA, May 2016. Available online:
7153 https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_hesd_final_508.pdf
- 7154 Ericson Jogsten I, Nadal M, van Bavel B, Lindström G and Domingo JL, 2012. Per- and polyfluorinated
7155 compounds (PFCS) in house dust and indoor air in Catalonia, Spain: implications for human exposure.
7156 *Environment International*, 39, 172-180.
- 7157 Eriksen KT, Raaschou-Nielsen O, Sørensen M, Roursgaard M, Loft S and Møller P, 2010. Genotoxic
7158 potential of the perfluorinated chemicals PFOA, PFOS, PFBS, PFNA and PFHxA in human HepG2 cells.
7159 *Mutation Research-Genetic Toxicology and Environmental Mutagenesis*, 700, 39-43.
- 7160 Eriksen KT, Sørensen M, McLaughlin JK, Tjønneland A, Overvad K and Raaschou-Nielsen O, 2011.
7161 Determinants of plasma PFOA and PFOS levels among 652 Danish men. *Environmental Science &*
7162 *Technology*, 45, 8137–8143. <https://doi.org/10.1021/es100626h>
- 7163 Eriksson U and Kärman A, 2015. World-Wide Indoor Exposure to Polyfluoroalkyl Phosphate Esters
7164 (PAPs) and other PFASs in Household Dust. *Environmental Science & Technology*, 49, 14503-14511.
- 7165 Eriksson U, Kärman A, Rotander A, Mikkelsen B and Dam M, 2013. Perfluoroalkyl substances (PFASs)
7166 in food and water from Faroe Islands. *Environmental Science and Pollution Research*, 20, 7940-7948.
7167 doi:10.1007/s11356-013-1700-3
- 7168 Eriksson U, Roos A, Lind Y, Hope K, Ekblad A and Kärman A, 2016a. Comparison of PFASs contamination
7169 in the freshwater and terrestrial environments by analysis of eggs from osprey (*Pandion haliaetus*),

- 7170 tawny owl (*Strix aluco*), and common kestrel (*Falco tinnunculus*). Environmental Research, 149, 40-
7171 47. doi: 10.1016/j.envres.2016.04.03
- 7172 Eriksson U, Mueller JF, Toms LL, Hobson P and Kärrman A, 2017. Temporal trends of PFASs, PFCAs and
7173 selected precursors in Australian serum from 2002 to 2013. Environmental Pollution, 220(Pt A), 168-
7174 177. doi: 10.1016/j.envpol.2016.09.036
- 7175 Ernst A, Brix N, Lauridsen LLB, Olsen J, Parner ET, Liew Z, Olsen LH and Ramlau-Hansen CH, 2019.
7176 Exposure to Perfluoroalkyl Substances during Fetal Life and Pubertal Development in Boys and Girls
7177 from the Danish National Birth Cohort. Environmental Health Perspectives, 127, 17004. doi:
7178 10.1289/EHP3567.
- 7179 Eschauzier C, Raat KJ, Stuyfzand PJ and De Voogt P, 2013. Perfluorinated alkylated acids in groundwater
7180 and drinking water: identification, origin and mobility. Science of Total Environment, 458-460, 477-
7181 485. doi:10.1016/j.scitotenv.2013.04.066
- 7182 Fair PA, Driscoll E, Mollenhauer MAM, Bradshaw SG, Yun SH, Kannan K, Bossart GD, Keil DE and Peden-
7183 Adams MM, 2011. Effects of environmentally-relevant levels of perfluorooctane sulfonate on clinical
7184 parameters and immunological functions in B 6C 3F 1 mice. Journal of Immunotoxicology, 8, 17–29.
- 7185 Fang X, Zhang L, Feng Y, Zhao Y and Dai J, 2008. Immunotoxic effects of perfluorononanoic acid on
7186 BALB/c mice. Toxicological Sciences, 105, 312-321. doi:10.1093/toxsci/kfn127
- 7187 Fang X, Feng Y, Shi Z and Dai J, 2009. Alterations of cytokines and MAPK signaling pathways are related
7188 to the immunotoxic effect of perfluorononanoic acid. Toxicological Sciences, 108, 367-376.
7189 doi:10.1093/toxsci/kfp019
- 7190 Fang X, Gao G, Xue H, Zhang X and Wang H, 2012a. *In vitro* and *in vivo* studies of the toxic effects of
7191 perfluorononanoic acid on rat hepatocytes and Kupffer cells. Environmental Toxicology and
7192 Pharmacology, 34, 484-494.
- 7193 Fang X, Gao G, Xue H, Zhang X and Wang H, 2012b. Exposure of perfluorononanoic acid suppresses
7194 the hepatic insulin signal pathway and increases serum glucose in rats. Toxicology, 294, 109– 115.
- 7195 Fang S, Zhao S, Zhang Y, Zhong W and Zhu L, 2014. Distribution of perfluoroalkyl substances (PFASs)
7196 with isomer analysis among the tissues of aquatic organisms in Taihu Lake, China. Environmental
7197 Pollution, 193, 224-232. <https://doi.org/10.1016/j.envpol.2014.07.006>
- 7198 Fang X, Gao G, Zhang X and Wang H, 2015. Perfluorononanoic acid disturbed the metabolism of lipid
7199 in the liver of streptozotocin-induced diabetic rats. Toxicology Mechanisms and Methods, 25, 622-
7200 627.
- 7201 Fang X, Wu C, Li H, Yuan W and Wang X, 2018. Elevation of intracellular calcium and oxidative stress
7202 is involved in perfluorononanoic acid-induced neurotoxicity. Toxicology and Industrial Health, 34,
7203 139-145. doi: 10.1177/0748233717742262
- 7204 Fasano WJ, Kennedy GL, Szostek B, Farrar DG, Ward RJ, Haroun L and Hinderliter PM, 2005. Penetration
7205 of ammonium perfluorooctanoate through rat and human skin *in vitro*. Drug and Chemical
7206 Toxicology, 28, 79-90.
- 7207 Fasano WJ, Carpenter SC, Gannon SA, Snow TA, Stadler JC, Kennedy GL, Buck RC, Korzeniowski SH,
7208 Hinderliter PM and Kemper RA, 2006. Absorption, distribution, metabolism, and elimination of 8-2
7209 fluorotelomer alcohol in the rat. Toxicological Sciences, 91, 341-355. doi:10.1093/toxsci/kfj160
- 7210 Fei C, McLaughlin JK, Tarone RE and Olsen J, 2007. Perfluorinated chemicals and fetal growth: a study
7211 within the Danish National Birth Cohort. Environmental Health Perspectives, 115, 1677–1682.
7212 <https://doi.org/10.1289/ehp.10506>

- 7213 Fei C, McLaughlin JK, Lipworth L and Olsen J, 2008. Prenatal exposure to perfluorooctanoate (PFOA)
7214 and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy.
7215 Environmental Health Perspectives, 116, 1391-1395.
- 7216 Fei C, McLaughlin JK, Lipworth L and Olsen J, 2010a. Prenatal exposure to PFOA and PFOS and risk of
7217 hospitalization for infectious diseases in early childhood. Environmental Research, 110, 773-777.
7218 <https://doi.org/10.1016/j.envres.2010.08.004>
- 7219 Fei C and Olsen J, 2011. Prenatal exposure to perfluorinated chemicals and behavioral or coordination
7220 problems at age 7 years. Environmental Health Perspectives, 119, 573-578.
- 7221 Fei C, McLaughlin JK, Lipworth L and Olsen J, 2010. Prenatal exposure to PFOA and PFOS and risk of
7222 hospitalization for infectious diseases in early childhood. Environmental Research, 110, 773-777.
- 7223 Felizeter S, McLachlan MS and de Voogt P, 2012. Uptake of perfluorinated alkyl acids by hydroponically
7224 grown lettuce (*Lactuca sativa*). Environmental Science and Technology, 46, 11735-11743. doi:
7225 10.1021/es302398u
- 7226 Feng X, Cao X, Zhao S, Wang X, Hua X, Chen L and Chen L, 2017. Exposure of Pregnant Mice to
7227 Perfluorobutanesulfonate Causes Hypothyroxinemia and Developmental Abnormalities in Female
7228 Offspring. Toxicological Sciences, 155, 409-419. doi:10.1093/toxsci/kfw219
- 7229 Filgo AJ, Quist EM, Hoenerhoff MJ, Brix AE, Kissling GE and Fenton SE, 2015. Perfluorooctanoic Acid
7230 (PFOA)-induced Liver Lesions in Two Strains of Mice Following Developmental Exposures: PPAR α Is
7231 Not Required. Toxicologic Pathology, 43, 558-568.
- 7232 Finlay C, 2008. 8-2 Telomer B Alcohol: acute oral toxicity-fixed dose method. DuPont-6711.
- 7233 Fisher M, Arbuckle TE, Wade M and Haines DA, 2013. Do perfluoroalkyl substances affect metabolic
7234 function and plasma lipids? - Analysis of the 2007-2009, Canadian Health Measures Survey (CHMS)
7235 Cycle 1. Environmental Research, 121, 95-103. doi: 10.1016/j.envres.2012.11.006
- 7236 Fisher M, Arbuckle TE, Liang CL, LeBlanc A, Gaudreau E, Foster WG, Haines D, Davis K and Fraser WD,
7237 2016. Concentrations of persistent organic pollutants in maternal and cord blood from the maternal-
7238 infant research on environmental chemicals (MIREC) cohort study. Environmental Health, 15, 59.
7239 doi: 10.1186/s12940-016-0143-y.
- 7240 Fleisch AF, Rifas-Shiman SL, Mora AM, Calafat AM, Ye X, Luttmann-Gibson H, Gillman MW, Oken E and
7241 Sagiv SK, 2017. Early-Life Exposure to Perfluoroalkyl Substances and Childhood Metabolic Function.
7242 Environmental Health Perspectives, 125, 481-487. doi:10.1289/ehp303
- 7243 Fletcher T, Galloway TS, Melzer D, Holcroft P, Cipelli R, Pilling LC, Mondal D, Luster M and Harries LW,
7244 2013. Associations between PFOA, PFOS and changes in the expression of genes involved in
7245 cholesterol metabolism in humans. Environment International, 57-58, 2-10.
- 7246 Foreman JE, Chang SC, Ehresman DJ, Butenhoff JL, Anderson CR, Palkar PS, Kang BH, Gonzalez FJ and
7247 Peters JM, 2009. Differential hepatic effects of perfluorobutyrate mediated by mouse and human
7248 PPAR- α . Toxicological Sciences, 110, 204-211.
- 7249 Franken C, Koppen G, Lambrechts N, Govarts E, Bruckers L, Den Hond E, Loots I, Nelen V, Sioen I,
7250 Nawrot TS, Baeyens W, Van Larebeke N, Boonen F, Ooms D, Wevers M, Jacobs G, Covaci A,
7251 Schettgen T and Schoeters G, 2017. Environmental exposure to human carcinogens in teenagers
7252 and the association with DNA damage. Environmental Research, 152, 165-174.
7253 doi:10.1016/j.envres.2016.10.012
- 7254 Framtiden i våre hender, online. Om Framtiden i våre henders kartlegging av PFAS i kosmetikk, mars
7255 2018. Available online:
7256 https://www.framtiden.no/bilder/dokumenter/bakgrunn_kosmetikk_mars2018.pdf

- 7257 Franko J, Meade BJ, Frasch HF, Barbero AM and Anderson SE, 2012. Dermal penetration potential of
7258 perfluorooctanoic acid (PFOA) in human and mouse skin. *Journal of Toxicology and Environmental*
7259 *Health A*, 75, 50-62.
- 7260 Frawley RP, Smith M, Cesta MF, Hayes-Bouknight S, Blystone C, Kissling GE, Harris S and Germolec D,
7261 2018. Immunotoxic and hepatotoxic effects of perfluoro-n-decanoic acid (PFDA) on female Harlan
7262 Sprague-Dawley rats and B6C3F1/N mice when administered by oral gavage for 28 days. *Journal of*
7263 *Immunotoxicology*, 15, 41-52. doi:10.1080/1547691x.2018.1445145
- 7264 Freberg BI, Haug LS, Olsen R, Daae HL, Hersson M, Thomsen C, Thorud S, Becher G, Molander P and
7265 Ellingsen DG, 2010. Occupational exposure to airborne perfluorinated compounds during professional
7266 ski waxing. *Environmental Science & Technology*, 44, 7723-7728.
7267 <https://doi.org/10.1021/es102033k>
- 7268 Frisbee SJ, Brooks AP Jr, Maher A, Flensburg P, Arnold S, Fletcher T, Steenland K, Shankar A, Knox SS,
7269 Pollard C, Halverson JA, Vieira VM, Jin C, Leyden KM and Ducatman AM, 2009. The C8 health project:
7270 design, methods, and participants. *Environmental Health Perspectives*, 117, 1873-1882.
- 7271 Fromme H, Schlummer M, Möller A, Gruber L, Wolz G and Ungewiss J 2007. Exposure of an adult
7272 population to perfluorinated substances using duplicate diet portions and biomonitoring data.
7273 *Environment Science and Technology*, 41, 7928-7933
- 7274 Fromme H, Tittlemier SA, Völkel W, Wilhelm M and Twardella D. 2009. Perfluorinated compounds -
7275 Exposure assessment for the general population in western countries. *International Journal of*
7276 *Hygiene and Environmental Health*, 212, 239-270.
- 7277 Fromme H, Mosch C, Morovitz M, Alba-Alejandre I, Boehmer S, Kiranoglu M, Faber F, Hannibal I, Genzel-
7278 Boroviczény O, Koletzko B and Völkel W, 2010. Pre- and postnatal exposure to perfluorinated
7279 compounds (PFCs). *Environmental Science & Technology*, 44, 7123-7129.
- 7280 Fromme H, Dreyer A, Dietrich S, Fembacher L, Lahrz T and Völkel W, 2015. Neutral polyfluorinated
7281 compounds in indoor air in Germany-the LUPE 4 study. *Chemosphere*, 139, 572-578.
- 7282 Fromme H, Wöckner M, Roscher E and Völkel W, 2017. ADONA and perfluoroalkylated substances in
7283 plasma samples of German blood donors living in South Germany. *International Journal of Hygiene*
7284 *and Environmental Health*, 220 (2 Pt B), 455-460.
7285 <https://www.ncbi.nlm.nih.gov/pubmed/28073630>
- 7286 Fu J, Gao Y, Wang T, Liang Y, Zhang A, Wang Y and Jiang G, 2015. Elevated levels of perfluoroalkyl
7287 acids in family members of occupationally exposed workers: the importance of dust transfer.
7288 *Scientific Reports*, 5, 9313. doi: 10.1038/srep09313.
- 7289 Fu Y, Wang T, Fu Q, Wang P and Lu Y, 2014. Associations between serum concentrations of
7290 perfluoroalkyl acids and serum lipid levels in a Chinese population. *Ecotoxicology and Environmental*
7291 *Safety*, 106, 246-252.
- 7292 Fu Y, Wang T, Wang P, Fu Q and Lu Y, 2014. Effects of age, gender and region on serum concentrations
7293 of perfluorinated compounds in general population of Henan, China. *Chemosphere*, 110, 104-110.
7294 doi: 10.1016/j.chemosphere.2014.02.020
- 7295 Fujii Y, Harada KH and Koizumi A, 2013. Occurrence of perfluorinated carboxylic acids (PFCAs) in
7296 personal care products and compounding agents. *Chemosphere*, 93, 538-544.
7297 <https://doi.org/10.1016/j.chemosphere.2013.06.049>
- 7298 Fujii Y, Niisoe T, Harada KH, Uemoto S, Ogura Y, Takenaka K and Koizumi A, 2015. Toxicokinetics of
7299 perfluoroalkyl carboxylic acids with different carbon chain lengths in mice and humans. *Journal of*
7300 *Occupational Health*, 57, 1-12. doi:10.1539/joh.14-0136-OA

- 7301 Furdui VI, Stock NL, Ellis DA, Butt CM, Whittle DM, Crozier PW, Reiner EJ, Muir DC and Mabury SA,
7302 2007. Spatial distribution of perfluoroalkyl contaminants in lake trout from the Great Lakes.
7303 *Environmental Science & Technology*, 41, 1554-1559.
- 7304 Gaillard J, Veyrand B, Thomas M, Dauchy X, Boiteux V, Marchand P, Le Bizec B, Banas D and Feidt C,
7305 2017. Tissue Uptake, Distribution, and Elimination of Perfluoroalkyl Substances in Juvenile Perch
7306 through Perfluorooctane Sulfonamidoethanol Based Phosphate Diester Dietary Exposure.
7307 *Environmental Science & Technology*, 51, 7658-7666. doi:10.1021/acs.est.6b05598
- 7308 Galatius A, Bossi R, Sonne C, Riget FF, Kinze CC, Lockyer C, Teilmann J and Dietz R, 2013. PFAS profiles
7309 in three North Sea top predators: metabolic differences among species? *Environmental Science and*
7310 *Pollution Research*, 20, 8013-8020. doi:10.1007/s11356-013-1633-x
- 7311 Gallo V, Leonardi G, Brayne C, Armstrong B and Fletcher T, 2013. Serum perfluoroalkyl acids
7312 concentrations and memory impairment in a large cross-sectional study. *BMJ Open*, 3, e002414.
7313 doi:10.1136/bmjopen-2012-002414
- 7314 Gallo V, Leonardi G, Genser B, Lopez-Espinosa MJ, Frisbee SJ, Karlsson L, Ducatman AM and Fletcher
7315 T, 2012. Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) concentrations and
7316 liver function biomarkers in a population with elevated PFOA exposure. *Environmental Health*
7317 *Perspectives*, 120, 655-660.
- 7318 Gannon SA, Johnson T, Nabb DL, Serex TL, Buck RC and Loveless SE, 2011. Absorption, distribution,
7319 metabolism, and excretion of [1-14C]-perfluorohexanoate ([14C]-PFHx) in rats and mice. *Toxicology*,
7320 283, 55-62. <https://doi.org/10.1016/j.tox.2011.02.004>
- 7321 Gao Y, Fu J, Cao H, Wang Y, Zhang A, Liang Y, Wang T, Zhao C and Jiang G, 2015. Differential
7322 accumulation and elimination behavior of perfluoroalkyl Acid isomers in occupational workers in a
7323 manufactory in China. *Environmental Science & Technology*, 49, 6953-6962.
7324 doi:10.1021/acs.est.5b00778
- 7325 Gao K, Gao Y, Li Y, Fu J and Zhang A, 2016. A rapid and fully automatic method for the accurate
7326 determination of a wide carbon-chain range of per- and polyfluoroalkyl substances (C4-C18) in
7327 human serum. *Journal of Chromatography A*, pii: S0021-9673(16)31266-3.
- 7328 García-Valcárcela AI, Moleroa E, Escorialb MC, Chuecab MC and Tadeo JL, 2014. Uptake of
7329 perfluorinated compounds by plants grown in nutrient solution. *Science of the Total Environment*,
7330 472, 20-26.
- 7331 Gazouli M, Yao ZX, Boujrad N, Corton JC, Culty M and Papadopoulos V, 2002. Effect of peroxisome
7332 proliferators on Leydig cell peripheral-type benzodiazepine receptor gene expression, hormone-
7333 stimulated cholesterol transport, and steroidogenesis: Role of the peroxisome proliferator-activator
7334 receptor α . *Endocrinology*, 143, 2571–2583.
- 7335 Gebbink WA, Glynn A and Berger U, 2015. Temporal changes (1997-2012) of perfluoroalkyl acids and
7336 selected precursors (including isomers) in Swedish human serum. *Environmental Pollution*, 199, 166-
7337 173.
- 7338 Gebbink WA, Bignert A and Berger U, 2016. Perfluoroalkyl acids (PFAAs) and selected precursors in the
7339 Baltic seaenvironment: do precursors play a role in food web accumulation of PFAAs? *Environmental*
7340 *Science Technology*, 50, 6354–6362.
- 7341 Gewurtz SB, Backus SM, De Silva AO, Ahrens L, Armellin A, Evans M, Fraser S, Gledhill M, Guerra P,
7342 Harner T, Helm PA, Hung H, Khera N, Kim MG, King M, Lee SC, Letcher RJ, Martin P, Marvin C,
7343 McGoldrick DJ, Myers AL, Pelletier M, Pomeroy J, Reiner EJ, Rondeau M, Sauve MC, Sekela M, Shoeib
7344 M, Smith DW, Smyth SA, Struger J, Spry D, Syrgiannis J and Waltho J, 2013. Perfluoroalkyl acids in

- the Canadian environment: multi-media assessment of current status and trends. *Environment International*, 59, 183-200. doi:10.1016/j.envint.2013.05.008
- Ghisi R, Vamerali T and Manzetti, 2019. Accumulation of perfluorinated alkyl substances (PFAS) in agricultural plants: A review. *Environmental Research*, 169, 326-341, <https://doi.org/10.1016/j.envres.2018.10.023>
- Giesy JP and Kannan K, 2001. Global distribution of perfluorooctane sulfonate in wildlife. *Environmental Science & Technology*, 35, 1339-1342. <https://doi.org/10.1021/es001834k>
- Gleason JA, Post GB and Fagliano JA, 2015. Associations of perfluorinated chemical serum concentrations and biomarkers of liver function and uric acid in the US population (NHANES), 2007-2010. *Environmental Research*, 136, 8-14. doi: 10.1016/j.envres.2014.10.004
- Glynn A, Berger U, Bignert A, Ullah S, Aune M, Lignell S, and Darnerud PO, 2012. Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996-2010. *Environmental Science & Technology*, 46, 9071-9079.
- Gobelius L, Lewis J and Ahrens, L, 2017. Plant uptake of Per- and Polyfluoroalkyl Substances at a Contaminated Fire Training facility to Evaluate the Phytoremediation Potential of Various Plant Species. *Environmental Science & Technology*, 51, 12602-12610.
- Goeritz I, Falk S, Stahl T, Schafers C and Schlechtriem C, 2013. Biomagnification and tissue distribution of perfluoroalkyl substances (PFASs) in market-size rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry*, 32, 2078-2088. doi:10.1002/etc.2279
- Goldenberg RL, Culhane JF, Iams JD and Romero R, 2008. Epidemiology and causes of preterm birth. *The Lancet*, 371, 75-84.
- Goll V, Alexandre E, Viollon-Abadie C, Nicod L, Jaeck D and Richert L, 1999. Comparison of the effects of various peroxisome proliferators on peroxisomal enzyme activities, DNA synthesis, and apoptosis in rat and human hepatocyte cultures. *Toxicology and Applied Pharmacology*, 160, 21-32
- Gómez-Canela C, Fernandez-Sanjuan M, Farres M and Lacorte S, 2015. Factors affecting the accumulation of perfluoroalkyl substances in human blood. *Environmental Science and Pollution Research International*, 22, 1480-1486. <https://doi.org/10.1007/s11356-014-3439-x>
- Gomis MI, Vestergren R, Borg D and Cousins IT, 2018. Comparing the toxic potency *in vivo* of long-chain perfluoroalkyl acids and fluorinated alternatives. *Environment International*, 113, 1-9. doi: 10.1016/j.envint.2018.01.011.
- Gorrochategui E, Pérez-Albaladejo E, Casas J, Lacorte S and Porte C, 2014. Perfluorinated chemicals: Differential toxicity, inhibition of aromatase activity and alteration of cellular lipids in human placental cells. *Toxicology and Applied Pharmacology*, 277, 124-130. doi: 10.1016/j.taap.2014.03.012
- Goudarzi H, Miyashita C, Okada E, Kashino I, Kobayashi S, Chen CJ, Ito S, Araki A, Matsuura H, Ito YM and Kishi R, 2016. Effects of prenatal exposure to perfluoroalkyl acids on prevalence of allergic diseases among 4-year-old children. *Environment International*, 94, 124-132. doi: 10.1016/j.envint.2016.05.020
- Goudarzi H, Miyashita C, Okada E, Kashino I, Chen CJ, Ito S, Araki A, Kobayashi S, Matsuura H and Kishi R, 2017. Prenatal exposure to perfluoroalkyl acids and prevalence of infectious diseases up to 4 years of age. *Environment International*, 104, 132-138. <https://doi.org/10.1016/j.envint.2017.01.024>
- Govarts E, Iszatt N, Trnovec T, de Cock M, Eggesbo M, Murinova LP, van de Bor M, Guxens M, Chevrier C, Koppen G, Lamoree M, Hertz-Picciotto I, Lopez-Espinosa M-J, Lertxundi A, Grimalt JO, Torrent M, Goni-Irigoyen F, Vermeulen R, Legler J and Schoeters G, 2018. Prenatal exposure to endocrine disrupting chemicals and risk of being born small for gestational age: Pooled analysis of seven

- 7389 European birth cohorts. *Environmental International*, 115, 267-278.
7390 <https://doi.org/10.1016/j.envint.2018.03.017>
- 7391 Graber JM, Alexander C, Laumbach RJ, Black K, Strickland PO, Georgopoulos PG, Marshall EG, Shendell
7392 DG, Alderson D, Mi Z, Mascari M and Weisel CP, 2019. Per and polyfluoroalkyl substances (PFAS)
7393 blood levels after contamination of a community water supply and comparison with 2013-2014
7394 NHANES. *Journal of Exposure Science and Environmental Epidemiology*, 29, 172-182.
7395 doi:10.1038/s41370-018-0096-z.
- 7396 Grandjean P, Weihe P, Jorgensen PJ, Clarkson T, Cernichiari E and Videro T, 1992. Impact of maternal
7397 seafood diet on fetal exposure to mercury, selenium, and lead. *Archives of Environmental Health*,
7398 47, 185-195. doi:10.1080/00039896.1992.9938348
- 7399 Grandjean P, Bjerre KS, Weihe P and Steuerwald U, 2001. Birthweight in a fishing community:
7400 significance of essential fatty acids and marine food contaminants. *International Journal of*
7401 *Epidemiology*, 30, 1272-1278.
- 7402 Grandjean P, Andersen EW, Budtz-Jorgensen E, Nielsen F, Molbak K, Weihe P and Heilmann C, 2012.
7403 Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA*, 307,
7404 391-397. doi: 10.1001/jama.2011.2034
- 7405 Grandjean P, Heilmann C, Weihe P, Nielsen F, Mogensen UB, Timmermann A and Budtz-Jorgensen E,
7406 2017a. Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine
7407 antibody concentrations at age 5-years. *Journal of Immunotoxicology*, 14, 188-195.
7408 doi:10.1080/1547691x.2017.1360968
- 7409 Grandjean P, Heilmann C, Weihe P, Nielsen F, Mogensen UB and Budtz-Jorgensen E, 2017b. Serum
7410 Vaccine Antibody Concentrations in Adolescents Exposed to Perfluorinated Compounds.
7411 *Environmental Health Perspectives*, 125, 077018. doi:10.1289/ehp275
- 7412 Granum B, Haug LS, Namork E, Stølevik SB, Thomsen C, Aaberge IS, van Loveren H, Løvik M and
7413 Nygaard UC, 2013. Pre-natal exposure to perfluoroalkyl substances may be associated with altered
7414 vaccine antibody levels and immune-related health outcomes in early childhood. *Journal of*
7415 *Immunotoxicology*, 10, 373-379.
- 7416 Gremmel C, Fromel T and Knepper TP, 2016. Systematic determination of perfluoroalkyl and
7417 polyfluoroalkyl substances (PFASs) in outdoor jackets. *Chemosphere*, 160, 173-180.
7418 doi:10.1016/j.chemosphere.2016.06.043
- 7419 Gribble MO, Bartell SM, Kannan K, Wu Q, Fair PA and Kamen DL, 2015. Longitudinal measures of
7420 perfluoroalkyl substances (PFAS) in serum of Gullah African Americans in South Carolina: 2003-2013.
7421 *Environmental Research*, 143(Pt B), 82-88.
- 7422 Gump BB, Wu Q, Dumas AK and Kannan K, 2011. Perfluorochemical (PFC) exposure in children:
7423 associations with impaired response inhibition. *Environmental Science & Technology*. 45, 8151-8159.
- 7424 Guruge KS, Hikono H, Shimada N, Murakami K, Hasegawa J, Yeung LW, Yamanaka N and Yamashita N,
7425 2009. Effect of perfluorooctane sulfonate (PFOS) on influenza A virus-induced mortality in female
7426 B6C3F1 mice. *Journal of Toxicological Sciences*, 34, 687-691.
- 7427 Guruge KS, Noguchi M, Yoshioka K, Yamazaki E, Taniyasu S, Yoshioka M, Yamanaka N, Ikezawa M,
7428 Tanimura N, Sato M, Yamashita N and Kawaguchi H, 2016. Microminipigs as a new experimental
7429 animal model for toxicological studies: comparative pharmacokinetics of perfluoroalkyl acids. *Journal*
7430 *of Applied Toxicology*, 36, 68-75. doi:10.1002/jat.3145
- 7431 Gützkow KB, Haug LS, Thomsen C, Sabaredzovic A, Becher G and Brunborg G, 2012. Placental transfer
7432 of perfluorinated compounds is selective-a Norwegian Mother and Child sub-cohort study.
7433 *International Journal of Hygiene and Environmental Health*, 215, 216-219.

- 7434 Gyllenhammar I, Berger U, Sundström M, Lignell S, Aune M, Darnerud PO, Kotova N and Glynn A, 2013.
7435 Perfluoroalkyl Acids in Serum from Nursing Women Living in an Area in Sweden with Drinking Water
7436 Contamination. Nationell miljöövervakning på uppdrag av naturvårdsverket. Report NV-02409-12
- 7437 Gyllenhammar I, Berger U, Sundström M, McCleaf P, Eurén K, Eriksson S, Ahlgren S, Lignell S, Aune M,
7438 Kotova N and Glynn A, 2015. Influence of contaminated drinking water on perfluoroalkyl acid levels
7439 in human serum-A case study from Uppsala, Sweden. *Environmental Research*, 140, 673-683.
- 7440 Haarr A, Hylland K, Eckbo N, Gabrielsen GW, Herzke D, Bustnes JO, Blevin P, Chastel O, Moe B, Hanssen
7441 SA, Sagerup K and Borga K, 2017. DNA damage in Arctic seabirds: Baseline, sensitivity to a genotoxic
7442 stressor, and association with organohalogen contaminants. *Environmental Toxicology and*
7443 *Chemistry*, 37, 1084-1091. doi:10.1002/etc.4035
- 7444 Hadrup N, Mikael Pedersen, Kasper Skov, Niels Lund Hansen. Berthelsen LO, Kongsbak K, Boberg J,
7445 Dybdahl M, Hass U, Frandsen H and Vinggaard AM, 2016. Perfluorononanoic acid in combination
7446 with 14 chemicals exerts low-dose mixture effects in rats. *Archives of Toxicology*, 90, 661-675.
- 7447 Hagen DF, Belisle J, Johnson JD and Venkateswarlu P, 1981. Characterization of fluorinated metabolites
7448 by a gas chromatographic-helium microwaveplasma detector. The biotransformation of
7449 1H,1H,2H,2H-perfluorodecanol to perfluorooctanoate. *Analytical Biochemistry*, 118, 336-343.
- 7450 Haines DA and Murray J, 2012. Human biomonitoring of environmental chemicals--early results of the
7451 2007-2009 Canadian Health Measures Survey for males and females. *Hygiene and Environmental*
7452 *Health*, 215, 133-137.
- 7453 Halldorsson TI, Fei C, Olsen J, Lipworth L, McLaughlin JK and Olsen SF, 2008. Dietary predictors of
7454 perfluorinated chemicals: a study from the Danish National Birth Cohort. *Environmental Science &*
7455 *Technology*, 42, 8971-8977.
- 7456 Halldorsson TI, Rytter D, Haug LS, Bech BH, Danielsen I, Becher G, Henriksen TB and Olsen SF, 2012.
7457 Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective
7458 cohort study. *Environmental Health Perspectives*, 120, 668-673.
- 7459 Halsne R, Tandberg JJ, Lobert VH, Østby GC, Thoen E, Ropstad E and Verhaegen S, 2016. Effects of
7460 perfluorinated alkyl acids on cellular responses of MCF-10A mammary epithelial cells in monolayers
7461 and on acini formation in vitro. *Toxicology Letters*. 259, 95-107. doi: 10.1016/j.toxlet.2016.08.004
- 7462 Han X, Yang CH and Snajdr SI, 2008. Uptake of perfluorooctanoate in freshly isolated hepatocytes from
7463 male and female rats. *Toxicology Letters* 181, 81-86.
- 7464 Hamm MP, Cherry NM, Chan E, Martin JW and Burstyn I, 2010. Maternal exposure to perfluorinated
7465 acids and fetal growth. *Journal of Exposure Science and Environmental Epidemiology*, 20, 589-597.
7466 <https://doi.org/10.1038/jes.2009.57>
- 7467 Han X, Nabb DL, Russell MH, Kennedy GL and Rickard RW, 2012. Renal elimination of
7468 perfluorocarboxylates (PFCAs). *Chemical Research in Toxicology*, 25, 35-46.
7469 <https://doi.org/10.1021/tx200363w>
- 7470 Han R, Hu M, Zhong Q, Wan C, Liu L, Li F, Zhang F and Ding W, 2018a. Perfluorooctane sulphonate
7471 induces oxidative hepatic damage via mitochondria-dependent and NF-kappaB/TNF-alpha-mediated
7472 pathway. *Chemosphere*, 191, 1056-1064. doi:10.1016/j.chemosphere.2017.08.070
- 7473 Han R, Zhang F, Wan C, Liu L, Zhong Q and Ding W, 2018b. Effect of perfluorooctane sulphonate-
7474 induced Kupffer cell activation on hepatocyte proliferation through the NF-kappaB/TNF-alpha/IL-6-
7475 dependent pathway. *Chemosphere*, 200, 283-294. doi:10.1016/j.chemosphere.2018.02.137

- 7476 Hansen KJ, Clemen LA, Ellefson ME and Johnson HO, 2001. Compound-specific, quantitative
7477 characterization of organic fluorochemicals in biological matrices, *Environmental Science &*
7478 *Technology*, 35, 766-770.
- 7479 Hansen S, Vestergren R, Herzke D, Melhus M, Evenset A, Hanssen L, Brustad M and Sandanger TM,
7480 2016. Exposure to per- and polyfluoroalkyl substances through the consumption of fish from lakes
7481 affected by aqueous film-forming foam emissions - A combined epidemiological and exposure
7482 modeling approach. *The SAMINOR 2 Clinical Study. Environment International*, 94, 272-282.
- 7483 Hanssen L, Dudarev AA, Huber S, Odland JO, Nieboer E and Sandanger TM, 2013. Partition of
7484 perfluoroalkyl substances (PFASs) in whole blood and plasma, assessed in maternal and umbilical
7485 cord samples from inhabitants of arctic Russia and Uzbekistan. *Science of the Total Environment*,
7486 447, 430-437. doi:10.1016/j.scitotenv.2013.01.029
- 7487 Hardell E, Kärman A, van Bavel B, Bao J, Carlberg M and Hardell L, 2014. Case-control study on
7488 perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer. *Environment International*, 63, 35-
7489 39.
- 7490 Harrad S, de Wit CA, Abdallah MA, Bergh C, Björklund JA, Covaci A, Darnerud PO, de Boer J, Diamond
7491 M, Huber S, Leonards P, Mandalakis M, Ostman C, Haug LS, Thomsen C and Webster TF, 2010.
7492 Indoor contamination with hexabromocyclododecanes, polybrominated diphenyl ethers, and
7493 perfluoroalkyl compounds: an important exposure pathway for people? *Environmental Science &*
7494 *Technology*, 44, 3221-3231.
- 7495 Harris MW and Birnbaum LS, 1989a. Developmental toxicity of perfluorodecanoic acid in C57BL/6N mice.
7496 *Fundamental and Applied Toxicology*, 12, 442-448.
- 7497 Harris MW, Uraih LC, Birnbaum LS, 1989b. Acute toxicity of perfluorodecanoic acid in C57BL/6 mice
7498 differs from 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Fundamental and Applied Toxicology*, 13, 723-736.
7499 doi:10.1016/0272-0590(89)90330-8
- 7500 Hartman TJ, Calafat AM, Holmes AK, Marcus M, Northstone K, Flanders WD, Kato K and Taylor EV,
7501 2017. Prenatal Exposure to Perfluoroalkyl Substances and Body Fatness in Girls. *Child Obesity*, 13,
7502 222-230. doi:10.1089/chi.2016.0126
- 7503 Hasmall SC, James NH, Macdonald N, Gonzalez FJ, Peters JM and Roberts RA, 2000. Suppression of
7504 mouse hepatocyte apoptosis by peroxisome proliferators: role of PPAR α and TNF α . *Mutation*
7505 *Research*, 448, 193-200.
- 7506 Hasmall SC, James NH, Macdonald N, West D, Chevalier S, Cosulich SC and Roberts RA, 1999.
7507 Suppression of apoptosis and induction of DNA synthesis *in vitro* by the phthalate plasticizers
7508 monoethylhexylphthalate (MEHP) and diisononylphthalate (DINP): a comparison of rat and human
7509 hepatocytes *in vitro*. *Archives of Toxicology*, 73, 451-456.
- 7510 Haug LS, Thomsen C and Becher G, 2009 Time trends and the influence of age and gender on serum
7511 concentrations of perfluorinated compounds in archived human samples. *Environment and Science*
7512 *Technology*, 43, 2131-2136.
- 7513 Haug LS, Thomsen c, Brantsæter AL, Kvale HE, Haugen M, Becher G, Alexander J, Meltzer HM and
7514 Knutsen HK, 2010. Diet and particularly seafood are major sources of perfluorinated compounds in
7515 humans. *Environment International*, 36, 772-778.
- 7516 Haug LS, Huber S, Schlabach M, Becher G and Thomsen C, 2011a. Investigation on per- and
7517 polyfluorinated compounds in paired samples of house dust and indoor air from Norwegian homes.
7518 *Environmental Science & Technology*, 45, 7991-7998.

- 7519 Haug LS, Huber S, Becher G and Thomsen C, 2011b. Characterisation of human exposure pathways to
7520 perfluorinated compounds-comparing exposure estimates with biomarkers of exposure. *Environment*
7521 *International*, 37, 687-693.
- 7522 Heffernan AL, Cunningham TK, Drage DS, Alyward LL, Thompson K, Vijayasarathy S, Mueller JF, Atkin
7523 SL and Sathypalan T, 2018. Perfluorinated alkyl acids in the serum and follicular fluid of UK women
7524 with and without polycystic ovarian syndrome undergoing fertility treatment and associations with
7525 hormonal and metabolic parameters. *International Journal of Hygiene and Environmental Health*,
7526 221, 1068-1075.
- 7527 Heilmann C, Budtz-Jørgensen E, Nielsen F, Heinzow B, Weihe P and Grandjean P, 2010. Serum
7528 concentrations of antibodies against vaccine toxoids in children exposed perinatally to
7529 immunotoxicants. *Environmental Health Perspectives*, 118, 1434-1438. doi:10.1289/ehp.1001975
- 7530 Henderson WM and Smith MA, 2007. Perfluorooctanoic acid and perfluorononanoic acid in fetal and
7531 neonatal mice following in utero exposure to 8-2 fluorotelomer alcohol. *Toxicological Science*, 95,
7532 452-461. doi:10.1093/toxsci/kfl162
- 7533 Higgins CP and Luthy R G, 2006. Sorption of Perfluorinated Surfactants on Sediments. *Environmental*
7534 *Science & Technology*, 40, 23, 7251-7256.
- 7535 Hirata-Koizumi M, Fujii S, Furukawa M, Ono A and Hirose A, 2012. Repeated dose and
7536 reproductive/developmental toxicity of perfluorooctadecanoic acid in rats. *Journal of Toxicological*
7537 *Sciences*, 37, 63-79.
- 7538 Hirata-Koizumi M, Fujii S, Hina K, Matsumoto M, Takahashi M, Ono A and Hirose A, 2015. Repeated
7539 dose and reproductive/developmental toxicity of long-chain perfluoroalkyl carboxylic acids in rats:
7540 perfluorohexadecanoic acid and perfluorotetradecanoic acid. *Fundamental Toxicological Sciences*, 2,
7541 177-190.
- 7542 Hoffman K, Webster TF, Weisskopf MG, Weinberg J and Vieira VM, 2010. Exposure to polyfluoroalkyl
7543 chemicals and attention deficit/hyperactivity disorder in U.S. children 12-15 years of age.
7544 *Environmental Health Perspectives*, 118, 1762-1767.
- 7545 Hoivik DJ, Qualls CW Jr, Mirabile RC, Cariello NF, Kimbrough CL, Colton HM, Anderson SP, Santostefano
7546 MJ, Morgan RJ, Dahl RR, Brown AR, Zhao Z, Mudd PN Jr, Oliver WB Jr, Brown HR and Miller RT,
7547 2004. Fibrates induce hepatic peroxisome and mitochondrial proliferation without overt evidence of
7548 cellular proliferation and oxidative stress in cynomolgus monkeys. *Carcinogenesis*, 25, 1757-1769.
- 7549 Holmström KE, Johansson A-K, Bignert A, Lindberg P and Berger U, 2010. Temporal Trends of
7550 Perfluorinated Surfactants in Swedish Peregrine Falcon Eggs (*Falco peregrinus*), 1974–2007.
7551 *Environmental Science & Technology*, 44, 4083–4088.
- 7552 Hölzer J, Midasch O, Rauchfuss K, Kraft M, Reupert R, Angerer J, Kleeschulte P, Marschall N and Wilhelm
7553 M, 2008. Biomonitoring of perfluorinated compounds in children and adults exposed to
7554 perfluorooctanoate-contaminated drinking water. *Environmental Health Perspectives*, 116, 651-657.
- 7555 Houde M, De Silva AO, Muir DCG and Letcher RJ, 2011. Monitoring of perfluorinated compounds in
7556 aquatic biota: an updated review. *Environmental Science & Technology*, 45, 7962-7973.
- 7557 Houde M, Martin JW, Letcher RJ, Solomon KR and Muir DCG, 2006. Biological monitoring of
7558 polyfluoroalkyl substance: a review. *Environmental Science & Technology*, 40, 3463–3473.
- 7559 Hu XC, Dassuncao C, Zhang X, Grandjean P, Weihe P, Webster GM, Nielsen F and Sunderland EM, 2018.
7560 Can profiles of poly- and Perfluoroalkyl substances (PFASs) in human serum provide information on
7561 major exposure sources? *Environmental Health*, 17, 11. DOI 10.1186/s12940-018-0355-4

- 7562 Hu W, Jones PD, Upham BL, Trosko JE, Lau C and Giesy JP, 2002. Inhibition of gap junctional
7563 intercellular communication by perfluorinated compounds in rat liver and dolphin kidney epithelial
7564 cell lines in vitro and Sprague-Dawley rats *in vivo*. *Toxicological Sciences*, 68, 429-436.
- 7565 Huang M, Jiao J, Zhuang P, Chen X, Wang J and Zhang Y, 2018. Serum polyfluoroalkyl chemicals are
7566 associated with risk of cardiovascular diseases in national US population. *Environment International*,
7567 119, 37-46. doi: 10.1016/j.envint.2018.05.051
- 7568 Huang MC, Dzierlenga AL, Robinson VG, Waidyanatha S, DeVito MJ, Edifrid MA, Granville CA, Gibbs ST
7569 and Blystone CR, 2019. Toxicokinetics of perfluorobutane sulfonate (PFBS), perfluorohexane-1-
7570 sulphonic acid (PFHxS), and perfluorooctane sulfonic acid (PFOS) in male and female Hsd:Sprague
7571 Dawley SD rats after intravenous and gavage administration. *Toxicology Reports*, 6, 645-655. doi:
7572 10.1016/j.toxrep.2019.06.016
- 7573 Huck I, Beggs K and Apte U, 2018. Paradoxical Protective Effect of Perfluorooctanesulfonic Acid Against
7574 High-Fat Diet-Induced Hepatic Steatosis in Mice. *International Journal of Toxicology*, 37, 383-392.
7575 doi:10.1177/1091581818790934
- 7576 Hui Z, Li R and Chen L, 2017. The impact of exposure to environmental contaminant on hepatocellular
7577 lipid metabolism. *Gene*, 622, 67-71. doi:10.1016/j.gene.2017.04.024
- 7578 Hurley S, Goldberg D, Wang M, Park JS, Petreas M, Bernstein L, Anton-Culver H, Nelson DO and
7579 Reynolds P, 2018. Breast cancer risk and serum levels of per- and poly-fluoroalkyl substances: a
7580 case-control study nested in the California Teachers Study. *Environmental Health Perspectives*, 1 -
7581 Abstracts of the International Society for Environmental Epidemiology (ISEE),
7582 doi:10.1289/isesisee.2018.004.02.40
- 7583 Huybrechts I, Sioen I, Boon PE, Ruprich J, Lafay L, Turrini A, Amiano P, Hirvonen T, De Neve M, Arcella
7584 D, Moschandreas J, Westerlund A, Ribas-Barba L, Hilbig A, Papoutsou S, Christensen T, Oltarzewski
7585 M, Virtanen S, Rehurkova I, Azpiri M, Sette S, Kersting M, Walkiewicz A, Serra-Majem L, Volatier JL,
7586 Trolle E, Tornaritis M, Busk L, Kafatos A, Fabiansson S, De Henauw S and Van Klaveren JD,
7587 2011. Dietary exposure assessments for children in Europe (the EXPOCHI project): rationale,
7588 methods and design. *Archives of Public Health*, 69, 1169-1184.
- 7589 IARC (International Agency for Research on Cancer), 2016. Monograph Perfluorooctanoic acid. Available
7590 online: <http://monographs.iarc.fr/ENG/Monographs/vol110/mono110-01.pdf>
- 7591 Impinen A, Nygaard UC, Lodrup Carlsen KC, Mowinckel P, Carlsen KH, Haug LS and Granum B, 2018.
7592 Prenatal exposure to perfluoroalkyl substances (PFASs) associated with respiratory tract infections but
7593 not allergy- and asthma-related health outcomes in childhood. *Environmental Research*, 160, 518-
7594 523. doi: 10.1016/j.envres.2017.10.012
- 7595 Impinen A, Longnecker MP, Nygaard UC, London SJ, Ferguson KK, Haug LS and Granum B, 2019.
7596 Maternal levels of perfluoroalkyl substances (PFASs) during pregnancy and childhood allergy and
7597 asthma related outcomes and infections in the Norwegian Mother and Child (MoBa) cohort.
7598 *Environment International*, 124, 462-472.
- 7599 Ingelido AM, Marra V, Abballe A, Valentini S, Iacovella N, Barbieri P, Porpora MG, Domenico Ad and De
7600 Felip E, 2010. Perfluorooctanesulfonate and perfluorooctanoic acid exposures of the Italian general
7601 population. *Chemosphere*, 80(10), 1125-1130.
- 7602 Ingelido AM, Abballe A, Gemma S, Dellatte E, Iacovella N, De Angelis G, Zampaglioni F, Marra V, Miniero
7603 R, Valentini S, Russo F, Vazzoler M, Testai E and De Felip E, 2018. Biomonitoring of perfluorinated
7604 compounds in adults exposed to contaminated drinking water in the Veneto Region, Italy.
7605 *Environment International*, 110, 149-159. doi:10.1016/j.envint.2017.10.026

- 7606 Ito Y, Yamanoshita O, Kurata Y, Kamijima M, Aoyama T and Nakajima T, 2007a. Induction of peroxisome
7607 proliferator-activated receptor alpha (PPARα)-related enzymes by di(2-ethylhexyl) phthalate (DEHP)
7608 treatment in mice and rats, but not marmosets. *Archives of Toxicology*, 81, 219–226.
- 7609 Ito Y, Yamanoshita O, Asaeda N, Tagawa Y, Lee CH, Aoyama T, Ichihara G, Furuhashi K, Kamijima M,
7610 Gonzalez F J, and Nakajima T, 2007b. Di(2-ethylhexyl)phthalate induces hepatic tumorigenesis
7611 through a peroxisome proliferator-activated receptor alpha-independent pathway. *Journal of*
7612 *Occupational Health*, 49, 172–182.
- 7613 Iwai H, 2011. Toxicokinetics of ammonium perfluorohexanoate. *Drug and Chemical Toxicology*, 34, 341-
7614 346. doi:10.3109/01480545.2011.585162
- 7615 Iwai H and Hoberman AM, 2014. Oral (Gavage) Combined Developmental and Perinatal/Postnatal
7616 Reproduction Toxicity Study of Ammonium Salt of Perfluorinated Hexanoic Acid in Mice. *International*
7617 *Journal of Toxicology*, 33, 219-237. doi:10.1177/1091581814529449
- 7618 Iwai H, Hoberman AM, Goodrum PE, Mendelsohn E and Anderson JK, 2019, Addendum to Iwai and
7619 Hoberman (2014)—Reassessment of Developmental Toxicity of PFHxA in Mice. *International Journal*
7620 *of Toxicology*, 38, 183-191.
- 7621 Iwase Y, Kudo N, Toyama T, Tamura M, Mitsumoto A and Kawashima Y, 2006. Effects of 8-2
7622 fluorotelomer alcohol on oleic acid formation in the liver of rats. *Biological and Pharmaceutical*
7623 *Bulletin*, 29, 1740-1746.
- 7624 Iwabuchi K, Senzaki N, Mazawa D, Sato I, Hara M, Ueda F, Liu W and Tsuda S, 2017. Tissue
7625 toxicokinetics of perfluoro compounds with single and chronic low doses in male rats. *Journal of*
7626 *Toxicological Sciences*, 42, 301-317. doi:10.2131/jts.42.301
- 7627 James NH, Soames AR and Roberts RA, 1998. Suppression of hepatocyte apoptosis and induction of
7628 DNA synthesis by the rat and mouse hepatocarcinogen diethylhexylphthalate (DEHP) and the mouse
7629 hepatocarcinogen 1,4-dichlorobenzene (DCB). *Archives of Toxicology*, 72, 784–790.
- 7630 Jolly RA, Goldstein KM, Wei T, Gao H, Chen P, Huang S, Colet JM, Ryan TP, Thomas CE and Estrem ST,
7631 2005. Pooling samples within microarray studies: a comparative analysis of rat liver transcription
7632 response to prototypical toxicants. *Physiological Genomics*, 22, 346–355.
- 7633 Jahnke A and Berger U, 2009. Trace analysis of per- and polyfluorinated alkyl substances in various
7634 matrices—How do current methods perform? *Journal of Chromatography A*, 1216, 410–421.
7635 <https://doi.org/10.1016/j.chroma.2008.08.098>
- 7636 Jain RB, 2013. Association between thyroid profile and perfluoroalkyl acids: data from NHANES 2007-
7637 2008. *Environmental Research*, 126, 51-59. doi: 10.1016/j.envres.2013.08.006
- 7638 Jain RB, 2019. Concentration of selected liver enzymes across the stages of glomerular function: the
7639 associations with PFOA and PFOS. *Heliyon*, 5, e02168. doi: 10.1016/j.heliyon.2019.e02168
- 7640 Jain RB and Ducatman A, 2019. Roles of gender and obesity in defining correlations between
7641 perfluoroalkyl substances and lipid/lipoproteins. *Science of the Total Environment*, 653, 74-81.
- 7642 Jain RB, 2014. Contribution of diet and other factors to the levels of selected polyfluorinated compounds:
7643 data from NHANES 2003-2008. *International Journal of Hygiene and Environmental Health*, 217, 52-
7644 61.
- 7645 Jain RB and Ducatman A, 2019. Selective Associations of Recent Low Concentrations of Perfluoroalkyl
7646 Substances with Liver Function Biomarkers: NHANES 2011 to 2014 Data on US Adults Aged ≥20
7647 Years. *Journal of Occupational and Environmental Medicine*, 61, 293-302. doi:
7648 10.1097/jom.0000000000001532

- 7649 Jakobsson K, Kronholm Diab K, Lindh C, Persson B and Jönsson B, 2014. Exponering för perfluorerade
7650 ämnen (PFAS) i dricksvatten i Ronneby kommun. Rapport 8/2014. Available online:
7651 [https://sodrasjukvardsregionen.se/download/exponering-for-perfluorerade-amnen-pfas-i-](https://sodrasjukvardsregionen.se/download/exponering-for-perfluorerade-amnen-pfas-i-dricksvatten-i-ronneby-kommun/)
7652 [dricksvatten-i-ronneby-kommun/](https://sodrasjukvardsregionen.se/download/exponering-for-perfluorerade-amnen-pfas-i-dricksvatten-i-ronneby-kommun/)
- 7653 Jensen MS, Nørgaard-Pedersen B, Toft G, Hougaard DM, Bonde JP, Cohen A, Thulstrup AM, Ivell R,
7654 Anand-Ivell R, Lindh CH and Jönsson BA, 2012. Phthalates and perfluorooctanesulfonic acid in human
7655 amniotic fluid: temporal trends and timing of amniocentesis in pregnancy. *Environmental Health*
7656 *Perspectives*, 120, 897-903.
- 7657 Jensen TK, Andersen LB, Kyhl HB, Nielsen F, Christesen HT and Grandjean P, 2015. Association between
7658 perfluorinated compound exposure and miscarriage in Danish pregnant women. *PLoS ONE*, 10,
7659 e0123496. <https://doi.org/10.1371/journal.pone.0123496>
- 7660 Ji B, Bi Y, Simeone D, Mortensen RM and Logsdon CD, 2001. Human pancreatic acinar cells lack
7661 functional responses to cholecystokinin and gastrin. *Gastroenterology*, 121, 1380–1390.
- 7662 Ji K, Kim S, Kho Y, Paek D, Sakong J, Ha J, Kim S and Choi K, 2012. Serum concentrations of major
7663 perfluorinated compounds among the general population in Korea: dietary sources and potential
7664 impact on thyroid hormones. *Environment International*, 45, 78-85. doi:
7665 10.1016/j.envint.2012.03.007
- 7666 Jin H, Zhang Y, Jiang W, Zhu L and Martin JW, 2016. Isomer-Specific Distribution of Perfluoroalkyl
7667 Substances in Blood. *Environmental Science & Technology*, 50, 7808-7815.
7668 doi:10.1021/acs.est.6b01698
- 7669 Jo A, Ji K and Choi K, 2014. Endocrine disruption effects of long-term exposure to perfluorodecanoic
7670 acid (PFDA) and perfluorotridecanoic acid (PFTrDA) in zebrafish (*Danio rerio*) and related
7671 mechanisms. *Chemosphere*, 108, 360-366.
- 7672 Joensen UN, Bossi R, Leffers H, Jensen AA, Skakkebaek NE and Jorgensen N, 2009. Do prefluoroalkyl
7673 compounds ompair human semen quality? *Environmental Health Perspectives*, 117, 923-927.
- 7674 Joensen UN, Veyrand B, Antignac JP, Blomberg Jensen M, Petersen JH, Marchand P, Skakkebaek NE,
7675 Andersson AM, Le Bizec B and Jørgensen N, 2013. PFOS (perfluorooctanesulfonate) in serum is
7676 negatively associated with testosterone levels, but not with semen quality, in healthy men. *Human*
7677 *Reproduction*, 28, 599-608.
- 7678 Johansson JH, Berger U, Vestergren R, Cousin IT, Bignert A, Glynn A and Darnerud PO, 2014. Temporal
7679 trends (1999e2010) of perfluoroalkyl acids in commonlyconsumed food items. *Environmental*
7680 *Pollution*, 188, 102-108. <http://dx.doi.org/10.1016/j.envpol.2014.01.026>
- 7681 Jørgensen KT, Specht IO, Lenters V, Bach CC, Rylander L, Jönsson BAG, Lindh CH, Giwercman A,
7682 Heederik D, Toft G, Bonde JP, 2014. Perfluoroalkyl substances and time to pregnancy in couples
7683 from Greenland, Poland and Ukraine. *Environmental Health*, 13, 116. [https://doi.org/10.1186/1476-](https://doi.org/10.1186/1476-069X-13-116)
7684 [069X-13-116](https://doi.org/10.1186/1476-069X-13-116)
- 7685 Jogsten IE, Perello G, Llebaria X, Bigas E, Marti-Cid R, Karrman A and Domingo JL, 2009. Exposure to
7686 perfluorinated compounds in Catalonia, Spain, through consumption of various raw and cooked
7687 foodstuffs, including packaged food. *Food and Chemical Toxicology*, 47, 1577-1583.
7688 doi:10.1016/j.fct.2009.04.004
- 7689 Johansson N, Fredriksson A and Eriksson P, 2008. Neonatal exposure to perfluorooctane sulfonate
7690 (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice.
7691 *Neurotoxicology*, 29, 160-169. doi:10.1016/j.neuro.2007.10.008

- 7692 Kannan K, Corsolini S, Falandysz J, Fillmann G, Kumar KS, Loganathan BG, Mohd MA, Olivero J, Van
7693 Wouwe N, Yang KH and Aldous KM, 2004. Perfluorooctanesulfonate and Related Fluorochemicals in
7694 Human Blood from Several Countries. *Environmental Science & Technology*, 38, 4489-4495.
- 7695 Johnson PI, Sutton P, Atchley DS, Koustas E, Lam J, Sen S, Robinson KA, Axelrad DA and Woodruff TJ,
7696 2014, The Navigation Guide-Evidence-Based Medicine Meets Environmental Health: Systematic
7697 Review of Human Evidence for PFOA Effects on Fetal Growth. *Environmental Health Perspectives*,
7698 122, 1028-1039.
- 7699 Karagas MR, Choi AL, Oken E, Horvat M, Schoeny R, Kamai E, Cowell W, Grandjean P and Korrick S,
7700 2012. Evidence on the human health effects of low-level methylmercury exposure. *Environmental*
7701 *Health Perspectives*, 120, 799-806. doi:10.1289/ehp.1104494
- 7702 Karlsen M, Grandjean P, Weihe P, Steuerwald U, Oulhote Y and Valvi D, 2016. Early-life exposures to
7703 persistent organic pollutants in relation to overweight in preschool children. *Reproductive Toxicology*,
7704 68, 145-153. doi:10.1016/j.reprotox.2016.08.002
- 7705 Kärman A, Ericson I, van Bavel B, Darnerud PO, Aune M, Glynn A, Lignell S and Lindström G, 2007.
7706 Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum
7707 and a temporal trend, 1996-2004, in Sweden. *Environmental Health Perspectives*, 115, 226-230.
- 7708 Kärman A, van Bavel B, Järnberg U, Hardell L and Lindström G, 2006. Perfluorinated chemicals in
7709 relation to other persistent organic pollutants in human blood. *Chemosphere*, 64, 1582-1591.
7710 doi:10.1016/j.chemosphere.2005.11.040
- 7711 Kataria A, Trachtman H, Malaga-Dieguez L and Trasande L, 2015. Association between perfluoroalkyl
7712 acids and kidney function in a cross-sectional study of adolescents. *Environmental Health*, 14, 89.
7713 doi: 10.1186/s12940-015-0077-9
- 7714 Kato K, Wong LY, Jia LT, Kuklenyik Z and Calafat AM, 2011. Trends in exposure to polyfluoroalkyl
7715 chemicals in the U.S. Population: 1999-2008. *Environment Science and Technology*, 45, 8037-8345.
- 7716 Kato, K, Wong LY, Chen A, Dunbar C, Webster GM, Lanphear BP and Calafat AM, 2014. Changes in
7717 serum concentrations of maternal poly- and perfluoroalkyl substances over the course of pregnancy
7718 and predictors of exposure in a multiethnic cohort of Cincinnati, Ohio pregnant women during
7719 2003–2006. *Environmental Science & Technology*, 48, 9600–9608.
- 7720 Kato K, Ye X and Calafat AM, 2015a. PFASs in the general population. In: *Toxicological Effects of*
7721 *Perfluoroalkyl and Polyfluoroalkyl Substances*. Series: Molecular and Integrative Toxicology. De Witt
7722 JC (ed.). Springer International Publishing Switzerland, 51-76. DOI 10.1007/978-3-319-15518-0_3
- 7723 Kato H, Fujii S, Takahashi M, Matsumoto M, Hirata-Koizumi M, Ono A and Hirose A, 2015b. Repeated
7724 dose and reproductive/developmental toxicity of perfluorododecanoic acid in rats. *Environmental*
7725 *Toxicology*, 30, 1244-1263. doi:10.1002/tox.21996
- 7726 Kawabata K, Matsuzaki H, Nukui S, Okazaki M, Sakai A, Kawashima Y and Kudo N, 2017.
7727 Perfluorododecanoic Acid Induces Cognitive Deficit in Adult Rats. *Toxicological Sciences*, 157, 421-
7728 428. doi:10.1093/toxsci/kfx058
- 7729 Kawamoto K, Sato I, Tsuda S, Yoshida M, Yaegashi K, Saito N, Liu W and Jin Y, 2011. Ultrasonic-induced
7730 tonic convulsion in rats after subchronic exposure to perfluorooctane sulfonate (PFOS). *Journal of*
7731 *Toxicological Sciences*, 36, 55-62.
- 7732 Kawashima Y, Kobayashi H, Miura H and Kozuka H, 1995. Characterization of hepatic responses of rat
7733 to administration of perfluorooctanoic and perfluorodecanoic acids at low levels. *Toxicology*, 99, 169-
7734 178.

- 7735 Kersten S and Stienstra R, 2017. The role and regulation of the peroxisome proliferator activated
7736 receptor alpha in human liver. *Biochimie*, 136, 75–84. <https://doi.org/10.1016/j.biochi.2016.12.019>
- 7737 Keil DE, Mehlmann T, Butterworth L and Peden-Adams MM, 2008. Gestational exposure to
7738 perfluorooctane sulfonate suppresses immune function in B6C3F1 mice. *Toxicological Sciences*, 103,
7739 77-85.
- 7740 Kelly BC, Ikonomou MG, Blair JD, Surridge B, Hoover D, Grace R and Gobas FAPC, 2009. Perfluoroalkyl
7741 Contaminants in an Arctic Marine Food Web: trophic Magnification and Wildlife Exposure.
7742 *Environmental Science & Technology*, 43, 4037–4043.
- 7743 Kerger BD, Copeland TL and DeCaprio AP, 2011. Tenuous dose-response correlations for common
7744 disease states: case study of cholesterol and perfluorooctanoate/sulfonate (PFOA/PFOS) in the C8
7745 Health Project. *Drug and Chemical Toxicology*, 34, 396-404.
- 7746 Khalil N, Chen A, Lee M, Czerwinski SA, Ebert JR, DeWitt JC and Kannan K, 2016. Association of
7747 Perfluoroalkyl Substances, Bone Mineral Density, and Osteoporosis in the U.S. Population in NHANES
7748 2009-2010. *Environmental Health Perspectives*, 124, 81-87.
- 7749 Kielsen K, Shamim Z, Ryder LP, Nielsen F, Grandjean P, Budtz-Jorgensen E and Heilmann C, 2016.
7750 Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to
7751 perfluorinated alkylates. *Journal of Immunotoxicology*, 13, 270-273. doi:
7752 10.3109/1547691x.2015.1067259
- 7753 Kim S, Choi K, Ji K, Seo J, Kho Y, Park J, Kim S, Park S, Hwang I, Jeon J, Yang H and Giesy JP, 2011a.
7754 Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid
7755 hormones. *Environmental Science & Technology*, 45, 7465-7472.
- 7756 Kim SK, Lee KT, Kang CS, Tao L, Kannan K, Kim KR, Kim CK, Lee JS, Park PS, Yoo YW, Ha JY, Shin YS
7757 and Lee JH, 2011b. Distribution of perfluorochemicals between sera and milk from the same mothers
7758 and implications for prenatal and postnatal exposures. *Environmental Pollution*, 159, 169-174.
- 7759 Kim DH, Lee MY and Oh JE, 2014. Perfluorinated compounds in serum and urine samples from children
7760 aged 5-13 years in South Korea. *Environmental Pollution*, 192, 171-178.
- 7761 Kim DH, Kim UJ, Kim HY, Choi SD and Oh JE, 2016. Perfluoroalkyl substances in serum from South
7762 Korean infants with congenital hypothyroidism and healthy infants—Its relationship with thyroid
7763 hormones. *Environmental Research*, 147, 399–404. <https://doi.org/10.1016/j.envres.2016.02.037>
- 7764 Kim SJ, Shin H, Lee YB and Cho HY, 2018. Sex-specific risk assessment of PFHxS using a physiologically
7765 based pharmacokinetic model. *Archives of Toxicology*, 92, 1113-1131. doi:10.1007/s00204-017-
7766 2116-5
- 7767 Kissa E, 2001. Fluorinated surfactants and repellents, 2nd Edition. Surfactant Science Series, Volume 97.
7768 Marcel Dekker Inc., New York, 623 pp. ISBN: 0-8247-0472-X
- 7769 Klaunig JE, Shinohara M, Iwai H, Chengelis CP, Kirkpatrick JB, Wang Z and Bruner RH, 2015. Evaluation
7770 of the chronic toxicity and carcinogenicity of perfluorohexanoic acid (PFHxA) in Sprague-Dawley rats.
7771 *Toxicologic Pathology*, 43, 209-220.
- 7772 Kleszczyński K, Gardzielewski P, Mulkiewicz E, Stepnowski P and Składanowski AC, 2007. Analysis of
7773 structure-cytotoxicity *in vitro* relationship (SAR) for perfluorinated carboxylic acids. *Toxicology in*
7774 *Vitro*, 21, 1206-1211.
- 7775 Knepper TP and Lange FT (Eds), 2011. Polyfluorinated Chemicals and Transformation Products,
7776 Handbook of Environmental Chemistry, Springer Verlag, Vol 17, ISBN 978-3-642-21871-2

- 7777 Knox SS, Jackson T, Javins B, Frisbee SJ, Shankar A and Ducatman AM, 2011. Implications of early
7778 menopause in women exposed to perfluorocarbons. *Journal of Clinical Endocrinology & Metabolism*,
7779 96, 1747-1753.
- 7780 Köhrle J, 2008. Environment and endocrinology: the case of thyroidology. *Annals of Endocrinology*, 69,
7781 116–122.
- 7782 Kotthoff M, Müller J, Jüriling H, Schlummer M and Fiedler D, 2015. Perfluoroalkyl and polyfluoroalkyl
7783 substances in consumer products. *Environmental Science and Pollution Research*, 22, 14546-14549.
7784 doi: 10.1007/s11356-015-4202-7
- 7785 Koustas E, Lam J, Sutton P, Johnson PI, Atchley DS, Sen S, Robinson KA, Axelrad DA and Woodruff TJ,
7786 2014. The Navigation Guide - evidence-based medicine meets environmental health: systematic
7787 review of nonhuman evidence for PFOA effects on fetal growth. *Environmental Health Perspectives*,
7788 122, 1015–1027.
- 7789 Kowalczyk J, Ehlers S, Oberhausen A, Tischer M, Furst P, Schafft H and Lahrssen-Wiederholt M, 2013.
7790 Absorption, distribution, and milk secretion of the perfluoroalkyl acids PFBS, PFHxS, PFOS, and PFOA
7791 by dairy cows fed naturally contaminated feed. *Journal of Agricultural and Food Chemistry*, 61, 2903-
7792 2912. doi:10.1021/jf304680j
- 7793 Kratzer J, Ahrens L, Roos A, Bäcklin BM and Ebinghaus R, 2011. Temporal trends of polyfluoroalkyl
7794 compounds (PFCs) in liver tissue of grey seals (*Halichoerus grypus*) from the Baltic Sea, 1974–2008.
7795 *Chemosphere*, 85, 253–261.
- 7796 Kraugerud M, Zimmer KE, Ropstad E and Verhaegen S, 2011. Perfluorinated compounds differentially
7797 affect steroidogenesis and viability in the human adrenocortical carcinoma (H295R) *in vitro* cell assay.
7798 *Environmental Science & Technology*, 44, 9550-9556. doi: 10.1021/es1021922.
- 7799 Kudo N, Bandai N, Suzuki E, Katakura M and Kawashima Y, 2000. Induction by perfluorinated fatty acids
7800 with different carbon chain length of peroxisomal beta-oxidation in the liver of rats. *Chemico-
7801 Biological Interactions*, 124, 119-132.
- 7802 Kudo N, Suzuki E, Katakura M, Ohmori K, Noshiro R and Kawashima Y, 2001. Comparison of the
7803 elimination between perfluorinated fatty acids with different carbon chain length in rats. *Chemico-
7804 Biological Interactions*, 134, 203-216.
- 7805 Kudo N, Suzuki-Nakajima E, Mitsumoto A and Kawashima Y, 2006. Responses of the Liver to
7806 Perfluorinated Fatty Acids with Different Carbon Chain Length in Male and Female Mice: In Relation
7807 to Induction of Hepatomegaly, Peroxisomal beta-oxidation and Microsomal 1-
7808 Acylglycerophosphocholine Acyltransferase. *Biological and Pharmaceutical Bulletin*, 29, 1952-1957.
7809 doi:10.1248/bpb.29.1952
- 7810 Kielsen K, Shamim Z, Ryder LP, Nielsen F, Grandjean P, Budtz-Jørgensen E and Heilmann C, 2016.
7811 Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to
7812 perfluorinated alkylates. *Journal of Immunotoxicology*, 13, 270–273.
7813 <https://doi.org/10.3109/1547691X.2015.1067259>
- 7814 Kurata Y, Kidachi F, Yokoyama M, Toyota N, Tsuchitani M and Katoh M, 1998. Subchronic toxicity of
7815 di(2-ethylhexyl)phthalate in common marmosets: lack of hepatic peroxisome proliferation, testicular
7816 atrophy, or pancreatic acinar cell hyperplasia. *Toxicological Sciences*, 42, 49–56.
- 7817 Kwon EJ, Shin JS, Kim BM, Shah-Kulkarni S, Park H, Kho YL, Park EA, Kim YJ and Ha EH, 2016. Prenatal
7818 Exposure to Perfluorinated Compounds Affects Birth Weight Through GSTM1 Polymorphism. *Journal
7819 of Occupational and Environmental Medicine*, 58, e198–e205.
7820 <https://doi.org/10.1097/JOM.0000000000000739>.

- 7821 Lacina O, Hradkova P, Pulkrabova J and Hajslova J, 2011. Simple, high throughput ultra-high
7822 performance liquid chromatography/tandem mass spectrometry trace analysis of perfluorinated
7823 alkylated substances in food of animal origin: Milk and fish. *Journal of Chromatography A*, 1218,
7824 4312–4321
- 7825 Laden F, Neas LM, Spiegelman D, Hankinson SE, Willett WC, Ireland K, Wolff MS and Junter DJ, 1999.
7826 Predictors of plasma concentrations of DDE and PCBs in a group of U.S. women. *Environmental*
7827 *Health Perspectives*, 107, 75-81. doi: 10.1289/ehp.9910775
- 7828 Ladics GS, Kennedy GL, O'Connor J, Everds N, Malley LA, Frame SR, Gannon S, Jung R, Roth T, Iwai H
7829 and Shin-ya S, 2008. 90-Day Oral Gavage Toxicity Study of 8-2 Fluorotelomer Alcohol in Rats. *Drug*
7830 *and Chemical Toxicology*, 31, 189-216. doi:10.1080/01480540701873103
- 7831 Lai KP, Ng AHM, Wan HL, Wong AYM, Leung CCT, Li R and Wong CKC, 2018. Dietary Exposure to the
7832 Environmental Chemical, PFOS on the Diversity of Gut Microbiota, Associated with the Development
7833 of Metabolic Syndrome. *Frontiers in Microbiology*, 9, 2552. doi: 10.3389/fmicb.2018.02552
- 7834 Laitinen JA, Koponen J, Koikkalainen J and Kiviranta H, 2014. Firefighters' exposure to perfluoroalkyl
7835 acids and 2-butoxyethanol present in firefighting foams. *Toxicology Letters*, 231, 227-232.
- 7836 Land M, de Wit CA, Cousins IT, Herzke D, Johansson J and Martin JW, 2015. What is the effect of
7837 phasing out long-chain per- and polyfluoroalkyl substances on the concentrations of perfluoroalkyl
7838 acids and their precursors in the environment? A systematic review protocol. *Environmental Evidence*,
7839 4(3), 1–13. doi:10.1186/s13750-017-0114-y
- 7840 Land M, de Wit CA, Bignert A, Cousins IT, Herzke D, Johansson JH and Martin JW, 2018. What is the
7841 effect of phasing out long-chain per- and polyfluoroalkyl substances on the concentrations of
7842 perfluoroalkyl acids and their precursors in the environment? A systematic review. *Environmental*
7843 *Evidence*, 7, 4. doi:10.1186/s13750-017-0114-y
- 7844 Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, Canfield RL, Dietrich KN,
7845 Bornschein R, Greene T, Rothenberg SJ, Needleman HL, Schnaas L, Wasserman G, Graziano J and
7846 Roberts R, 2005. Low-level environmental lead exposure and children's intellectual function: an
7847 international pooled analysis. *Environmental Health Perspectives*, 113, 894-899.
- 7848 Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, Butenhoff JL and Stevenson LA,
7849 2003. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse: II. Postnatal
7850 evaluation. *Toxicological Sciences*, 74, 382–392.
- 7851 Lauritzen HB, Larose TL, Øien T, Odland JØ, van de Bor M, Jacobsen GW and Sandanger TM, 2016.
7852 Factors Associated with Maternal Serum Levels of Perfluoroalkyl Substances and Organochlorines: A
7853 Descriptive Study of Parous Women in Norway and Sweden. *PLoS One*, 11(11):e0166127. doi:
7854 10.1371/journal.pone.0166127.
- 7855 Lee H, D'Eon J and Mabury SA, 2010. Biodegradation of polyfluoroalkyl phosphates as a source of
7856 perfluorinated acids to the environment. *Environmental Science & Technology*, 44, 3305-3310. doi:
7857 10.1021/es9028183.
- 7858 Lee I and Viberg H, 2013. A single neonatal exposure to perfluorohexane sulfonate (PFHxS) affects the
7859 levels of important neuroproteins in the developing mouse brain. *Neurotoxicology*, 37, 190-196.
7860 doi:10.1016/j.neuro.2013.05.007
- 7861 Lee SS, Pineau T, Drago J, Lee EJ, Owens JW, Kroetz DL, Fernandez-Salguero PM, Westphal H and
7862 Gonzalez FJ, 1995. Targeted disruption of the alpha isoform of the peroxisome proliferator-activated
7863 receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators.
7864 *Molecular & Cellular Toxicology*, 15, 3012-3022.

- 7865 Lee JK, Lee S, Choi YA, Jin ML, Kim YY, Kang BC, Kim MJ, Dhakal H, Lee SR, Kim SU, Khang S and Kim
7866 SH, 2018. Perfluorooctane sulfonate exacerbates mast cell-mediated allergic inflammation by the
7867 release of histamine. *Molecular & Cellular Toxicology*, 14,173-181. DOI 10.1007/s13273-018-0019-z
- 7868 Lefebvre DE, Curran I, Armstrong C, Coady L, Parenteau M, Liston V, Barker M, Aziz S, Rutherford K,
7869 Bellon- Gagnon P, Shenton J, Mehta R and Bondy G, 2008. Immunomodulatory Effects of Dietary
7870 Potassium Perfluorooctane Sulfonate (PFOS) Exposure in Adult Sprague-Dawley Rats. *Journal of*
7871 *Toxicology and Environmental Health-Part A-Current Issues*, 71, 1516–1525.
- 7872 Lenters V, Portengen L, Rignell-Hydbom A, Jonsson BA, Lindh CH, Piersma AH, Toft G, Bonde JP,
7873 Heederik D, Rylander L and Vermeulen R, 2016. Prenatal Phthalate, Perfluoroalkyl Acid, and
7874 Organochlorine Exposures and Term Birth Weight in Three Birth Cohorts: Multi-Pollutant Models
7875 Based on Elastic Net Regression. *Environmental Health Perspectives*, 124, 365-372.
7876 doi:10.1289/ehp.1408933
- 7877 Letcher RJ, Su G, Moore JN, Williams LL, Martin PA, de Solla SR and Bowerman WW, 2015.
7878 Perfluorinated sulfonate and carboxylate compounds and precursors in herring gull eggs from across
7879 the Laurentian Great Lakes of North America: Temporal and recent spatial comparisons and exposure
7880 implications. *Science of the Total Environment*, 538, 468-477. doi:10.1016/j.scitotenv.2015.08.083
- 7881 Lewis RC, Johns LE and Meeker JD, 2015. Serum Biomarkers of Exposure to Perfluoroalkyl Substances
7882 in Relation to Serum Testosterone and Measures of Thyroid Function among Adults and Adolescents
7883 from NHANES 2011-2012. *International Journal of Environmental Research and Public Health*, 12,
7884 6098-6114. doi: 10.3390/ijerph120606098
- 7885 Li Y, Ramdhan DH, Naito H, Yamagishi N, Ito Y, Hayashi Y, Yanagiba Y, Okamura A, Tamada H, Gonzalez
7886 FJ and Nakajima T, 2011. Ammonium perfluorooctanoate may cause testosterone reduction by
7887 adversely affecting testis in relation to PPAR α . *Toxicology Letters*, 205, 265–272
- 7888 Li J, Guo F, Wang Y, Zhang J, Zhong Y, Zhao Y and Wu Y, 2013. Can nail, hair and urine be used for
7889 biomonitoring of human exposure to perfluorooctane sulfonate and perfluorooctanoic acid?
7890 *Environmental International*, 53, 47-52.
- 7891 Li Y, Cheng Y, Xie Z and Zeng F, 2017. Perfluorinated alkyl substances in serum of the southern Chinese
7892 general population and potential impact on thyroid hormones. *Scientific Reports*, 7:43380. doi:
7893 10.1038/srep43380
- 7894 Li Y, Fletcher T, Mucs D, Scott K, Lindh CH, Tallving P and Jakobsson K, 2018a. Half-lives of PFOS,
7895 PFHxS and PFOA after end of exposure to contaminated drinking water. *Occupational and*
7896 *Environmental Medicine*, 75, 46-51. doi: 10.1136/oemed-2017-104651
- 7897 Li D, Song P, Liu L and Wang X, 2018b. Perfluorooctanoic acid exposure during pregnancy alters the
7898 apoptosis of uterine cells in pregnant mice. *I International Journal of Clinical and Experimental*
7899 *Pathology*, 11, 5602-5611.
- 7900 Li CH, Ren X-M, Cao L-Y, Qina W-P and Guo L-H, 2019. Investigation of binding and activity of
7901 perfluoroalkyl substances to the human peroxisome proliferator-activated receptor β/δ .
7902 *Environmental Science: Processes & Impacts*, 21, 1908-1914.
- 7903 Li D, Zhang L, Zhang Y, Guan S, Gong X and Wang X, 2019. Maternal exposure to perfluorooctanoic
7904 acid (PFOA) causes liver toxicity through PPAR- α pathway and lowered histone acetylation in
7905 female offspring mice. *Environmental Science and Pollution Research*, 26, 18866–18875.
- 7906 Liang X, Xie G, Wu X, Su M and Yang B, 2019 Effect of prenatal PFOS exposure on liver cell function in
7907 neonatal mice. *Environmental Science and Pollution*, 26, 18240–18246.
7908 <https://doi.org/10.1007/s11356-019-05245-4>

- 7909 Lieder PH, Chang SC, York RG and Butenhoff JL, 2009a. Toxicological evaluation of potassium
7910 perfluorobutanesulfonate in a 90-day oral gavage study with Sprague-Dawley rats. *Toxicology*, 255,
7911 45-52. doi:10.1016/j.tox.2008.10.002
- 7912 Lieder PH, York RG, Hakes DC, Chang SC and Butenhoff JL, 2009b. A two-generation oral gavage
7913 reproduction study with potassium perfluorobutanesulfonate (K+PFBS) in Sprague Dawley rats.
7914 *Toxicology*, 259, 33-45.
- 7915 Lien GW, Huang CC, Shiu JS, Chen MH, Hsieh WS, Guo YL and Chen PC, 2016. Perfluoroalkyl substances
7916 in cord blood and attention deficit/hyperactivity disorder symptoms in seven-year-old children.
7917 *Chemosphere*, 156, 118-127. doi:10.1016/j.chemosphere.2016.04.102
- 7918 Liew Z, Ritz B, Bonefeld-Jørgensen EC, Henriksen TB, Nohr EA, Bech BH, Fei C, Bossi R, von Ehrenstein
7919 OS, Streja E, Uldall P and Olsen J, 2014. Prenatal exposure to perfluoroalkyl substances and the risk
7920 of congenital cerebral palsy in children. *American Journal of Epidemiology*, 180, 574-581.
7921 doi:10.1093/aje/kwu179
- 7922 Liew Z, Ritz B, von Ehrenstein OS, Bech BH, Nohr EA, Fei C, Bossi R, Henriksen TB, Bonefeld-Jørgensen
7923 EC and Olsen J, 2015. Attention deficit/hyperactivity disorder and childhood autism in association
7924 with prenatal exposure to perfluoroalkyl substances: a nested case-control study in the Danish
7925 National Birth Cohort. *Environmental Health Perspectives*, 123, 367-373.
- 7926 Lilja K, Norström K, Remberger M, Kaj L, Egelrud L, Junedahl E, Brorström-Lundén E, Ghebremeskel M
7927 and Schlabach M, 2009. Screening of Selected Hazardous Substances in the Eastern Baltic Marine
7928 Environment. IVL Swedish Environmental Research Institute Ltd. B1874, Project report NCM/
7929 HELCOM "Screening of hazardous substances in the eastern Baltic Sea", 57 p. Available online:
7930 <https://www.ivl.se/download/18.343dc99d14e8bb0f58b7598/1445517412208/B1874.pdf>
- 7931 Lin CY, Chen PC, Lin YC and Lin LY, 2009. Association among serum perfluoroalkyl chemicals, glucose
7932 homeostasis, and metabolic syndrome in adolescents and adults. *Diabetes Care*, 32, 702-707. doi:
7933 10.2337/dc08-1816
- 7934 Lin CY, Lin LY, Chiang CK, Wang WJ, Su YN, Hung KY and Chen PC, 2010. Investigation of the
7935 associations between low-dose serum perfluorinated chemicals and liver enzymes in US adults. *Am*
7936 *Journal of Gastroenterology*, 105, 1354-1363. doi: 10.1038/ajg.2009.707
- 7937 Lin C-Y, Lin L-Y, Wen T-W, Lien G-W, Chien K-L, Hsu SHJ, Liao C-C, Sung F-C, Chen P-C and Su T-C,
7938 2013b. Association between levels of serum perfluorooctane sulfate and carotid artery intima-media
7939 thickness in adolescents and young adults. *International Journal of Cardiology*, 168, 3309-3316.
7940 <https://doi.org/10.1016/j.ijcard.2013.04.042>
- 7941 Lin CY, Wen LL, Lin LY, Wen TW, Lien GW, Chen CY, Hsu SH, Chien KL, Sung FC, Chen PC and Su TC,
7942 2011. Associations between levels of serum perfluorinated chemicals and adiponectin in a young
7943 hypertension cohort in Taiwan. *Environmental Science & Technology*, 45, 10691-10698. doi:
7944 10.1021/es201964x
- 7945 Lin CY, Wen LL, Lin LY, Wen TW, Lien GW, Hsu SH, Chien KL, Liao CC, Sung FC, Chen PC and Su TC,
7946 2013a. The associations between serum perfluorinated chemicals and thyroid function in adolescents
7947 and young adults. *Journal of Hazardous Materials*, 244-245, 637-644. doi:
7948 10.1016/j.jhazmat.2012.10.049
- 7949 Lind DV, Priskorn L, Harmer Lassen T, Nielsen F, Boye Kyhl H, Møbjerg Kristensen D, Christesen HT,
7950 Jørgensen JS, Grandjean P and Kold Jensen T, 2017. Prenatal exposure to perfluoroalkyl substances
7951 and anogenital distance at 3 months of age in a Danish mother-child cohort. *Reproductive*
7952 *Toxicology*, 68, 200-206. doi: 10.1016/j.reprotox.2016.08.019

- 7953 Lin PD, Cardenas A, Hauser R, Gold DR, Kleinman KP, Hivert MF, Fleisch AF, Calafat AM, Webster TF,
7954 Horton ES and Oken E, 2019. Per- and polyfluoroalkyl substances and blood lipid levels in pre-diabetic
7955 adults-longitudinal analysis of the diabetes prevention program outcomes study. *Environment*
7956 *International*, 129, 343-353.
- 7957 Lind L, Zethelius B, Salihovic S, van Bavel B and Lind PM, 2014. Circulating levels of perfluoroalkyl
7958 substances and prevalent diabetes in the elderly. *Diabetologia*, 57, 473-479. doi: 10.1007/s00125-
7959 013-3126-3
- 7960 Lind PM, Salihovic S, van Bavel B and Lind L, 2017. Circulating levels of perfluoroalkyl substances
7961 (PFASs) and carotid artery Atherosclerosis. *Environmental Research*, 152, 157-164.
7962 <https://doi.org/10.1016/j.envres.2016.10.002>
- 7963 Lindeman B, Maass C, Duale N, Gützkow KB, Brunborg G and Andreassen A, 2012. Effects of per- and
7964 polyfluorinated compounds on adult rat testicular cells following *in vitro* exposure. *Reproductive*
7965 *Toxicology*, 33, 531-537.
- 7966 Lindstrom AB, Strynar MJ, Laurence and Libelo E, 2011. Polyfluorinated Compounds: Past, Present, and
7967 Future. *Environmental Science & Technology*, 45, 7954-7961.
- 7968 Liu J, Li J, Liu Y, Chan HM, Zhao Y, Cai Z and Wu Y, 2011 Comparison on gestation and lactation
7969 exposure of perfluorinated compounds for newborns. *Environment International*, 37, 1206-1212.
- 7970 Liu X, Guo Z, Krebs KA, Pope RH and Roache NF, 2014a. Concentrations and trends of perfluorinated
7971 chemicals in potential indoor sources from 2007 through 2011 in the US. *Chemosphere*, 98, 51-57.
- 7972 Liu C, Chang VW, Gin KY and Nguyen VT, 2014b. Genotoxicity of perfluorinated chemicals (PFCs) to the
7973 green mussel (*Perna viridis*). *Science of the Total Environment*, 487, 117-122.
7974 doi:10.1016/j.scitotenv.2014.04.017
- 7975 Liu Y, Pereira AS, Beeson S, Vestergren R, Berger U, Olsen GW, Glynn A and Martin JW, 2015. Temporal
7976 trends of perfluorooctanesulfonate isomer and enantiomer patterns in archived Swedish and
7977 American serum samples. *Environment International*, 75, 215-22.
- 7978 Liu H, Zhang H, Cui R, Guo X, Wang D and Dai J, 2016. Activation of peroxisome proliferator-activated
7979 receptor α ameliorates perfluorododecanoic acid-induced production of reactive oxygen species in
7980 rat liver. *Archives of Toxicology*, 90, 1383-1397.
- 7981 Liu X, Fang M, Xu F and Chen D, 2019. Characterization of the binding of per- and poly-fluorinated.
7982 *Trends in Analytical Chemistry*, 116, 177-185. <https://doi.org/10.1016/j.trac.2019.05.017>
- 7983 Loccisano AE, Campbell JL Jr, Andersen ME and Clewell HJ, 2011. Evaluation and prediction of
7984 pharmacokinetics of PFOA and PFOS in the monkey and human using a PBPK model. *Regulatory*
7985 *Toxicology and Pharmacology*, 59, 157-175. <https://doi.org/10.1016/j.yrtph.2010.12.004>
- 7986 Löfstedt Gilliam J, Leonel J, Cousins IT and Benskin JP, 2016. Is Ongoing Sulfluramid Use in South
7987 America a Significant Source of Perfluorooctanesulfonate (PFOS)? Production Inventories,
7988 Environmental Fate, and Local Occurrence. *Environmental Science & Technology*, 50, 653-659.
- 7989 Löfstrand K, Jorundsdottir H, Tomy G, Svavarsson J, Weihe P, Nygard T and Bergman K, 2008. Spatial
7990 trends of polyfluorinated compounds in guillemot (*Uria aalge*) eggs from North-Western Europe.
7991 *Chemosphere*, 72, 1475-1480. doi:10.1016/j.chemosphere.2008.05.011
- 7992 Long M, Ghisari M and Bonefeld-Jørgensen EC, 2013. Effects of perfluoroalkyl acids on the function of
7993 the thyroid hormone and the aryl hydrocarbon receptor. *Environmental Science and Pollution*
7994 *Research*, 20, 8045-8056. doi: 10.1007/s11356-013-1628-7

- 7995 Long M, Knudsen AK, Pedersen HS and Bonefeld-Jørgensen EC, 2015. Food intake and serum persistent
7996 organic pollutants in the Greenlandic pregnant women: The ACCEPT sub-study. *Science of the Total*
7997 *Environment*, 529, 198-212.
- 7998 Looker C, Luster MI, Calafat AM, Johnson VJ, Burleson GR, Burleson FG and Fletcher T, 2014. Influenza
7999 vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate.
8000 *Toxicological Sciences*, 138, 76–88. <https://doi.org/10.1093/toxsci/kft269>
- 8001 Lopez-Espinosa MJ, Mondal D, Armstrong B, Bloom MS and Fletcher T, 2012. Thyroid function and
8002 perfluoroalkyl acids in children living near a chemical plant. *Environmental Health Perspectives*, 120,
8003 1036-1041. doi: 10.1289/ehp.1104370
- 8004 Lopez-Espinosa MJ, Mondal D, Armstrong BG, Eskenazi B and Fletcher T, 2016. Perfluoroalkyl
8005 Substances, Sex Hormones, and Insulin-like Growth Factor-1 at 6-9 Years of Age: A Cross-Sectional
8006 Analysis within the C8 Health Project. *Environmental Health Perspectives*, 124, 1269–1275.
- 8007 Lorenzo M, Campo J, Farré M, Pérez F, Picó Y and Barceló D, 2016. Perfluoroalkyl substances in the
8008 Ebro and Guadalquivir river basins (Spain). *Science of the Total Environment*, 540, 191-199.
8009 <https://doi.org/10.1016/j.scitotenv.2015.07.045>
- 8010 Loveless SE, Hoban D, Sykes G, Frame SR and Everds NE, 2008. Evaluation of the immune system in
8011 rats and mice administered linear ammonium perfluorooctanoate. *Toxicological Sciences*, 105, 86–
8012 96.
- 8013 Loveless SE, Slezak B, Serex T, Lewis J, Mukerji P, O'Connor JC, Donner EM and Frame SR, Korzeniowski
8014 SH and Buck RC, 2009. Toxicological evaluation of sodium perfluorohexanoate. *Toxicology*, 264, 32-
8015 44
- 8016 Lu Z, Lu R, Zheng H, Yan J, Song L, Wang J, Yang H and Cai M, 2018. Risk exposure assessment of per-
8017 and polyfluoroalkyl substances (PFASs) in drinking water and atmosphere in central eastern China.
8018 *Environmental Science and Pollution Research*, 25, 9311–9320. [https://doi.org/10.1007/s11356-](https://doi.org/10.1007/s11356-017-0950-x)
8019 [017-0950-x](https://doi.org/10.1007/s11356-017-0950-x)
- 8020 Luebker DJ, Hansen KJ, Bass NM, Butenhoff JL and Seacat AM, 2002. Interactions of fluorochemicals
8021 with rat liver fatty acid-binding protein. *Toxicology*, 176, 175-185.
- 8022 Luo M, Tan Z, Dai M, Song D, Lin J, Xie M, Yang J, Sun L, Wei D, Zhao J, Gonzalez FJ and Liu A, 2017.
8023 Dual action of peroxisome proliferator-activated receptor alpha in perfluorodecanoic acid-induced
8024 hepatotoxicity. *Archives of Toxicology*, 91, 897-907. doi:10.1007/s00204-016-1779-7
- 8025 Luster MI, Portier C, Pait DG, Rosenthal GJ, Germolec DR, Corsini E, Blaylock BL, Pollock P, Kouchi Y
8026 Craig W, 1993. Risk assessment in immunotoxicology. II. Relationships between immune and host
8027 resistance tests. *Fundamental and Applied Toxicology*, 21, 71-82.
- 8028 Lv N, Zhao M, Han Y, Cui L, Zhong W, Wang C and Jiang Q, 2018. The roles of bone morphogenetic
8029 protein 2 in perfluorooctanoic acid induced developmental cardiotoxicity and l-carnitine mediated
8030 protection. *Toxicology and Applied Pharmacology*, 352, 68-76. doi:10.1016/j.taap.2018.05.028
- 8031 Ma X, Stoffregen DA, Wheelock GD, Rininger JA and Babish JG, 1997. Discordant hepatic expression of
8032 the cell division control enzyme p34cdc2 kinase, proliferating cell nuclear antigen, p53 tumor
8033 suppressor protein, and p21Waf1 cyclin-dependent kinase inhibitory protein after WY14,643 ([4-
8034 chloro-6-(2,3-xylidino)- 2-pyrimidinylthio]acetic acid) dosing to rats. *Molecular Pharmacology*, 51,
8035 69–78
- 8036 Ma Y, Sachdeva K, Liu J, Song X, Li Y, Yang D, Deng R, Chichester CO and Yan B, 2005. Clofibrate and
8037 perfluorodecanoate both upregulate the expression of the pregnane X receptor but oppositely affect
8038 its ligand-dependent induction on cytochrome P450 3A23. *Biochemical Pharmacology*, 69, 1363-
8039 1371.

- 8040 Macon MB, Villanueva LR, Tatum-Gibbs K, Zehr RD, Strynar MJ, Stanko JP, White SS, Helfant L and
8041 Fenton SE, 2011. Prenatal perfluorooctanoic acid exposure in CD-1 mice: Low-dose developmental
8042 effects and internal dosimetry. *Toxicological Sciences*, 122, 134–145.
- 8043 Maisonet M, Terrell ML, McGeehin MA, Christensen KY, Holmes A, Calafat AM and Marcus M, 2012.
8044 Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal
8045 growth in British girls. *Environmental Health Perspectives*, 120, 1432-1437.
8046 doi:10.1289/ehp.1003096
- 8047 Maher JM, Aleksunes LM, Dieter MZ, Tanaka Y, Peters JM, Manautou JE and Klaassen CD, 2008. Nrf2-
8048 and PPAR alpha-mediated regulation of hepatic Mrp transporters after exposure to perfluorooctanoic
8049 acid and perfluorodecanoic acid. *Toxicological Sciences*, 106, 319-328. doi:10.1093/toxsci/kfn177
- 8050 Mallet CR, Lu Z and Mazzeo JR, 2004. A study of ion suppression effects in electron spray ionization from
8051 mobile phase additives and solid-phase extracts. *Rapid Communications in Mass Spectrometry*, 18,
8052 49-58.
- 8053 Mancini FR, Cano-Sancho G, Gambaretti J, Marchand P, Bouteron-Ruault MC, Severi G, Arveux P, Antignac
8054 JP and Kvaskoff M, 2019. Perfluorinated alkylated substances serum concentration and breast cancer
8055 risk: Evidence from a nested case-control study in the French E3N cohort. *International Journal of*
8056 *Cancer*, 146, 917-928. doi: 10.1002/ijc.32357
- 8057 Manzano-Salgado CB, Casas M, Lopez-Espinosa MJ, Ballester F, Iniguez C, Martinez D, Costa O, Santa-
8058 Marina L, Pereda-Pereda E, Schettgen T, Sunyer J and Vrijheid M, 2017a. Prenatal exposure to
8059 perfluoroalkyl substances and birth outcomes in a Spanish birth cohort. *Environment International*,
8060 108, 278-284. doi:10.1016/j.envint.2017.09.006
- 8061 Manzano-Salgado CB, Casas M, Lopez-Espinosa MJ, Ballester F, Iniguez C, Martinez D, Romaguera D,
8062 Fernández-Barrés S, Santa-Marina L, Basterretxea M, Schettgen T, Valvi D, Vioque J, Sunyer J and
8063 Vrijheid M, 2017b Prenatal Exposure to Perfluoroalkyl Substances and Cardiometabolic Risk in
8064 Children from the Spanish INMA Birth Cohort Study. *Environmental Health Perspectives*, 125,
8065 097018. doi: 10.1289/EHP1330
- 8066 Manzano-Salgado CB, Granum B, Lopez-Espinosa MJ, Ballester F, Iñiguez C, Gascón M, Martínez D,
8067 Guxens M, Basterretxea M, Zabaleta C, Schettgen T, Sunyer J, Vrijheid M and Casas M, 2019.
8068 Prenatal exposure to perfluoroalkyl substances, immune-related outcomes, and lung function in
8069 children from a Spanish birth cohort study. *International Journal of Hygiene and Environmental*
8070 *Health*, 222, 945-954.
- 8071 Maras M, Vanparys C, Muylle F, Robbens J, Berger U, Barber JL, Blust R and De Coen W, 2006. Estrogen-
8072 like properties of fluorotelomer alcohols as revealed by MCF-7 breast cancer cell proliferation.
8073 *Environmental Health Perspectives*, 114, 100-105.
- 8074 Marks KJ, Cutler AJ, Jeddy Z, Northstone K, Kato K and Hartman TJ, 2019. Maternal serum
8075 concentrations of perfluoroalkyl substances and birth size in British boys. *International Journal of*
8076 *Hygiene and Environmental Health*, 222, 889-895. doi: 10.1016/j.ijheh.2019.03.008.
- 8077 Martin JW, Mabury SA, Solomon KR and Muir DCG, 2003a. Dietary Accumulation of Perfluorinated Acids
8078 in Juvenile Rainbow Trout (*Oncorhynchus Mykiss*). *Environmental Toxicology and Chemistry*, 22,
8079 189–195.
- 8080 Martin JW, Mabury SA, Solomon KR and Muir DCG, 2003b. Bioconcentration and Tissue Distribution of
8081 Perfluorinated Acids in Rainbow Trout (*Oncorhynchus Mykiss*). *Environmental Toxicology and*
8082 *Chemistry*, 22, 196–204.
- 8083 Martin JW, Kannan K, Berger U, de Voogt P, Field J, Franklin J, Giesy JP, Harner T, Muir DC, Scott B,
8084 Kaiser M, Jarnberg U, Jones KC, Mabury SA, Schroeder H, Simcik M, Sottani C, van Bavel B, Karrman

- 8085 A, Lindstrom G and van Leeuwen SP, 2004. Analytical challenges hamper perfluoroalkyl research.
8086 Environmental Science & Technology 38, 248A–255A.
- 8087 Martin JW, Mabury SA and O'Brien PJ, 2005. Metabolic products and pathways of fluorotelomer alcohols
8088 in isolated rat hepatocytes. Chemical-Biological Interactions, 155, 165–180.
8089 doi:10.1016/j.cbi.2005.06.007
- 8090 Martin JW, Ellis DA, Mabury SA, Hurley MD and Wallington TJ, 2006. Atmospheric chemistry of
8091 perfluoroalkanesulfonamides: kinetic and product studies of the OH radical and Cl atom initiated
8092 oxidation of N-ethyl perfluorobutanesulfonamide. Environmental Science & Technology, 40, 864–872.
- 8093 Martin JW, Asher BJ, Beesoon S, Benskin JP and Ross MS, 2010. PFOS or PreFOS? Are perfluorooctane
8094 sulfonate precursors (PreFOS) important determinants of human and environmental perfluorooctane
8095 sulfonate (PFOS) exposure? Journal of Environmental Monitoring, 12, 1979–2004.
- 8096 Martinson HA, Lyons TR, Giles ED, Borges VF and Schedin P, 2013. Developmental windows of breast
8097 cancer risk provide opportunities for targeted chemoprevention. Experimental Cell Research, 319,
8098 1671–1678. doi: 10.1016/j.yexcr.2013.04.018
- 8099 Mashayekhi V, Tehrani KH, Hashemzaei M, Tabrizian K, Shahraki J and Hosseini MJ, 2015. Mechanistic
8100 approach for the toxic effects of perfluorooctanoic acid on isolated rat liver and brain mitochondria.
8101 Human and Experimental Toxicology, 34, 985–996.
- 8102 Mastrantonio M, Bai E, Uccelli R, Cordiano V, Screpanti A and Crosignani P, 2018. Drinking water
8103 contamination from perfluoroalkyl substances (PFAS): an ecological mortality study in the Veneto
8104 Region, Italy. European Journal of Public Health, 28, 180–185. doi: 10.1093/eurpub/ckx066
- 8105 Mattsson K, Rignell-Hydbom A, Holmberg S, Thelin A, Jonsson BA, Lindh CH, Sehlstedt A and Rylander
8106 L, 2015. Levels of perfluoroalkyl substances and risk of coronary heart disease: Findings from a
8107 population-based longitudinal study. Environmental Research, 142, 148–154. doi:
8108 10.1016/j.envres.2015.06.033
- 8109 MDH (Minnesota Department of Health), 2012. Perfluorochemical contamination in southern
8110 Washington county, northern Dakota county, and southeastern Ramsey county, Minnesota. US DHHS
8111 ATSDR, Atlanta. Available online:
8112 <http://www.health.state.mn.us/divs/eh/hazardous/topics/pfcs/pha/woodbury/pha3m0112.pdf>
- 8113 Melzer D, Rice N, Depledge MH, Henley WE and Galloway TS, 2010. Association between serum
8114 perfluorooctanoic acid (PFOA) and thyroid disease in the U.S. National Health and Nutrition
8115 Examination Survey. Environmental Health Perspectives, 118, 686–692.
- 8116 Meng Q, Inour K, Ritz B, Olsen J and Liew Z, 2018. Prenatal Exposure to Perfluoroalkyl Substances and
8117 Birth Outcomes: an updated Analysis from the Danish National Birth Cohort. International Journal of
8118 Environmental Research and Public Health, 15, 1832. doi:10.3390/ijerph15091832
- 8119 Merten C, Ferrari P, Bakker M, Boss A, Hearty A, Leclercq C, Lindtner O, Tlustos C, Verger P, Volatier JL
8120 and Arcella D, 2011. Methodological characteristics of the national dietary surveys carried out in the
8121 European Union as included in the European Food Safety Authority (EFSA) Comprehensive European
8122 Food Consumption Database. Food Additives and Contaminants Part A, 28, 975–995.
- 8123 Michigan Science Advisory Workgroup (2019). Health-based drinking water value recommendations for
8124 PFAS in Michigan. Available online: [https://www.michigan.gov/documents/pfasresponse/Health-](https://www.michigan.gov/documents/pfasresponse/Health-Based_Drinking_Water_Value_Recommendations_for_PFAS_in_Michigan_Report_659258_7.pdf)
8125 [Based Drinking Water Value Recommendations for PFAS in Michigan Report 659258 7.pdf](https://www.michigan.gov/documents/pfasresponse/Health-Based_Drinking_Water_Value_Recommendations_for_PFAS_in_Michigan_Report_659258_7.pdf)
- 8126 Midgett K, Peden-Adams MM, Gilkeson GS and Kamen DL, 2015. In vitro evaluation of the effects of
8127 perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) on IL-2 production in human
8128 T-cells. Journal of Applied Toxicology, 35, 459–465.

- 8129 Minata M, Harada KH, Karrman A, Hitomi T, Hirose M, Murata M, Gonzalez FJ and Koizumi A, 2010.
8130 Role of peroxisome proliferator-activated receptor- α in hepatobiliary injury induced by
8131 ammonium perfluorooctanoate in mouse liver. *Industrial Health*, 48, 96-107.
- 8132 Minnesota Department of Health, 2012a. Perfluorochemical contamination in southern Washington
8133 county, northern Dakota county, and southeastern Ramsey county, Minnesota. US DHHS ATSDR,
8134 Atlanta. Available online:
8135 <http://www.health.state.mn.us/divs/eh/hazardous/topics/pfcs/pha/woodbury/pha3m0112.pdf>
8136 .Accessed
- 8137 Miralles-Marco A and Harrad S, 2015. Perfluorooctane sulfonate: a review of human exposure,
8138 biomonitoring and the environmental forensics utility of its chirality and isomer distribution.
8139 *Environment International*, 77, 148-159.
- 8140 Möller A, Ahrens L, Surm R, Westerveld J, van der Wielen F, Ebinghaus R and de Voogt P, 2010.
8141 Distribution and sources of polyfluoroalkyl substances (PFAS) in the River Rhine watershed.
8142 *Environmental Pollution*, 158, 3243-3250. doi: 10.1016/j.envpol.2010.07.019
- 8143 Mollenhauer MAM, Bradshaw SG, Fair PA, McGuinn WD and Peden-Adams MM, 2011. Effects of
8144 perfluorooctane sulfonate (PFOS) exposure on markers of inflammation in female B6C3F1 mice.
8145 *Journal of Environmental Science and Health Part A-Toxic/Hazardous Substances & Environmental*
8146 *Engineering*, 46, 97-108.
- 8147 Mondal D, Weldon RH, Armstrong BG, Gibson LJ, Lopez-Espinosa MJ, Shin HM and Fletcher T, 2014.
8148 Breastfeeding: a potential excretion route for mothers and implications for infant exposure to
8149 perfluoroalkyl acids. *Environmental Health Perspectives*, 122, 187-192. doi:10.1289/ehp.1306613
- 8150 Mørck TA, Nielsen F, Nielsen JKS, Siersma VD, Grandjean P and Knudsen LE, 2015. PFAS concentrations
8151 in plasma samples from Danish school children and their mothers. *Chemosphere*, 129, 203-209.
8152 <https://doi.org/10.1016/j.chemosphere.2014.07.018>
- 8153 Mora AM, Oken E, Rifas-Shiman SL, Webster TF, Gillman MW, Calafat AM, Ye X and Sagiv SK, 2017.
8154 Prenatal Exposure to Perfluoroalkyl Substances and Adiposity in Early and Mid-Childhood.
8155 *Environmental Health Perspectives*, 125, 467-473. doi:10.1289/ehp246
- 8156 Mulkiewicz E, Jastorff B, Składanowski AC, Kleszczyński K and Stepnowski P, 2007. Evaluation of the
8157 acute toxicity of perfluorinated carboxylic acids using eukaryotic cell lines, bacteria and enzymatic
8158 assays. *Environmental Toxicology and Pharmacology*, 23, 279-285.
- 8159 Munoz G, Giraudel JL, Botta F, Lestremat F, Devier MH, Budzinski H and Labadie P, 2015. Spatial
8160 distribution and partitioning behavior of selected poly- and perfluoroalkyl substances in freshwater
8161 ecosystems: a French nationwide survey. *Science of the Total Environment*, 517, 48-56.
8162 doi:10.1016/j.scitotenv.2015.02.043
- 8163 Mueller CE, De Silva AO, Small J, Williamson M, Wang X, Morris A, Katz S, Gamberg M and Muir DCG,
8164 2011. Biomagnification of Perfluorinated Compounds in a Remote Terrestrial Food Chain: Lichen-
8165 Caribou-Wolf. *Environmental Science & Technology*, 45, 8665-8673.
- 8166 Mylchreest E, Munley SM and Kennedy GL Jr, 2005. Evaluation of the developmental toxicity of 8-2
8167 telomer B alcohol. *Drug and Chemical Toxicology*, 28, 315-328. doi:10.1081/dct-200064491
- 8168 Ma X, Stoffregen DA, Wheelock GD, Rininger JA and Babish JG, 1997. Discordant hepatic expression of
8169 the cell division control enzyme p34cdc2 kinase, proliferating cell nuclear antigen, p53 tumor
8170 suppressor protein, and p21Waf1 cyclin-dependent kinase inhibitory protein after WY14,643 ([4-
8171 chloro-6-(2,3-xylidino)- 2-pyrimidinylthio]acetic acid) dosing to rats. *Molecular Pharmacology*, 51,
8172 69-78

- 8173 Nabb DL, Szostek B, Himmelstein MW, Mawn MP, Gargas ML, Sweeney LM, Stadler GC, Buck RC and
8174 Fasano WJ, 2007. *In vitro* metabolism of 8-2 fluorotelomer alcohol: Interspecies comparisons and
8175 metabolic pathway refinement. *Toxicological Sciences*, 100, 333-344.
- 8176 Nakagawa T, Ramdhan DH, Tanaka N, Naito H, Tamada H, Ito Y, Li Y, Hayashi Y, Yamagishi N, Yanagiba
8177 Y, Aoyama T, Gonzalez FJ and Nakajima T, 2012. Modulation of ammonium perfluorooctanoate-
8178 induced hepatic damage by genetically different PPAR α in mice. *Archives of Toxicology*, 86, 63-74.
8179 <https://doi.org/10.1007/s00204-011-0704-3>
- 8180 Naile JE, Wiseman S, Bachtold K, Jones PD and Giesy JP, 2012. Transcriptional effects of perfluorinated
8181 compounds in rat hepatoma cells. *Chemosphere*, 86, 270-277.
- 8182 Nakamura T, Ito Y, Yanagiba Y, Ramdhan DH, Kono Y, Naito H, Hayashi Y, Li Y, Aoyama T, Gonzalez
8183 FJ and Nakajima T, 2009. Microgram-order ammonium perfluorooctanoate may activate mouse
8184 peroxisome proliferator-activated receptor α , but not human PPAR α . *Toxicology*, 265, 27-
8185 33.
- 8186 Negri E, Metruccio F, Guercio V, Tosti L, Benfenati E, Bonzi R, La Vecchia C and Moretto A, 2017.
8187 Exposure to PFOA and PFOS and fetal growth: a critical merging of toxicological and epidemiological
8188 data. *Critical Reviews in Toxicology*, 47, 489-515.
- 8189 Nelson JW, Hatch EE and Webster TF, 2010. Exposure to polyfluoroalkyl chemicals and cholesterol, body
8190 weight, and insulin resistance in the general U.S. population. *Environmental Health Perspectives*,
8191 118, 197-202. doi: 10.1289/ehp.0901165
- 8192 Nelson JW, Scammell MK, Hatch EE and Webster TF, 2012. Social disparities in exposures to bisphenol
8193 A and polyfluoroalkyl chemicals: a cross-sectional study within NHANES 2003-2006. *Environmental*
8194 *Health*, 11, 10. doi: 10.1186/1476-069X-11-10
- 8195 Nian M, Li Q-Q, Bloom M, Qian Z, Syberg KM, Vaughn MG, Wang S-Q, Wei Q, Zeeshan M, Gurram N,
8196 Chu C, Wang J, Tian Y-P, Hu L-W, Liu K-K, Yang B-Y, Liu R-Q, Feng D, Zeng X-W and Dong G-H,
8197 2019. Liver function biomarkers disorder is associated with exposure to perfluoroalkyl acids in adults:
8198 Isomers of C8 Health Project in China. *Environmental Research*, 172, 81-88. doi:
8199 <https://doi.org/10.1016/j.envres.2019.02.013>
- 8200 Ng CA and Hungerbühler K, 2014. Bioaccumulation of Perfluorinated Alkyl Acids: Observations and
8201 Models. *Environmental Science & Technology*, 48, 4637-4648. doi:10.1021/es404008g
- 8202 NICNAS (National Industrial Chemicals Notification and Assessment Scheme), 2005. Potassium
8203 perfluorobutane sulfonate: Hazard assessment. Australia. Available online:
8204 [http://www.nicnas.gov.au/Publications/CAR/Other/Potassium Perfluorobutane Sulfonate PDF.pdf](http://www.nicnas.gov.au/Publications/CAR/Other/Potassium%20Perfluorobutane%20Sulfonate%20PDF.pdf)
- 8205 Nilsson H, Kärrman A, Rotander A, van Bavel B, Lindström G and Westberg H, 2010. Inhalation Exposure
8206 to Fluorotelomer Alcohols Yield Perfluorocarboxylates in Human Blood? *Environmental Science &*
8207 *Technology*, 44, 7717-7722. doi:10.1021/es101951t
- 8208 Nilsson H, Karrman A, Rotander A, van Bavel B, Lindstrom G and Westberg H, 2013. Professional ski
8209 waxers' exposure to PFAS and aerosol concentrations in gas phase and different particle size
8210 fractions. *Environmental Science: Processes & Impacts*, 15, 814-822. doi:10.1039/c3em30739e
- 8211 Noorlander CW, van Leeuwen SPJ, te Biesebeek JD, Mengelers MJB and Zeilmaker MJ, 2011. Levels of
8212 Perfluorinated Compounds in Food and Dietary Intake of PFOS and PFOA in The Netherlands. *Journal*
8213 *of Agriculture and Food Chemistry*, 59, 7496-7505.
- 8214 Nøst TH, Vestergren R, Berg V, Nieboer E, Odland JØ and Sandanger TM, 2014. Repeated
8215 measurements of per- and polyfluoroalkyl substances (PFASs) from 1979 to 2007 in males from
8216 Northern Norway: assessing time trends, compound correlations and relations to age/birth cohort.
8217 *Environment International*, 67, 43-53.

- 8218 Norden M, Berger U and Engwall M, 2013. High levels of perfluoroalkyl acids in eggs and embryo livers
8219 of great cormorant (*Phalacrocorax carbo sinensis*) and herring gull (*Larus argentatus*) from Lake
8220 Vanern, Sweden. Environmental Science and Pollution Research, 20, 8021-8030.
8221 doi:10.1007/s11356-013-1567-3
- 8222 NTP (National Toxicology Program), 2019a. NTP technical report on the toxicity studies of perfluoroalkyl
8223 carboxylates (perfluorohexanoic acid, perfluorooctanoic acid, perfluorononanoic acid, and
8224 perfluorodecanoic acid) administered by gavage to sprague dawley (Hsd:Sprague Dawley SD) rats.
8225 NTP TOX 97. Research Triangle Park, North Carolina, USA. Available online: <http://ntp.niehs.nih.gov>
- 8226 NTP (National Toxicology Program), 2019b. NTP technical report on the toxicity studies of perfluoroalkyl
8227 sulfonates (perfluorobutane sulfonic acid, perfluorohexane sulfonate potassium salt, and
8228 perfluorooctane sulfonic acid) administered by gavage to sprague dawley (Hsd:Sprague Dawley SD)
8229 rats. NTP TOX 96. Research Triangle Park, North Carolina, USA. Available online:
8230 <http://ntp.niehs.nih.gov>
- 8231 Numata J, Kowalczyk J, Adolphs J, Ehlers S, Schafft H, Fuerst P, Müller-Graf C, Lahrssen-Wiederholt M
8232 and Greiner M, 2014. Toxicokinetics of seven perfluoroalkyl sulfonic and carboxylic acids in pigs fed
8233 a contaminated diet. Journal of Agricultural and Food Chemistry, 62, 6861–6870.
8234 <https://doi.org/10.1021/jf405827u>
- 8235 Nyberg E, Awad R, Bignert A, Ek C, Sallsten G and Benskin JP, 2018. Inter-individual, inter-city, and
8236 temporal trends of per- and polyfluoroalkyl substances in human milk from Swedish mothers between
8237 1972 and 2016. Environmental Science: Processes & Impacts, 20, 1136-1147.
- 8238 Obourn JD, Frame SR, Bell RH Jr, Longnecker DS, Elliott GS and Cook JC, 1997. Mechanisms for the
8239 pancreatic oncogenic effects of the peroxisome proliferator Wyeth-14,643. Toxicology and Applied
8240 Pharmacology, 145, 425–436.
- 8241 Ode A, Rylander L, Lindh CH, Källén K, Jönsson BA, Gustafsson P, Olofsson P, Ivarsson SA and Rignell-
8242 Hydbom A, 2013. Determinants of maternal and fetal exposure and temporal trends of perfluorinated
8243 compounds. Environmental Science and Pollution Research, 20, 7970-7978.
- 8244 Ode A, Källén K, Gustafsson P, Rylander L, Jönsson BA, Olofsson P, Ivarsson SA, Lindh CH and Rignell-
8245 Hydbom A, 2014. Fetal exposure to perfluorinated compounds and attention deficit hyperactivity
8246 disorder in childhood. PLoS One, 9(4):e95891
- 8247 OECD (The Organisation for Economic Co-operation and Development), 2018. Toward a New
8248 Comprehensive Global Database of Per- and Polyfluoroalkyl Substances (PFASs): Summary Report
8249 on Updating the OECD 2007 List of Per- and Polyfluoroalkyl Substances (PFASs). Series on Risk
8250 Management No. 39. Available online:
8251 [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV-JM-](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV-JM-MONO(2018)7&doclanguage=en)
8252 [MONO\(2018\)7&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV-JM-MONO(2018)7&doclanguage=en)
- 8253 Ohmori K, Kudo N, Katayama K and Kawashima Y, 2003. Comparison of the toxicokinetics between
8254 perfluorocarboxylic acids with different carbon chain length. Toxicology, 184, 135-140.
- 8255 Okada E, Sasaki S, Saijo Y, Washino N, Miyashita C, Kobayashi S, Konishi K, Ito YM, Ito R, Nakata A,
8256 Iwasaki Y, Saito K, Nakazawa H and Kishi R, 2012. Prenatal exposure to perfluorinated chemicals
8257 and relationship with allergies and infectious diseases in infants. Environmental Research, 112, 118–
8258 125. <https://doi.org/10.1016/j.envres.2011.10.003>
- 8259 Okada E, Kashino I, Matsuura H, Sasaki S, Miyashita C, Yamamoto J, Ikeno T, Ito YM, Matsumura T,
8260 Tamakoshi A and Kishi R, 2013. Temporal trends of perfluoroalkyl acids in plasma samples of
8261 pregnant women in Hokkaido, Japan, 2003-2011. Environment International, 60, 89-96.

- 8262 Okada E, Sasaki S, Kashino I, Matsuura H, Miyashita C, Kobayashi S, Itoh K, Ikeno T, Tamakoshi A and
8263 Kishi R, 2014. Prenatal exposure to perfluoroalkyl acids and allergic diseases in early childhood.
8264 *Environment International*, 65, 127-134. doi: 10.1016/j.envint.2014.01.007
- 8265 Olsen GW, 2015. PFAS biomonitoring in Higher Exposed Populations. *Toxicological Effects of*
8266 *Perfluoroalkyl and Polyfluoroalkyl Substances*, Molecular and Integrative Toxicology. Springer
8267 International Publishing, Switzerland DOI 10.1007/978-3-319-15518-0_4
- 8268 Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL and Zobel LR, 2007. Half-
8269 life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and
8270 perfluorooctanoate in retired fluorochemical production workers. *Environmental Health Perspectives*,
8271 115, 1298-1305.
- 8272 Olsen GW, Butenhoff JL and Zobel LR, 2009. Perfluoroalkyl chemicals and human fetal development:
8273 an epidemiologic review with clinical and toxicological perspectives. *Reproductive Toxicology*, 27,
8274 212-230. doi:10.1016/j.reprotox.2009.02.001
- 8275 Oulhote Y, Steuerwald U, Debes F, Weihe P and Grandjean P, 2016. Behavioral difficulties in 7-year old
8276 children in relation to developmental exposure to perfluorinated alkyl substances. *Environment*
8277 *International*, 97, 237-245. doi:10.1016/j.envint.2016.09.015
- 8278 Pachkowski B, Post GB and Stern AH, 2019. The derivation of a Reference Dose (RfD) for
8279 perfluorooctane sulfonate (PFOS) based on immune suppression. *Environmental Research*, 171, 452-
8280 469. doi: 10.1016/j.envres.2018.08.004.
- 8281 Papadopoulou E, Sabaredzovic A, Namork E, Nygaard UC, Granum B and Haug LS, 2016. Exposure of
8282 Norwegian toddlers to perfluoroalkyl substances (PFAS): The association with breastfeeding and
8283 maternal PFAS concentrations. *Environment International*, 94, 687-694.
- 8284 Papadopoulou E, Poothong S, Koekkoek J, Lacattini L, Padilla-Sanchez JA, Haugen M, Herzke D,
8285 Valdersnes S, Maage A, Cousins IT, Leonards PEG and Haug LS, 2017. Estimating human exposure
8286 to perfluoroalkyl acids via solid food and drinks: Implementation and comparison of different dietary
8287 assessment methods. *Environmental Research*, 158, 269-276.
8288 <http://dx.doi.org/10.1016/j.envres.2017.06.011>
- 8289 Parzefall W, Berger W, Kainzbauer E, Teufelhofer O, Schulte-Hermann R and Thurman RG, 2001.
8290 Peroxisome proliferators do not increase DNA synthesis in purified rat hepatocytes. *Carcinogenesis*,
8291 22, 519-523.
- 8292 Peden-Adams MM, Keller JM, Eudaly JG, Berger J, Gilkeson GS and Keil DE, 2008. Suppression of
8293 humoral immunity in mice following exposure to perfluorooctane sulfonate. *Toxicological Sciences*,
8294 104, 144-154.
- 8295 Peng H, Zhang S, Sun J, Zhang Z, Giesy JP and Hu J, 2014. Isomer-Specific Accumulation of
8296 Perfluorooctanesulfonate from (N-Ethyl perfluorooctanesulfonamido) ethanol-based Phosphate
8297 Diester in Japanese Medaka (*Oryzias latipes*). *Environmental Science & Technology*, 48, 1058-1066.
- 8298 Pennings JL, Jennen DG, Nygaard UC, Namork E, Haug LS, van Loveren H and Granum B, 2016. Cord
8299 blood gene expression supports that prenatal exposure to perfluoroalkyl substances causes
8300 depressed immune functionality in early childhood. *Journal of Immunotoxicology*, 13, 173-180.
- 8301 Pérez F, Nadal M, Navarro-Ortega A, Fabrega F, Domingo JL, Barcelo D and Farre M, 2013. Accumulation
8302 of perfluoroalkyl substances in human tissues. *Environment International*, 59, 354-362.
8303 doi:10.1016/j.envint.2013.06.004
- 8304 Perrone CE, Shao L and Williams GM, 1998. Effect of rodent hepatocarcinogenic peroxisome proliferators
8305 on fatty acyl-CoA oxidase, DNA synthesis, and apoptosis in cultured human and rat hepatocytes.
8306 *Toxicology and Applied Pharmacology*, 150, 277-286.

- 8307 Persson S, Rotander A, Karrman A, van Bavel B and Magnusson U, 2013. Perfluoroalkyl acids in subarctic
8308 wild male mink (*Neovison vison*) in relation to age, season and geographical area. *Environment*
8309 *International*, 59, 425-430. doi:10.1016/j.envint.2013.06.025
- 8310 Pierozan P, Jerneren F and Karlsson O, 2018a. Perfluorooctanoic acid (PFOA) exposure promotes
8311 proliferation, migration and invasion potential in human breast epithelial cells. *Archives of Toxicology*,
8312 92, 1729-1739. <https://doi.org/10.1007/s00204-018-2181-4>
- 8313 Pierozan P and Karlsson O, 2018b. PFOS induces proliferation, cell-cycle progression, and malignant
8314 phenotype in human breast epithelial cells. *Archives of Toxicology*, 92, 705-716. doi: 10.1007/s00204-
8315 017-2077-8.
- 8316 Pizzurro DM, Seeley M, Kerper LE and Beck BD, 2019. Interspecies differences in perfluoroalkyl
8317 substances (PFAS) toxicokinetics and application to health-based criteria *Regulatory Toxicology and*
8318 *Pharmacology*, 106, 239-250.
- 8319 Poonthong S, Thomsen C, Padilla-Sanchez JA, Papadopoulou E and Haug LS, 2017. Distribution of Novel
8320 and Well-Known Poly- and Perfluoroalkyl Substances (PFASs) in Human Serum, Plasma, and Whole
8321 Blood. *Environmental Science & Technology*, 51, 13388-13396. doi: 10.1021/acs.est.7b03299.
- 8322 Poonthong S, Papadopoulou E, Padilla-Sánchez JA, Thomsen C and Haug LS, 2020. Multiple pathways of
8323 human exposure to poly- and perfluoroalkyl substances (PFASs): From external exposure to human
8324 blood. *Environment International*, 134, 105244. doi: 10.1016/j.envint.2019.105244
- 8325 Pope CA 3rd, Burnett RT, Turner MC, Cohen A, Krewski D, Jerrett M, Gapstur SM and Thun MJ, 2011.
8326 Lung cancer and cardiovascular disease mortality associated with ambient air pollution and cigarette
8327 smoke: shape of the exposure-response relationships. *Environmental Health Perspectives*, 119,
8328 1616-1621. doi: 10.1289/ehp.1103639.
- 8329 Porpora MG, Lucchini R, Abballe A, Ingelido AM, Valentini S, Fuggetta E, Cardi V, Ticino A, Marra V,
8330 Fulgenzi AR and De Felip E, 2013. Placental transfer of persistent organic pollutants: a preliminary
8331 study on mother-newborn pairs. *International Journal of Environmental Research and Public Health*,
8332 10, 699-711.
- 8333 Post GB, Cohn PD and Cooper KR, 2012. Perfluorooctanoic acid (PFOA), an emerging drinking water
8334 contaminant: a critical review of recent literature. *Environmental Research*, 116, 93-117.
- 8335 Power MC, Webster TF, Baccarelli AA and Weisskopf MG, 2013. Cross-sectional association between
8336 polyfluoroalkyl chemicals and cognitive limitation in the National Health and Nutrition Examination
8337 Survey. *Neuroepidemiology*, 40, 125-132.
- 8338 Powley CR, George SW, Ryan TW and Buck RC, 2005. Matrix effect free analytical methods for
8339 determination of perfluorinated carboxylic acids in environmental matrices. *Analytical Chemistry*, 77,
8340 6353-6358.
- 8341 Predieri B, Iughetti L, Guerranti C, Bruzzi P, Perra G and Focardi SE, 2015. High Levels of Perfluorooctane
8342 Sulfonate in Children at the Onset of Diabetes. *International Journal of Endocrinology*, 234358, 7 pp.
8343 <https://doi.org/10.1155/2015/234358>
- 8344 Prevedouros K, Cousins IT, Buck RC and Korzeniowski SH, 2006. Sources, fate and transport of
8345 perfluorocarboxylates. *Environmental Science & Technology*, 40, 32-44.
- 8346 Pugh G Jr, Isenberg JS, Kamendulis LM, Ackley DC, Clare LJ, Brown R, Lington AW, Smith JH and Klaunig
8347 JE, 2000. Effects of di-isononyl phthalate, di-2-ethylhexyl phthalate, and clofibrate in cynomolgus
8348 monkeys. *Toxicological Sciences*, 56, 181-188.
- 8349 Qazi MR, Xia Z, Bogdanska J, Chang SC, Ehresman DJ, Butenhoff JL, Nelson BD, DePierre JW and Abedi-
8350 Valugerdi M, 2009b. The atrophy and changes in the cellular compositions of the thymus and spleen

- 8351 observed in mice subjected to short-term exposure to perfluorooctanesulfonate are high dose
8352 phenomena mediated in part by peroxisome proliferator-activated receptor- α (PPAR α).
8353 *Toxicology*, 260, 68–76.
- 8354 Qazi MR, Bogdanska J, Butenhoff JL, Nelson BD, DePierre JW and Abedi-Valugerdi M, 2009a. High-dose,
8355 short-term exposure of mice to perfluorooctanesulfonate (PFOS) or perfluorooctanoate (PFOA)
8356 affects the number of circulating neutrophils differently, but enhances the inflammatory responses
8357 of macrophages to lipopolysaccharide (LPS) in a similar fashion. *Toxicology*, 262, 207–214.
- 8358 Qazi MR, Nelson BD, DePierre JW and Abedi-Valugerdi M, 2010. 28-Day dietary exposure of mice to a
8359 low total dose (7 mg/kg) of perfluorooctanesulfonate (PFOS) alters neither the cellular compositions
8360 of the thymus and spleen nor humoral immune responses: Does the route of administration play a
8361 pivotal role in PFOS-induced immunotoxicity? *Toxicology*, 267, 132–139.
- 8362 Qazi MR, Dean Nelson B, DePierre JW and Abedi-Valugerdi M, 2012. High-dose dietary exposure of mice
8363 to perfluorooctanoate or perfluorooctane sulfonate exerts toxic effects on myeloid and B-lymphoid
8364 cells in the bone marrow and these effects are partially dependent on reduced food consumption.
8365 *Food and Chemical Toxicology*, 50, 2955–2963.
- 8366 Qin XD, Qian Z, Vaughn MG, Huang J, Ward P, Zeng XW, Zhou Y, Zhu Y, Yuan P, Li M, Bai Z, Paul G,
8367 Hao YT, Chen W, Chen PC, Dong GH and Lee YL, 2016. Positive associations of serum perfluoroalkyl
8368 substances with uric acid and hyperuricemia in children from Taiwan. *Environmental Pollution*, 212,
8369 519–524. doi: 10.1016/j.envpol.2016.02.050
- 8370 Qin XD, Qian ZM, Dharmage SC, Perret J, Geiger SD, Rigdon SE, Howard S, Zeng XW, Hu LW, Yang BY,
8371 Zhou Y, Li M, Xu SL, Bao WW, Zhang YZ, Yuan P, Wang J, Zhang C, Tian YP, Nian M, Xiao X, Chen
8372 W, Lee YL and Dong GH, 2017. Association of perfluoroalkyl substances exposure with impaired lung
8373 function in children. *Environmental Research*, 155, 15–21. doi: 10.1016/j.envres.2017.01.025
- 8374 Quaak I, de Cock M, de Boer M, Lamoree M, Leonards P and van de Bor M, 2016. Prenatal Exposure to
8375 Perfluoroalkyl Substances and Behavioral Development in Children. *International Journal of*
8376 *Environmental Research and Public Health*, 13. doi:10.3390/ijerph13050511
- 8377 Quiñones O and Snyder SA, 2009. Occurrence of Perfluoroalkyl Carboxylates and Sulfonates in Drinking
8378 Water Utilities and Related Waters from the United States. *Environmental Science & Technology*, 43,
8379 9089–9095. doi:10.1021/es9024707
- 8380 Ramhøj L, Hass U, Boberg J, Scholze M, Christiansen S, Nielsen F and Axelstad M, 2018. Perfluorohexane
8381 Sulfonate (PFHxS) and a Mixture of Endocrine Disruptors Reduce Thyroxine Levels and Cause
8382 Antiandrogenic Effects in Rats. *Toxicological Sciences*, 163, 579–591. doi:10.1093/toxsci/kfy055
- 8383 Rand AA, Rooney JP, Butt CM, Meyer JN and Mabury SA, 2014. Cellular toxicity associated with exposure
8384 to perfluorinated carboxylates (PFCAs) and their metabolic precursors. *Chemical Research in*
8385 *Toxicology*, 27, 42–50.
- 8386 Rantakokko P, Mannisto V, Airaksinen R, Koponen J, Viluksela M, Kiviranta H and Pihlajamäki J, 2015.
8387 Persistent organic pollutants and non-alcoholic fatty liver disease in morbidly obese patients: a cohort
8388 study. *Environmental Health*, 14, 79. doi: 10.1186/s12940-015-0066-z
- 8389 Rayne S and Forest K, 2009. Congener-specific organic carbon-normalized soil and sediment-water
8390 partitioning coefficients for the C1 through C8 perfluoroalkyl carboxylic and sulfonic acids. *Journal of*
8391 *Environmental Science and Health Part A Toxic/Hazardous Substances & Environmental Engineering*,
8392 44, 1374–1387. doi:10.1080/10934520903217229
- 8393 Reistad T, Fonnum F and Mariussen E, 2013. Perfluoroalkylated compounds induce cell death and
8394 formation of reactive oxygen species in cultured cerebellar granule cells. *Toxicology Letters*, 218,
8395 56–60.

- 8396 Ren H, Vallanat B, Nelson DM, Yeung LW, Guruge KS, Lam PK, Lehman-McKeeman LD and Corton JC,
8397 2009. Evidence for the involvement of xenobiotic-responsive nuclear receptors in transcriptional
8398 effects upon perfluoroalkyl acid exposure in diverse species. *Reproductive Toxicology*, 27, 266-277.
- 8399 Ren XM, Zhang YF, Guo LH, Qin ZF, Lv QY and Zhang LY, 2015. Structure-activity relations in binding
8400 of perfluoroalkyl compounds to human thyroid hormone T3 receptor. *Archives of Toxicology*, 89,
8401 233–242.
- 8402 Ren XM, Qin WP, Cao LY, Zhang J, Yang Y, Wan B and Guo LH, 2016. Binding interactions of
8403 perfluoroalkyl substances with thyroid hormone transport proteins and potential toxicological
8404 implications. *Toxicology*, 366-367, 32-42. <http://dx.doi.org/10.1016/j.tox.2016.08.011>
- 8405 Rasoulpour RJ, Terry C, LeBaron MJ, Stebbins K, Ellis-Hutchings RG and Billington R, 2013. Mode-of-
8406 action and human relevance framework analysis for rat Leydig cell tumors associated with sulfoxaflo.
8407 *Critical Reviews in Toxicology*, 25-44. doi: 10.3109/10408444.2014.910750
- 8408 Riddell N, Arsenault G, Benskin JP, Chittim B, Martin JW, McAlees A and McCrindle R, 2009. Branched
8409 perfluorooctane sulfonate isomer quantification and characterization in blood serum samples by
8410 HPLC/ESI-MS(/MS). *Environmental Science & Technology*, 43, 7902–7908.
8411 <https://doi.org/10.1021/es901261v>
- 8412 Robledo CA, Yeung E, Mendola P, Sundaram R, Maisog J, Sweeney AM, Barr DB and Louis GM, 2015.
8413 Preconception maternal and paternal exposure to persistent organic pollutants and birth size: the
8414 LIFE study. *Environmental Health Perspectives*, 123, 88–94. <https://doi.org/10.1289/ehp.1308016>
- 8415 Rockwell CE, Turley AE, Cheng X, Fields PE and Klaassen CD, 2013. Acute Immunotoxic Effects of
8416 Perfluorononanoic Acid (PFNA) in C57BL/6 Mice. *Clinical and Experimental Pharmacology*, Suppl 4.
8417 doi:10.4172/2161-1459.s4-002
- 8418 Rockwell CE, Turley AE, Cheng X, Fields PE and Klaassen CD, 2017. Persistent alterations in immune
8419 cell populations and function from a single dose of perfluorononanoic acid (PFNA) in C57Bl/6 mice.
8420 *Food and Chemical Toxicology*, 100, 24-33. doi:10.1016/j.fct.2016.12.004
- 8421 Rogers JM, Ellis-Hutchings RG, Grey BE, Zucker RM, Norwood J Jr, Grace CE, Gordon CJ and Lau C,
8422 2014. Elevated blood pressure in offspring of rats exposed to diverse chemicals during pregnancy.
8423 *Toxicological Sciences*, 137, 436-446. doi:10.1093/toxsci/kft248
- 8424 Rolfe M, James NH and Roberts RA, 1997. Tumour necrosis factor alpha (TNF-alpha) suppresses
8425 apoptosis and induces S-phase in rodent hepatocytes: a mediator of the hepatocarcinogenicity of
8426 peroxisome proliferators? *Carcinogenesis* 18, 2277–2280.
- 8427 Roos A, Berger U, Jarnberg U, van Dijk J and Bignert A, 2013. Increasing concentrations of perfluoroalkyl
8428 acids in Scandinavian otters (*Lutra lutra*) between 1972 and 2011: a new threat to the otter
8429 population? *Environmental Science & Technology*, 47, 11757-11765. doi:10.1021/es401485t
- 8430 Rose MI, Rusyn I, Bojes HK, Belyea J, Cattley RC and Thurman RG, 2000. Role of Kupffer cells and
8431 oxidants in signaling peroxisome proliferator-induced hepatocyte proliferation. *Mutation Research*,
8432 448, 179–192.
- 8433 Rosen MB, Das KP, Rooney J, Abbott B, Lau C and Corton JC, 2017. PPARalpha-independent
8434 transcriptional targets of perfluoroalkyl acids revealed by transcript profiling. *Toxicology*, 387, 95-
8435 107. doi:10.1016/j.tox.2017.05.013
- 8436 Rosen MB, Lee JS, Ren H, Vallanat B, Liu J, Waalkes MP, Abbott BD, Lau C and Corton JC, 2008.
8437 Toxicogenomic dissection of the perfluorooctanoic acid transcript profile in mouse liver: evidence for
8438 the involvement of nuclear receptors PPAR alpha and CAR. *Toxicological Sciences*, 103, 46-56.

- 8439 Rosenmai AK, Taxvig C, Svingen T, Trier X, van Vugt-Lussenburg BM, Pedersen M, Lesné L, Jégou B
8440 and Vinggaard AM, 2016. Fluorinated alkyl substances and technical mixtures used in food paper-
8441 packaging exhibit endocrine-related activity in vitro. *Andrology*, 4, 662–672.
- 8442 Rosenmai AK, Ahrens L, le Godec T, Lundqvist J and Oskarsson, 2018. Relationship between
8443 peroxisome proliferator-activated receptor alpha activity and cellular concentration of 14 perfluoroalkyl
8444 substances in HepG2 cells. *Journal of applied Toxicology*, 38, 219-226. DOI: 10.1002/jat.3515
- 8445 Ross MS, Wong CS and Martin JW, 2012. Isomer-specific biotransformation of perfluorooctane
8446 sulfonamide in Sprague-Dawley rats. *Environmental Science & Technology*, 46, 3196-3203.
8447 doi:10.1021/es204028v
- 8448 Rotander A, Toms LM, Aylward L, Kay M and Mueller JF, 2015. Elevated levels of PFOS and PFHxS in
8449 firefighters exposed to aqueous film forming foam (AFFF). *Environment International*, 82, 28-34.
- 8450 Route WT, Russell RE, Lindstrom AB, Strynar MJ and Key RL, 2014. Spatial and Temporal Patterns in
8451 Concentrations of Perfluorinated Compounds in Bald Eagle Nestlings in the Upper Midwestern United
8452 States. *Environmental Science & Technology*, 48, 6653-6660. doi:10.1021/es501055d
- 8453 Rubarth J, Dreyer A, Guse N, Einax JW and Ebinghaus R, 2011. Perfluorinated compounds in red-
8454 throated divers from the German Baltic Sea: new findings from their distribution in 10 different
8455 tissues. *Environmental Chemistry*, 8, 419-428. doi:10.1071/EN10142
- 8456 Rush EL, Singer AB, Longnecker MP, Haug LS, Sabaredzovic A, Symanski E and Whitworth KW, 2018.
8457 Oral contraceptive use as a determinant of plasma concentrations of perfluoroalkyl substances
8458 among women in the Norwegian Mother and Child Cohort (MoBa) study. *Environment International*,
8459 112, 156-164. doi:10.1016/j.envint.2017.12.015
- 8460 Russell MH, Nilsson H and Buck RC, 2013. Elimination kinetics of perfluorohexanoic acid in humans and
8461 comparison with mouse, rat and monkey. *Chemosphere*, 93, 2419-2425.
8462 doi:10.1016/j.chemosphere.2013.08.060
- 8463 Russo J and Russo IH, 1996. Experimentally induced mammary tumors in rats. *Breast Cancer Research*
8464 and Treatment, 39(1), 7-20.
- 8465 Rylander C, Phi DT, Odland JØ and Sandanger TM, 2009. Perfluorinated compounds in delivering women
8466 from south central Vietnam. *Journal of Environmental Monitoring*, 11, 2002-2008. doi:
8467 10.1039/b908551c
- 8468 Sagiv SK, Rifas-Shiman SL, Webster TF, Mora AM, Harris MH, Calafat AM, Ye X, Gillman MW and Oken
8469 E, 2015. Sociodemographic and Perinatal Predictors of Early Pregnancy Per- and Polyfluoroalkyl
8470 Substance (PFAS) Concentrations. *Environmental Science Technology*, 49, 11849-11858.
- 8471 Saito N, Harada K, Inoue K, Sasaki K, Yoshinaga T and Koizumi A, 2004. Perfluorooctanoate and
8472 perfluorooctanesulfonate concentrations in surface water in Japan. *Journal of Occupational Health*,
8473 46, 49–59.
- 8474 Salihovic S, Nilsson H, Hagberg J and Lindström G, 2013. Trends in the analysis of persistent organic
8475 pollutants (POPs) in human blood. *TrAC Trends in Analytical Chemistry*, 46, 129–138.
8476 <https://doi.org/10.1016/j.trac.2012.06.009>
- 8477 Salihovic S, Stubleski J, Karrman A, Larsson A, Fall T, Lind L and Lind PM, 2018. Changes in markers of
8478 liver function in relation to changes in perfluoroalkyl substances - A longitudinal study. *Environment*
8479 *International*, 117, 196-203. doi: 10.1016/j.envint.2018.04.052
- 8480 Sato I, Kawamoto K, Nishikawa Y, Tsuda S, Yoshida M, Yaegashi K, Saito N, Liu W and Jin Y, 2009.
8481 Neurotoxicity of perfluorooctane sulfonate (PFOS) in rats and mice after single oral exposure. *Journal*
8482 *of Toxicological Sciences*, 34, 569-574.

- 8483 Savitz DA, 2007. Guest Editorial: Biomarkers of Perfluorinated Chemicals and Birth Weight.
8484 Environmental Health Perspectives, 115, A528–A529. <https://doi.org/10.1289/ehp.10923>.
- 8485 Schaidt LA, Balan SA, Blum A, Andrews DQ, Strynar MJ, Dickinson ME, Lunderberg DM, Lang JR and
8486 Peaslee GF, 2017. Fluorinated Compounds in U.S. Fast Food Packaging. Environmental Science &
8487 Technology Letters, 4, 105–111. doi:10.1021/acs.estlett.6b00435
- 8488 Scher DP, Kelly JE, Huset CA, Barry KM, Hoffbeck RW, Yingling VL and Messing RB, 2018. Occurrence
8489 of perfluoroalkyl substances (PFAS) in garden produce at homes with a history of PFAS-contaminated
8490 drinking water. Chemosphere, 196, 548–555. doi:10.1016/j.chemosphere.2017.12.179
- 8491 Schröter-Kermani C, Müller J, Jüriling H, Conrad A and Schulte C, 2013. Retrospective monitoring of
8492 perfluorocarboxylates and perfluorosulfonates in human plasma archived by the German
8493 Environmental Specimen Bank. International Journal of Hygiene and Environmental Health, 216,
8494 633–640.
- 8495 Seo SH, Son MH, Choi SD, Lee DH and Chang YS, 2018. Influence of exposure to perfluoroalkyl
8496 substances (PFASs) on the Korean general population: 10-year trend and health effects. Environment
8497 International, 113, 149–161. doi: 10.1016/j.envint.2018.01.025
- 8498 Shabalina IG, Kramarova TV, Mattsson CL, Petrovic N, Rahman Qazi M, Csikasz RI, Chang SC, Butenhoff
8499 J, DePierre JW, Cannon B and Nedergaard J, 2015. The Environmental Pollutants Perfluorooctane
8500 Sulfonate and Perfluorooctanoic Acid Upregulate Uncoupling Protein 1 (UCP1) in Brown-Fat
8501 Mitochondria Through a UCP1-Dependent Reduction in Food Intake. Journal of Toxicological
8502 Sciences, 146, 334–343.
- 8503 Shah YM, Morimura K, Yang Q, Tanabe T, Takagi M and Gonzalez FJ, 2007. Peroxisome proliferator-
8504 activated receptor alpha regulates a microRNA-mediated signaling cascade responsible for
8505 hepatocellular proliferation. Molecular and Cellular Biology, 27, 4238–4247.
- 8506 Shah-Kulkarni S, Kim BM, Hong YC, Kim HS, Kwon EJ, Park H, Kim YJ and Ha EH, 2016. Prenatal
8507 exposure to perfluorinated compounds affects thyroid hormone levels in newborn girls. Environment
8508 International, 94, 607–613. doi: 10.1016/j.envint.2016.06.024
- 8509 Shaw D, Lee R and Roberts RA, 2002. Species differences in response to the phthalate plasticizer
8510 monoisononylphthalate (MINP) *in vitro*: a comparison of rat and human hepatocytes. Archives of
8511 Toxicology, 76, 344–350.
- 8512 Shaw SD, Berger ML, Harris JH, Yun SH, Wu Q, Liao C, Blum A, Stefani A and Kannan K, 2013. Persistent
8513 organic pollutants including polychlorinated and polybrominated dibenzo-*p*-dioxins and
8514 dibenzofurans in firefighters from Northern California. Chemosphere, 91, 1386–1394.
- 8515 Shelly, C., Grandjean, P., Oulhote, Y., Plomgaard, P., Frikk-Schmidt, R., Nielsen, F., Zmirou-Navier, D.,
8516 Weihe, P. and Valvi, D, 2019. Early life exposures to perfluoroalkyl substances in relation to adipokine
8517 hormone levels at birth and during childhood. J Clin Endocrinol Metab, 104, 5338–5348. doi:
8518 10.1210/jc.2019-00385
- 8519 Shi Z, Zhang H, Ding L, Feng Y, Xu M and Dai J, 2009a. The effect of perfluorododecanoic acid on
8520 endocrine status, sex hormones and expression of steroidogenic genes in pubertal female rats.
8521 Reproductive Toxicology, 27, 352–359.
- 8522 Shi Z, Ding L, Zhang H, Feng Y, Xu M and Dai J, 2009b. Chronic exposure to perfluorododecanoic acid
8523 disrupts testicular steroidogenesis and the expression of related genes in male rats. Toxicology
8524 Letters, 188, 192–200.
- 8525 Shi Y, Yang L, Li J, Lai J, Wang Y, Zhao Y and Wu Y, 2017. Occurrence of perfluoroalkyl substances in
8526 cord serum and association with growth indicators in newborns from Beijing. Chemosphere, 169,
8527 396–402. <https://doi.org/10.1016/j.chemosphere.2016.11.050>

- 8528 Shipley JM, Hurst CH, Tanaka SS, DeRoos FL, Butenhoff JL, Seacat AM and Waxman DJ, 2004. trans-
8529 activation of PPARalpha and induction of PPARalpha target genes by perfluorooctane-based
8530 chemicals. *Toxicological Sciences*, 80, 151-160.
- 8531 Shoeib M, Harner T and Vlahos P, 2006. Perfluorinated Chemicals in the Arctic Atmosphere.
8532 *Environmental Science & Technology*, 40, 7577-7583. doi:10.1021/es0618999
- 8533 Shoeib M, Harner T, G MW and Lee SC, 2011. Indoor sources of poly- and perfluorinated compounds
8534 (PFCS) in Vancouver, Canada: implications for human exposure. *Environmental Science &*
8535 *Technology*, 45, 7999-8005. doi:10.1021/es103562v
- 8536 Sinclair E, Kim SK, Akinleye HB and Kannan K. 2007. Quantitation of gas-phase perfluoroalkyl surfactants
8537 and fluorotelomer alcohols released from nonstick cookware and microwave popcorn bags.
8538 *Environmental Science & Technology* 41, 1180-1185.
- 8539 Singer AB, Whitworth KW, Haug LS, Sabaredzovic A, Impinen A, Papadopoulou E and Longnecker MP,
8540 2018. Menstrual cycle characteristics as determinants of plasma concentrations of perfluoroalkyl
8541 substances (PFASs) in the Norwegian Mother and Child Cohort (MoBa study). *Environmental*
8542 *Research*, 166, 78-85. <https://doi.org/10.1016/j.envres.2018.05.019>
- 8543 Singh S and Singh SK, 2019a. Effect of gestational exposure to perfluorononanoic acid on neonatal mice
8544 testes. *Journal of Applied Toxicology*, 39, 1663-1671. doi: 10.1002/jat.3883
- 8545 Singh S and Singh SK, 2019b. Chronic exposure to perfluorononanoic acid impairs spermatogenesis,
8546 steroidogenesis and fertility in male mice. *Journal of Applied Toxicology*, 39, 420-431.
8547 doi:10.1002/jat.3733
- 8548 Singh S and Singh SK, 2019c. Acute exposure to perfluorononanoic acid in prepubertal mice: Effect on
8549 germ cell dynamics and an insight into the possible mechanisms of its inhibitory action on testicular
8550 functions. *Ecotoxicology and Environmental Safety*, 183, 109499. doi:
8551 10.1016/j.ecoenv.2019.109499
- 8552 Singh S and Singh SK, 2019d. Prepubertal exposure to perfluorononanoic acid interferes with
8553 spermatogenesis and steroidogenesis in male mice. *Ecotoxicology and Environmental Safety*, 170,
8554 590-599. doi: 10.1016/j.ecoenv.2018.12.034
- 8555 Skutlarek D, Exner M and Färber H, 2006. Perfluorinated surfactants in surface and drinking waters.
8556 *Environmental Science and Pollution Research*, 13, 299-307.
- 8557 Sznajder-Katarzyńska K, Surma M, Cieřlik E and Wiczowski W, 2018. The perfluoroalkyl substances
8558 (PFASs) contamination of fruits and vegetables. *Food Additives & Contaminants: Part A*, 35, 1776-
8559 1786. <https://doi.org/10.1080/19440049.2018.1502477>
- 8560 Sochorová L, Hanzlíková L, Černá M, Drgáčová A, Fialová A, Svarcova A, Gramblička T and Pulkrabová
8561 J, 2017. Perfluorinated alkylated substances and brominated flame retardants in serum of the Czech
8562 adult population. *International Journal of Hygiene and Environmental Health*, 220, 235-243.
8563 doi:10.1016/j.ijheh.2016.09.003
- 8564 Song M, Kim YJ, Park YK and Ryu JC, 2012. Changes in thyroid peroxidase activity in response to various
8565 chemicals. *Journal of Environmental Monitoring*, 14, 2121-2126. doi: 10.1039/c2em30106g
- 8566 Song P, Li D, Wang X and Zhong X, 2018. Effects of perfluorooctanoic acid exposure during pregnancy
8567 on the reproduction and development of male offspring mice. *Andrologia*, 50:e13059. PMID:
8568 29862542. DOI: 10.1111/and.13059
- 8569 Song X, Tang S, Zhu H, Chen Z, Zang Z, Zhang Y, Niu X, Wang X, Yin H, Zeng F and He C, 2018.
8570 Biomonitoring PFAAs in blood and semen samples: Investigation of a potential link between PFAAs

- 8571 exposure and semen mobility in China. *Environment International.*, 113, 50-54.
8572 <https://doi.org/10.1016/j.envint.2018.01.010>
- 8573 Stahl T, Heyn J, Thiele H, Hühner J, Failing K, Georgii S and Brunn H, 2009. Carryover of
8574 Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) from Soil to Plants. *Archives of*
8575 *Environmental Contamination and Toxicology*, 57, 289–298.
- 8576 Starling AP, Engel SM, Whitworth KW, Richardson DB, Stuebe AM, Daniels JL, Haug LS, Eggesbo M,
8577 Becher G, Sabareedzovic A, Thomsen C, Wilson RE, Travlos GS, Hoppin JA, Baird DD and Longnecker
8578 MP, 2014. Perfluoroalkyl substances and lipid concentrations in plasma during pregnancy among
8579 women in the Norwegian Mother and Child Cohort Study. *Environment International*, 62, 104-112.
8580 doi: 10.1016/j.envint.2013.10.004
- 8581 Steenland K, Barry V and Savitz D, 2018. Serum perfluorooctanoic acid (PFOA) and birthweight: an
8582 updated meta-analysis with bias analysis. *Epidemiology*, 29, 765-776. DOI:
8583 10.1097/EDE.0000000000000903
- 8584 Steenland K, Tinker S, Frisbee S, Ducatman A and Vaccarino V, 2009. Association of perfluorooctanoic
8585 acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant.
8586 *American Journal of Epidemiology*, 170, 1268-1278.
- 8587 Steenland K and Woskie S, 2012. Cohort mortality study of workers exposed to perfluorooctanoic acid.
8588 *American Journal of Epidemiology*, 176, 909-917.
- 8589 Stein CR and Savitz DA, 2011. Serum perfluorinated compound concentration and attention
8590 deficit/hyperactivity disorder in children 5-18 years of age. *Environment Health Perspectives*, 119,
8591 1466-1471.
- 8592 Stein CR, Ge Y, Wolff MS, Ye X, Calafat AM, Kraus T and Moran TM, 2016b. Perfluoroalkyl substance
8593 serum concentrations and immune response to FluMist vaccination among healthy adults.
8594 *Environmental Research*, 149, 171-178. doi: 10.1016/j.envres.2016.05.020
- 8595 Stein CR, McGovern KJ, Pajak AM, Maglione PJ and Wolff MS, 2016a. Perfluoroalkyl and polyfluoroalkyl
8596 substances and indicators of immune function in children aged 12-19 y: National Health and Nutrition
8597 Examination Survey. *Pediatric Research*, 79, 348-357. doi: 10.1038/pr.2015.213
- 8598 Stein CR, Savitz DA and Bellinger DC, 2013. Perfluorooctanoate and neuropsychological outcomes in
8599 children. *Epidemiology*, 24, 590-599. doi: 10.1097/EDE.0b013e3182944432
- 8600 Stein CR, Savitz DA and Bellinger DC, 2014. Perfluorooctanoate exposure in a highly exposed community
8601 and parent and teacher reports of behaviour in 6-12-year-old children. *Paediatric and Perinatal*
8602 *Epidemiology*, 28, 146-156.
- 8603 Stein CR, Wolff MS, Calafat AM, Kato K and Engel SM, 2012. Comparison of polyfluoroalkyl compound
8604 concentrations in maternal serum and amniotic fluid: a pilot study. *Reproductive Toxicology*, 34,
8605 312-316.
- 8606 Stock NL, Muir DCG and Mabury SA, 2010. Perfluoroalkyl compounds. In: Harrad S (ed.). *Persistent*
8607 *organic pollutants*. Wiley, Chichester, UK, 25–70.
- 8608 Stubleski J, Salihovic S, Lind L, Lind PM, van Bavel B and Kärrman A, 2016. Changes in serum levels of
8609 perfluoroalkyl substances during a 10-year follow-up period in a large population-based cohort.
8610 *Environment International*, 95, 86-92.
- 8611 Su TC, Kuo CC, Hwang JJ, Lien GW, Chen MF and Chen PC, 2016. Serum perfluorinated chemicals,
8612 glucose homeostasis and the risk of diabetes in working-aged Taiwanese adults. *Environment*
8613 *International*, 88, 15-22. doi: 10.1016/j.envint.2015.11.016

- 8614 Su M, Liang X, Xu X, Wu X and Yang B, 2019. Hepatoprotective benefits of vitamin C against
8615 perfluorooctane sulfonate induced liver damage in mice through suppressing inflammatory reaction
8616 and ER stress. *Environmental Toxicology and Pharmacology*, 65, 60-65,
8617 <https://doi.org/10.1016/j.etap.2018.12.004>
- 8618 Sundström M, Ehresman DJ, Bignert A, Butenhoff JL, Olsen GW, Chang SC and Bergman A, 2011. A
8619 temporal trend study (1972-2008) of perfluorooctanesulfonate, perfluorohexanesulfonate, and
8620 perfluorooctanoate in pooled human milk samples from Stockholm, Sweden. *Environment*
8621 *International*, 37, 178-83.
- 8622 Sundström M, Chang SC, Noker PE, Gorman GS, Hart JA, Ehresman DJ, Bergman A and Butenhoff JL,
8623 2012. Comparative pharmacokinetics of perfluorohexanesulfonate (PFHxS) in rats, mice, and
8624 monkeys. *Reproductive Toxicology*, 33, 441-451. doi:10.1016/j.reprotox.2011.07.004
- 8625 Suo C, Fan Z and Qiu J, 2017. Perfluorooctane sulfonate affects intestinal immunity against bacterial
8626 infection. *Scientific Reports*, 7, 5166. DOI:10.1038/s41598-017-04091-z
- 8627 Susmann H, Schaidler LA, Rodgers KM and Rudel RA, 2019. Dietary Habits Related to Food Packaging
8628 and Population Exposure to PFASs. *Environmental Health Perspectives*, 127, 107003-107010.
8629 <https://doi.org/10.1289/EHP4092>
- 8630 Swedish Chemical Agency, 2015. Occurrence and use of highly fluorinated substances and alternatives.
8631 Report from a government assignment. Report 7/15. Available online:
8632 [https://www.kemi.se/en/global/rapporter/2015/report-7-15-occurrence-and-use-of-highly-](https://www.kemi.se/en/global/rapporter/2015/report-7-15-occurrence-and-use-of-highly-fluorinated-substances-and-alternatives.pdf)
8633 [fluorinated-substances-and-alternatives.pdf](https://www.kemi.se/en/global/rapporter/2015/report-7-15-occurrence-and-use-of-highly-fluorinated-substances-and-alternatives.pdf)
- 8634 Swedish EPA (Swedish Environmental Protection Agency), 2012. Environmental and Health Risk
8635 Assessment of Perfluoroalkylated and Polyfluoroalkylated Substances (PFASs) in Sweden. Report
8636 6513, September 2012, 139 pp. Available online:
8637 <http://www.naturvardsverket.se/Documents/publikationer6400/978-91-620-6513-3.pdf?pid=3822>
- 8638 Symonds ME, Pope M and Budge H, 2015. The Ontogeny of Brown Adipose Tissue. *Annual Review of*
8639 *Nutrition*, 35, 295-320.
- 8640 Takahashi M, Ishida S, Hirata-Koizumi M, Ono A and Hirose A, 2014. Repeated dose and
8641 reproductive/developmental toxicity of perfluoroundecanoic acid in rats. *Journal of Toxicological*
8642 *Sciences Kato*, 39, 97-108.
- 8643 Tan X, Xie G, Sun X, Li Q, Zhong W, Qiao P, Sun X, Jia W and Zhou Z, 2013. High fat diet feeding
8644 exaggerates perfluorooctanoic acid-induced liver injury in mice via modulating multiple metabolic
8645 pathways. *PLoS One*, 8(4):e61409.
- 8646 Taniyasu S, Senthilkumar K, Yamazaki E, Yeung LW, Guruge KS, Kannan K and Yamashita N, 2013.
8647 Perfluoroalkyl substances in the blood of wild rats and mice from 47 prefectures in Japan: use of
8648 samples from nationwide specimen bank. *Archives of Environmental Contamination and Toxicology*,
8649 65, 149-170. doi:10.1007/s00244-013-9878-4
- 8650 Tao L, Kannan K, Aldous KM, Mauer MP and Eadon GA, 2008. Biomonitoring of perfluorochemicals in
8651 plasma of New York State personnel responding to the World Trade Center disaster. *Environmental*
8652 *Science & Technology*, 42, 3472-3478.
- 8653 Tateno C, Yamamoto T, Utoh R, Yamasaki C, Ishida Y, Myoken Y, Oofusa K, Okada M, Tsutsui N and
8654 Yoshizato K, 2015. Chimeric mice with hepatocyte-humanized liver as an appropriate model to study
8655 human peroxisome proliferator-activated receptor- α . *Toxicologic Pathology*, 43, 233-248.
- 8656 Tatum-Gibbs K, Wambaugh JF, Das KP, Zehr RD, Strynar MJ, Lindstrom AB, Delinsky A and Lau C, 2011.
8657 Comparative pharmacokinetics of perfluorononanoic acid in rat and mouse. *Toxicology*, 281, 48-55.
8658 doi:10.1016/j.tox.2011.01.003

- 8659 Taylor KW, Hoffman K, Thayer KA and Daniels JL, 2014. Polyfluoroalkyl chemicals and menopause
8660 among women 20-65 years of age (NHANES). *Environmental Health Perspectives*, 122, 145–150.
8661 <https://doi.org/10.1289/ehp.1306707>
- 8662 Theurich MA, Davanzo R, Busck-Rasmussen M, Diaz-Gomez NM, Brennan C, Kylberg E, Boerug A,
8663 McHugh L, Weikart C, Abraham K and Koletzko B, 2019. Breastfeeding Rates and Programs in
8664 Europe: A Survey of 11 National Breastfeeding Committees and Representatives. *JPGN*, 68, 400–407.
- 8665 Thomas M, Bayha C, Klein K, Müller S, Weiss TS, Schwab M and Zanger U, 2015. The truncated splice
8666 variant of peroxisome proliferator-activated receptor alpha, PPAR α -tr, autonomously regulates
8667 proliferative and pro-inflammatory genes. *BMC Cancer*, 15, 488. doi: 10.1186/s12885-015-1500-x
- 8668 Thompson J, Lorber M, Toms LL, Kato K, Calafat AM and Mueller JF, 2010. Use of simple pharmacokinetic
8669 modeling to characterize exposure of Australians to perfluorooctanoic acid and perfluorooctane
8670 sulfonic acid. *Environment International*, 36, 390–397. doi: 10.1016/j.envint.2010.02.008
- 8671 Thomsen C, Haug LS, Stigum H, Frøshaug M, Broadwell SL and Becher G, 2011. Changes in
8672 concentrations of perfluorinated compounds, polybrominated diphenyl ethers, and polychlorinated
8673 biphenyls in Norwegian breast-milk during twelve months of lactation. *Toxicology Letters*, 205, 62–
8674 68.
- 8675 Timmermann CAG, Budtz-Jørgensen E, Petersen MS, Weihe P, Steuerwald U, Nielsen F, Jensen TK and
8676 Grandjean P, 2016. Shorter duration of breastfeeding at elevated exposures to perfluoroalkyl
8677 substances. *Reproductive Toxicology*, 68, 164–170. <https://doi.org/10.1016/j.reprotox.2016.07.010>
- 8678 Timmermann CAG, Budtz-Jørgensen E, Jensen TK, Osuna CE, Petersen MS, Steuerwald U, Nielsen F,
8679 Poulsen LK, Weihe P and Grandjean P, 2017. Association between perfluoroalkyl substance exposure
8680 and asthma and allergic disease in children as modified by MMR vaccination. *Journal of*
8681 *Immunotoxicology*, 14, 39–49. doi: 10.1080/1547691X.2016.1254306
- 8682 Tittlemier SA, Pepper K and Edwards L, 2006. Concentrations perfluorooctane sulfonamides in Canadian
8683 total diet study composite food samples collected between 1992 and 2004. *Journal of Agricultural*
8684 *and Food Chemistry*, 54, 8385–8389.
- 8685 Toft G, Jonsson BA, Lindh CH, Giwercman A, Spano M, Heederik D, Lenters V, Vermeulen R, Rylander
8686 L, Pedersen HS, Ludwicki JK, Zvezdai V and Bonde JP, 2012. Exposure to perfluorinated compounds
8687 and human semen quality in Arctic and European populations. *Human Reproduction*, 27, 2532–2540.
8688 <https://doi.org/10.1093/humrep/des185>
- 8689 Toms LM, Thompson J, Rotander A, Hobson P, Calafat AM, Kato K, Ye X, Broomhall S, Harden F and
8690 Mueller JF, 2014. Decline in perfluorooctane sulfonate and perfluorooctanoate serum concentrations
8691 in an Australian population from 2002 to 2011. *Environment International*, 71, 74–80.
- 8692 Tomy GT, Tittlemier SA, Palace VP, Budakowski WR, Braekevelt E, Brinkworth L and Friesen K, 2004.
8693 Biotransformation of N-ethyl perfluorooctanesulfonamide by rainbow trout (*Onchorhynchus mykiss*)
8694 liver microsomes. *Environmental Science & Technology* 38, 758–762.
8695 <https://doi.org/10.1021/es034550j>
- 8696 Trier X, Granby K and Christensen JH, 2011. Polyfluorinated surfactants (PFS) in paper and board
8697 coating for food packaging. *Environmental Science and Pollution Research*, 18, 1108–1120.
- 8698 Trudel D, Horowitz L, Wormuth M, Scheringer M, Cousins IT and Hungerbühler K, 2008. Estimating
8699 consumer exposure to PFOS and PFOA. *Risk Analysis*, 28, 251–269.
- 8700 Tucker DK, Macon MB, Strynar MJ, Dagnino S, Andersen E and Fenton SE, 2015. The mammary gland
8701 is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid
8702 (PFOA) exposure. *Reproductive Toxicology*, 54, 26–36.
8703 <https://doi.org/10.1016/j.reprotox.2014.12.002>

- 8704 Uhl M, 2016. Unpublished report Um-MuKi Bratislava-Vienna study. Personal communication from Maria
8705 Uhl (Umweltbundesamt, Austria) to Line S. Haug. Email 30 August 2016.
- 8706 Ullah S, Huber S, Bignert A and Berger U, 2014. Temporal trends of perfluoroalkane sulfonic acids and
8707 their sulfonamide-based precursors in herring from the Swedish west coast 1991-2011 including
8708 isomer-specific considerations. *Environment International*, 65, 63-72.
8709 doi:10.1016/j.envint.2014.01.005
- 8710 Urbanek-Olejnik K, Liszewska M, Winczura A and Kostka G, 2016. Changes of c-Myc and DNMT1 mRNA
8711 and protein levels in the rat livers induced by dibutyl phthalate treatment. *Toxicology & Industrial
8712 Health*, 32, 801-808.
- 8713 US EPA (US Environmental Protection Agency), 2002. Telomer Research Program, 2002. Telomer
8714 Research Program Update, Presented to the USEPA OPPT, November 25, 2002; U.S. Environmental
8715 Protection Agency public docket AR226-1141, Washington, DC.
- 8716 Van Esterik JC, Bastos Sales L, Dolle ME, Hakansson H, Herlin M, Legler J and van der Ven LT, 2016.
8717 Programming of metabolic effects in C57BL/6JxFVB mice by *in utero* and lactational exposure to
8718 perfluorooctanoic acid. *Archives of Toxicology*, 90, 701-715. <https://doi.org/10.1007/s00204-015-1488-7>
8719
- 8720 van Leeuwen SPJ, van Velzen MJM, Swart CP, van der Veen I, Traag WA and de Boer J, 2009.
8721 Halogenated contaminants in farmed salmon, trout, tilapia, pangasius, and shrimp. *Environmental
8722 Science & Technology*, 43, 4009-4015.
- 8723 Van Loveren H, Van Amsterdam JGC, Vandebriel RJ, Kimman TG, Rümke HC, Steerenberg PS and Vos
8724 JG, 2001. Vaccine-Induced Antibody Responses as Parameters of the Influence of Endogenous and
8725 environmental Factors. *Environmental Health Perspectives*, 109, 757-764.
- 8726 Vanparys C, Maras M, Lenjou M, Robbens J, Van Bockstaele D, Blust R and De Coen W, 2006. Flow
8727 cytometric cell cycle analysis allows for rapid screening of estrogenicity in MCF-7 breast cancer cells.
8728 *Toxicology In Vitro*, 20, 1238-1248.
- 8729 Vélez MP, Arbuckle TE and Fraser WD, 2015. Maternal exposure to perfluorinated chemicals and reduced
8730 fecundity: the MIREC study. *Human Reproduction*, 30, 701-709,
8731 <https://doi.org/10.1093/humrep/deu350>
- 8732 Verner MA, Loccisano AE, Morken NH, Yoon M, Wu H, McDougall R, Maisonet M, Marcus M, Kishi R,
8733 Miyashita C, Chen MH, Hsieh WS, Andersen ME, Clewell HJ 3rd and Longnecker MP, 2015.
8734 Associations of Perfluoroalkyl Substances (PFAS) with Lower Birth Weight: An Evaluation of Potential
8735 Confounding by Glomerular Filtration Rate Using a Physiologically Based Pharmacokinetic Model
8736 (PBPK). *Environmental Health Perspectives*, 123, 1317-1324. <https://doi.org/10.1289/ehp.1408837>
- 8737 Verner MA, Ngueta G, Jensen ET, Fromme H, Völkel W, Nygaard UC, Granum B and Longnecker MP,
8738 2016. A Simple Pharmacokinetic Model of Prenatal and Postnatal Exposure to Perfluoroalkyl
8739 Substances (PFASs). *Environmental Science & Technology*, 50, 978-986.
- 8740 Vestergaard S, Nielsen F, Andersson AM, Hjøllund NH, Grandjean P, Andersen HR and Jensen TK, 2012.
8741 Association between perfluorinated compounds and time to pregnancy in a prospective cohort of
8742 Danish couples attempting to conceive. *Human Reproduction*, 27(3), 873-880.
8743 <https://doi.org/10.1093/humrep/der450>
- 8744 Vestergren R and Cousins IT, 2009. Tracking the pathways of human exposure to perfluorocarboxylates.
8745 *Environmental Science & Technology*, 43, 5565-5575. DOI: 10.1021/es900228k
- 8746 Vestergren R, Ullah S, Cousins IT and Berger U, 2012. A matrix effect-free method for reliable
8747 quantification of perfluoroalkyl carboxylic acids and perfluoroalkane sulfonic acids at low parts per
8748 trillion levels in dietary samples. *Journal of Chromatography A*, 1237, 64-71.

- 8749 Vetvicka V and Vetvickova J, 2013. Reversal of perfluorooctanesulfonate-induced immunotoxicity by a
8750 glucan-resveratrol-vitamin C combination. *Oriental Pharmacy and Experimental Medicine*, 13, 77–
8751 84.
- 8752 Viberg H, Lee I and Eriksson P, 2013. Adult dose-dependent behavioral and cognitive disturbances after
8753 a single neonatal PFHxS dose. *Toxicology*, 304, 185-191. doi:10.1016/j.tox.2012.12.013
- 8754 Viswakarma N, Jia Y, Bai L, Vluggens A, Borensztajn J, Xu J and Reddy JK., 2010. Coactivators in PPAR-
8755 Regulated Gene Expression. *PPAR Research*, pii: 250126. doi: 10.1155/2010/250126
- 8756 Vongphachan V, Cassone CG, Wu D, Chiu S, Crump D and Kennedy SW, 2011. Effects of perfluoroalkyl
8757 compounds on mRNA expression levels of thyroid hormone-responsive genes in primary cultures of
8758 avian neuronal cells. *Toxicological Sciences*, 120, 392–402. doi: 10.1093/toxsci/kfq395
- 8759 Vorkamp K, Nielsen F, Kyhl HB, Husby S, Nielsen LB, Barington T, Andersson AM and Jensen TK, 2014.
8760 Polybrominated diphenyl ethers and perfluoroalkyl substances in serum of pregnant women: levels,
8761 correlations, and potential health implications. *Archives of Environmental Contamination and*
8762 *Toxicology*, 67, 9-20.
- 8763 Vuong AM, Yolton K, Webster GM, Sjodin A, Calafat AM, Braun JM, Dietrich KN, Lanphear BP and Chen
8764 A, 2016. Prenatal polybrominated diphenyl ether and perfluoroalkyl substance exposures and
8765 executive function in school-age children. *Environmental Research*, 147, 556-564.
8766 doi:10.1016/j.envres.2016.01.008
- 8767 Wallace KB, Kissling GE, Melnick RL and Blystone CR, 2013. Structure-activity relationships for
8768 perfluoroalkane-induced in vitro interference with rat liver mitochondrial respiration. *Toxicology*
8769 *Letters*, 222, 257-264.
- 8770 Walters MW and Wallace KB, 2010. Urea cycle gene expression is suppressed by PFOA treatment in
8771 rats. *Toxicology Letters*, 197(1), 46-50.
- 8772 Wan HT, Zhao YG, Wei X, Hui KY, Giesy JP and Wong CK, 2012. PFOS-induced hepatic steatosis, the
8773 mechanistic actions on β -oxidation and lipid transport. *Biochimica et Biophysica Acta*, 1820, 1092-
8774 1101.
- 8775 Wan HT, Leung PY, Zhao YG, Wei X, Wong MH and Wong CK, 2013. Blood plasma concentrations of
8776 endocrine disrupting chemicals in Hong Kong populations. *Journal of Hazardous Materials*, 261, 763-
8777 769.
- 8778 Wang N, Szostek B, Buck RC, Folsom PW, Sulecki LM and Gannon JT, 2009. 8-2 Fluorotelomer alcohol
8779 aerobic soil biodegradation: Pathways, metabolites, and metabolite yields. *Chemosphere*, 75, 1089–
8780 1096.
- 8781 Wang IJ, Hsieh WS, Chen CY, Fletcher T, Lien GW, Chiang HL, Chiang CF, Wu TN and Chen PC, 2011.
8782 The effect of prenatal perfluorinated chemicals exposures on pediatric atopy. *Environmental*
8783 *Research*, 111, 785–791.
- 8784 Wang Z, Cousins IT, Scheringer M and Hungerbuhler K, 2013a. Fluorinated alternatives to long-chain
8785 perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSA) and their potential
8786 precursors. *Environment International*, 60, 242-248.
- 8787 Wang Y, Starling AP, Haug LS, Eggesbo M, Becher G, Thomsen C, Travlos G, King D, Hoppin JA, Rogan
8788 WJ and Longnecker MP, 2013b. Association between perfluoroalkyl substances and thyroid
8789 stimulating hormone among pregnant women: a cross-sectional study. *Environmental Health*, 12,
8790 76. doi: 10.1186/1476-069x-12-76

- 8791 Wang Z, Cousins IT, Scheringer M, Buck RC and Hungerbühler K, 2014a. Global emission inventories
8792 for C₁₄–C₁₄ perfluoroalkyl carboxylic acid (PFCA) homologues from 1951 to 2030, Part I: production
8793 and emissions from quantifiable sources. *Environment International*, 70, 62-75.
- 8794 Wang Z, Cousins IT, Scheringer M, Buck RC and Hungerbühler K, 2014b. Global emission inventories
8795 for C₁₄–C₁₄ perfluoroalkyl carboxylic acid (PFCA) homologues from 1951 to 2030, Part II: The
8796 remaining pieces of the puzzle. *Environment International*, 69, 166-176.
- 8797 Wang L, Wang Y, Liang Y, Li J, Liu Y, Zhang J, Zhang A, Fu J and Jiang G, 2014c. PFOS induced lipid
8798 metabolism disturbances in BALB/c mice through inhibition of low density lipoproteins excretion.
8799 *Scientific Reports*, 4, 4582
- 8800 Wang Y, Rogan WJ, Chen PC, Lien GW, Chen HY, Tseng YC, Longnecker MP and Wang SL, 2014d.
8801 Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and
8802 cord thyroid hormones: Taiwan maternal and infant cohort study. *Environmental Health*
8803 *Perspectives*, 122, 529-534. doi:10.1289/ehp.1306925
- 8804 Wang Y, Adgent M, Su PH, Chen HY, Chen PC, Hsiung CA and Wang SL, 2016. Prenatal exposure to
8805 perfluorocarboxylic acids (PFCAs) and fetal and postnatal growth in the Taiwan Maternal and Infant
8806 Cohort Study. *Environmental Health Perspectives*, 124, 1794–1800.
8807 <https://doi.org/10.1289/ehp.1509998>
- 8808 Wang J, Shengmin Yan, Wei Zhang, Hongxia Zhang, and Jiayin Dai, 2015a. Integrated Proteomic and
8809 miRNA Transcriptional Analysis Reveals the Hepatotoxicity Mechanism of PFNA Exposure in Mice.
8810 *Journal of Proteome Research*, 14, 330–341
- 8811 Wang Y, Rogan WJ, Chen HY, Chen PC, Su PH, Chen HY and Wang SL, 2015b. Prenatal exposure to
8812 perfluoroalkyl substances and children's IQ: The Taiwan maternal and infant cohort study.
8813 *International Journal of Hygiene and Environmental Health*, 218, 639-644.
8814 doi:10.1016/j.ijheh.2015.07.002
- 8815 Wang Z, Boucher JM, Scheringer M, Cousins IT and Hungerbühler K, 2017a. Toward a Comprehensive
8816 Global Emission Inventory of C₄–C₁₀ Perfluoroalkanesulfonic Acids (PFASs) and Related Precursors:
8817 Focus on the Life Cycle of C₈-Based Products and Ongoing Industrial Transition. *Environmental*
8818 *Science & Technology*, 51, 4482–4493.
- 8819 Wang Z, DeWitt JC, Higgins CP and Cousins IT, 2017b. A Never-Ending Story of Per- and Polyfluoroalkyl
8820 Substances (PFASs)? *Environmental Science & Technology*, 51, 2508–2518.
- 8821 Wang L, Zhao F, Kan M, Wen Z, Zhou Y, Yu L and Liu H, 2017c. Effects of perfluorooctanoic acid on
8822 oxidative stress and PPARα and its related CYP4A1 gene expression in rat liver. *Wei Sheng Yan Jiu*,
8823 46, 802-806.
- 8824 Wang Y, Shi Y, Vestergren R, Zhou Z, Liang Y and Cai Y, 2018a. Using hair, nail and urine samples for
8825 human exposure assessment of legacy and emerging per- and polyfluoroalkyl substances. *Science*
8826 *of the Total Environment*, 636, 383-391.
- 8827 Wang X, Kong B, He B, Wei L, Zhu J, Jin Y, Shan Y, Wang W, Pan C and Fu Z, 2018b. 8:2 Fluorotelomer
8828 alcohol causes immunotoxicity and liver injury in adult male C57BL/6 mice. *Environmental*
8829 *Toxicology*, 34, 141-149.
- 8830 Wang H, Du H, Yang J, Jiang H, O K, Xu L, Liu S, Yi J, Qian X, Chen Y, Jiang Q and He G, 2019a. PFOS,
8831 PFOA, estrogen homeostasis, and birth size in Chinese infants. *Chemosphere*, 221, 349-355.
8832 <https://doi.org/10.1016/j.chemosphere.2019.01.061>
- 8833 Wang, W., Zhou, W., Wu, S., Liang, F., Li, Y., Zhang, J., Cui, L., Feng, Y. and Wang, Y, 2019b.
8834 Perfluoroalkyl substances exposure and risk of polycystic ovarian syndrome related infertility in

- 8835 Chinese women. *Environmental Pollution*, 247 (2019) 824e831.
8836 <https://doi.org/10.1016/j.envpol.2019.01.039>
- 8837 Washburn ST, Bingman TS, Braithwaite SK, Buck RC, Buxton LW, Clewell HJ, Haroun LA, Kester JE,
8838 Rickard RW and Shipp AM, 2005. Exposure assessment and risk characterization for
8839 perfluorooctanoate in selected consumer articles. *Environmental Science & Technology*, 39, 3904-
8840 3910.
- 8841 Watkins AM, Wood CR, Lin MT and Abbott BD, 2015. The effects of perfluorinated chemicals on
8842 adipocyte differentiation in vitro. *Molecular and Cellular Endocrinology* 400, 90–101.
- 8843 Watkins DJ, Josson J, Elston B, Bartell SM, Shin HM, Vieira VM, Savitz DA, Fletcher T and Wellenius GA,
8844 2013. Exposure to perfluoroalkyl acids and markers of kidney function among children and
8845 adolescents living near a chemical plant. *Environmental Health Perspectives*, 121, 625-630. doi:
8846 10.1289/ehp.1205838
- 8847 Webster GM, Rauch SA, Marie NS, Mattman A, Lanphear BP and Venners SA, 2016. Cross-Sectional
8848 Associations of Serum Perfluoroalkyl Acids and Thyroid Hormones in U.S. Adults: Variation According
8849 to TPOAb and Iodine Status (NHANES 2007-2008). *Environmental Health Perspectives*, 124, 935-
8850 942. doi: 10.1289/ehp.1409589
- 8851 Webster GM, Venners SA, Mattman A and Martin JW, 2014. Associations between perfluoroalkyl acids
8852 (PFASs) and maternal thyroid hormones in early pregnancy: a population-based cohort study.
8853 *Environmental Research*, 133, 338-347. doi: 10.1016/j.envres.2014.06.012
- 8854 Weihe P, Kato K, Calafat AM, Nielsen F, Wanigatunga AA, Needham LL and Grandjean P, 2008. Serum
8855 concentrations of polyfluoroalkyl compounds in Faroese whale meat consumers. *Environmental
8856 Science & Technology*, 42, 6291-6295.
- 8857 Weiss JM, Andersson PL, Lamoree MH, Leonards PE, van Leeuwen SP and Hamers T, 2009. Competitive
8858 binding of poly- and perfluorinated compounds to the thyroid hormone transport protein
8859 transthyretin. *Toxicological Sciences*, 109, 206–216. doi: 10.1093/toxsci/kfp05
- 8860 Wen LL, Lin LY, Su TC, Chen PC and Lin CY, 2013. Association between serum perfluorinated chemicals
8861 and thyroid function in U.S. adults: the National Health and Nutrition Examination Survey 2007-2010.
8862 *Journal of Clinical Endocrinology and Metabolism*, 98, E1456-1464.
- 8863 West D, James N, Holden P, Brindle R, Rolfe M and Roberts R, 1999. Role for tumour necrosis factor α
8864 (TNF α) receptor 1 (TNFR1) and interleukin 1 receptor (IL1R) in the suppression of apoptosis by
8865 peroxisome proliferators. *Hepatology*, 30, 1417–1424.
- 8866 White SS, Calafat AM, Kuklennyik Z, Villanueva L, Zehr RD, Helfant L, Strynar MJ, Lindstrom AB,
8867 Thibodeaux JR, Wood C and Fenton SE, 2007. Gestational PFOA exposure of mice is associated with
8868 altered mammary gland development in dams and female offspring. *Toxicological Sciences*, 96, 133–
8869 144.
- 8870 White SS, Kato K, Jia LT, Basden BJ, Calafat AM, Hines EP, Stanko JP, Wolf CJ, Abbott BD and Fenton
8871 SE, 2009. Effects of perfluorooctanoic acid on mouse mammary gland development and
8872 differentiation resulting from cross-foster and restricted gestational exposures. *Reproductive
8873 Toxicology*, 27, 289–298. <https://doi.org/10.1016/j.reprotox.2008.11.054>
- 8874 White SS, Stanko JP, Kato K, Calafat AM, Hines EP and Fenton SE, 2011. Gestational and chronic low-
8875 dose PFOA exposures and mammary gland growth and differentiation in three generations of CD-1
8876 mice. *Environmental Health Perspectives*, 119, 1070–1076. <https://doi.org/10.1289/ehp.1002741>
- 8877 WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2009.
8878 *Environmental Health Criteria 240. Principles and methods for the assessment of chemicals in food.*

- 8879 WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2012. Guidance
8880 for immunotoxicity risk assessment for chemicals. Available online:
8881 <http://www.inchem.org/documents/harmproj/harmproj/harmproj10.pdf>
- 8882 Wielsøe M, Long M, Ghisari M and Bonefeld-Jørgensen EC, 2015. Perfluoroalkylated substances (PFAS)
8883 affect oxidative stress biomarkers *in vitro*. Chemosphere, 129, 239–245.
8884 <https://doi.org/10.1016/j.chemosphere.2014.10.014>
- 8885 Wielsøe M, Kern P and Bonefeld-Jørgensen EC, 2017. Serum levels of environmental pollutants is a risk
8886 factor for breast cancer in Inuit: a case control study. Environmental Health, 16, 56. DOI:
8887 10.1186/s12940-017-0269-6
- 8888 WIL Research Laboratories, 2005. A combined 28-day repeated dose oral toxicity study with the
8889 reproduction/developmental toxicity screening test of perfluorohexanoic acid and 1H, 1H, 2H, 2H-
8890 tridecafluoro-1-octanol in rats, with recovery. Study number WIL-534001. Sponsor: AGC Chemical.
8891 Available online: <http://mail.agc-chemicals.com/file.jsp?id=file/6-2FTOH-5.pdf>
- 8892 Wilhelm M, Hölzer J, Dobler L, Rauchfuss K, Midasch O, Kraft M, Angerer J and Wiesmüller G, 2009.
8893 Preliminary observations on perfluorinated compounds in plasma samples (1977-2004) of young
8894 German adults from an area with perfluorooctanoate-contaminated drinking water. International
8895 Journal of Hygiene and Environmental Health, 212, 142-145.
- 8896 Wilhelm M, Wittsiepe J, Völkel W, Fromme H and Kasper-Sonnenberg M, 2015. Perfluoroalkyl acids in
8897 children and their mothers: Association with drinking water and time trends of inner exposures—
8898 Results of the Duisburg birth cohort and Bochum cohort studies. International Journal of Hygiene and
8899 Environmental Health, 218, 645–655. <https://doi.org/10.1016/j.ijheh.2015.07.001>
- 8900 Wilson J, Berntsen HF, Zimmer KE, Verhaegen S, Frizzell C, Ropstad E and Connolly L, 2016. Do
8901 persistent organic pollutants interact with the stress response? Individual compounds, and their
8902 mixtures, interaction with the glucocorticoid receptor. Toxicology Letters, 241, 121-132. doi:
8903 10.1016/j.toxlet.2015.11.014
- 8904 Wolf CJ, Schmid JE, Lau C and Abbott BD, 2012. Activation of mouse and human peroxisome
8905 proliferator-activated receptor-alpha (PPARα) by perfluoroalkyl acids (PFAAs): further investigation
8906 of C4-C12 compounds. Reproductive Toxicology, 33, 546-551. DOI: 10.1016/j.reprotox.2011.09.009
- 8907 Wolf CJ, Takacs ML, Schmid JE, Lau C and Abbott BD, 2008a. Activation of mouse and human
8908 peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups
8909 and chain lengths. Toxicological Sciences, 106, 162-171.
- 8910 Wolf DC, Moore T, Abbott BD, Rosen MB, Das KP, Zehr RD, Lindstrom AB, Strynar MJ and Lau C, 2008.
8911 Comparative hepatic effects of perfluorooctanoic acid and WY 14,643 in PPAR-alpha knockout and
8912 wild-type mice. Toxicologic Pathology, 36, 632-639.
- 8913 Wolf CJ, Rider CV, Lau C and Abbott BD, 2014. Evaluating the additivity of perfluoroalkyl acids in binary
8914 combinations on peroxisome proliferator-activated receptor-α activation. Toxicology, 316, 43–54.
8915 doi: 10.1016/j.tox.2013.12.002
- 8916 Wong F, MacLeod M, Mueller JF and Cousins IT, 2014. Enhanced elimination of perfluorooctane sulfonic
8917 acid by menstruating women: evidence from population-based pharmacokinetic modeling.
8918 Environmental Science & Technology, 48, 8807-8814.
- 8919 Worley RR, Moore SM, Tierney BC, Ye X, Calafat AM, Campbell S, Woudneh MB and Fisher J, 2017. Per-
8920 and polyfluoroalkyl substances in human serum and urine samples from a residentially exposed
8921 community. Environment International, 106, 135-143. doi:10.1016/j.envint.2017.06.007

- 8922 Wu X, Xie G, Xu X, Wu W and Yang B, 2018. Adverse bioeffect of perfluorooctanoic acid on liver
8923 metabolic function in mice. *Environmental Science and Pollution Research International*, 25, 4787-
8924 4793. doi:10.1007/s11356-017-0872-7
- 8925 Xie Y, Yang Q, Nelson BD and DePierre JW, 2002. Characterization of the adipose tissue atrophy induced
8926 by peroxisome proliferators in mice. *Lipids* 37, 139–146.
- 8927 Xie Y, Yang Q, Nelson BD and DePierre JW, 2003. The relationship between liver peroxisome
8928 proliferation and adipose tissue atrophy induced by peroxisome proliferator exposure and withdrawal
8929 in mice. *Biochemical Pharmacology*, 66, 749–756.
- 8930 Xie W, Wu Q, Kania-Korwel I, Tharappel JC, Telu S, Coleman MC, Glauert HP, Kannan K, Mariappan SV,
8931 Spitz DR, Weydert J and Lehmler HJ, 2009. Subacute exposure to N-ethyl
8932 perfluorooctanesulfonamidoethanol results in the formation of perfluorooctanesulfonate and alters
8933 superoxide dismutase activity in female rats. *Archives of Toxicology*, 83, 909-924.
8934 doi:10.1007/s00204-009-0450-y
- 8935 Xing J, Wang G, Zhao J, Wang E, Yin B, Fang D, Zhao J, Zhang H, Chen YQ and Chen W, 2016. Toxicity
8936 assessment of perfluorooctane sulfonate using acute and subchronic male C57BL/6J mouse models.
8937 *Environmental Pollution*, 210, 388-396.
- 8938 Xu L, Krenitsky DM, Seacat AM, Butenhoff JL and Anders MW, 2004. Biotransformation of N-Ethyl-N-(2-
8939 hydroxyethyl)perfluorooctane sulfonamide by Rat Liver Microsomes, Cytosol, and Slices and by
8940 Expressed Rat and Human Cytochromes P450. *Chemical Research in Toxicology*, 17, 767-775.
8941 doi:10.1021/tx034222x
- 8942 Yahia D, Tsukuba C, Yoshida M, Sato I and Tsuda S, 2008. Neonatal death of mice treated with
8943 perfluorooctane sulfonate. *Journal of Toxicological Sciences*, 33, 219–226.
- 8944 Yahia D, El-Nasser MA, Abedel-Latif M, Tsukuba C, Yoshida M, Sato I and Tsuda S, 2010. Effects of
8945 perfluorooctanoic acid (PFOA) exposure to pregnant mice on reproduction. *Journal of Toxicological
8946 Sciences*, 35, 527–533.
- 8947 Yahia D, Haruka I, Kagashi Y and Tsuda S, 2014. 8-Hydroxy-2'-deoxyguanosine as a biomarker of
8948 oxidative DNA damage induced by perfluorinated compounds in TK6 cells. *Environmental Toxicology*,
8949 31, 192–200. <https://doi.org/10.1002/tox.22034>
- 8950 Yamaguchi M, Arisawa K, Uemura H, Katsuura-Kamano S, Takami H, Sawachika F, Nakamoto M, Jutta
8951 T, Toda E, Mori K, Hasegawa M, Tanto M, Shima M, Sumiyoshi Y, Morinaga K, Kodama K, Suzuki T,
8952 Nagai M and Satoh H, 2013. Consumption of seafood, serum liver enzymes, and blood levels of PFOS
8953 and PFOA in the Japanese population. *Journal of Occupational Health*, 55, 184-194.
- 8954 Yang Q, Nagano T, Shah Y, Cheung C, Ito S and Gonzalez FJ, 2008. The PPAR alpha-humanized mouse:
8955 a model to investigate species differences in liver toxicity mediated by PPAR alpha. *Toxicological
8956 Sciences*, 101,132-139.
- 8957 Yang C, Tan YS, Harkema JR and Haslam SZ, 2009. Differential effects of peripubertal exposure to
8958 perfluorooctanoic acid on mammary gland development in C57Bl/6 and Balb/c mouse strains.
8959 *Reproductive Toxicology*, 27, 299–306.
- 8960 Yang CH, Glover KP and Han X, 2010. Characterization of cellular uptake of perfluorooctanoate via
8961 organic aniontransporting polypeptide 1A2, organic anion transporter 4, and urate transporter 1 for
8962 their potential roles in mediating human renal reabsorption of perfluorocarboxylates. *Toxicological
8963 Sciences*, 117, 294–302.
- 8964 Yang L, Li J, Lai J, Luan H, Cai Z, Wang Y, Zhao Y and Wu Y, 2016. Placental Transfer of Perfluoroalkyl
8965 Substances and Associations with Thyroid Hormones: Beijing Prenatal Exposure Study. *Scientific
8966 Reports*, 6, 21699. doi: 10.1038/srep21699

- 8967 Yang X, Wang L, Sun W and Xue Z, 2009. [Effects of perfluorooctane sulfonate on amino acid
8968 neurotransmitters and glutamine synthetase in rats]. [Article in Chinese]. Journal of Hygiene
8969 Research, 38, 19-21.
- 8970 Yao PL, Ehresman DJ, Rae JM, Chang SC, Frame SR, Butenhoff JL, Kennedy GL and Peters JM, 2014.
8971 Comparative *in vivo* and *in vitro* analysis of possible estrogenic effects of perfluorooctanoic acid.
8972 Toxicology, 326, 62-73.
- 8973 Yao, Q., Shi, R., Wang, C. F., Han, W. C., Gao, Y., Zhang, Y., Zhou, Y. J., Ding, G. D. and Tian, Y, 2019.
8974 Cord blood Per- and polyfluoroalkyl substances, placental steroidogenic enzyme, and cord blood
8975 reproductive hormone. Environment International, 129, 573-582.
8976 <https://doi.org/10.1016/j.envint.2019.03.047>
- 8977 Ye L, Zhao B, Cai XH, Chu Y, Li C and Ge RS, 2012. The inhibitory effects of perfluoroalkyl substances
8978 on human and rat 11 β -hydroxysteroid dehydrogenase 1. Chemico-Biological Interactions, 195, 114-
8979 118. doi: 10.1016/j.cbi.2011.11.007
- 8980 Yeung LW, Robinson SJ, Koschorreck J and Mabury SA, 2013a. Part I. A temporal study of PFCAs and
8981 their precursors in human plasma from two German cities 1982-2009. Environmental Science &
8982 Technology, 47, 3865-3874.
- 8983 Yeung LW, Robinson SJ, Koschorreck J and Mabury SA, 2013b. Part II. A temporal study of PFOS and
8984 its precursors in human plasma from two German cities in 1982-2009. Environmental Science &
8985 Technology, 47, 3875-3882.
- 8986 Young CJ and Mabury SA, 2010. Atmospheric perfluorinated acid precursors: chemistry, occurrence,
8987 and impacts. Reviews of Environmental Contamination and Toxicology, 208, 1-109.
- 8988 Yu N, Wang X, Zhang B, Yang J, Li M, Li J, Shi W, Wei S and Yu H, 2015. Distribution of perfluorooctane
8989 sulfonate isomers and predicted risk of thyroid hormonal perturbation in drinking water. Water
8990 Research, 76, 171-180. doi: 10.1016/j.watres.2015.02.047
- 8991 Yu WG, Liu W and Jin YH, 2009. Effects of perfluorooctane sulfonate on rat thyroid hormone biosynthesis
8992 and metabolism. Environmental Toxicology and Chemistry, 28, 990-996.
- 8993 Yu WG, Liu W, Liu L and Jin YH, 2011. Perfluorooctane sulfonate increased hepatic expression of OAPT2
8994 and MRP2 in rats. Archives of Toxicology, 85, 613-621. doi:10.1007/s00204-010-0613-x
- 8995 Zabaleta I, Negreira N, Bizkarguenaga E, Prieto A, Covaci A and Zuloaga O, 2017. Screening and
8996 identification of per- and polyfluoroalkyl substances in microwave popcorn bags. Food Chemistry,
8997 230, 497-506. doi:10.1016/j.foodchem.2017.03.074
- 8998 Zarei MH, Hosseini Shirazi SF, Aghvami M and Pourahmad J, 2018. Perfluorooctanesulfonate (PFOS)
8999 Induces Apoptosis Signaling and Proteolysis in Human Lymphocytes through ROS Mediated
9000 Mitochondrial Dysfunction and Lysosomal Membrane Labialization. Iranian Journal of Pharmaceutical
9001 Research, 17, 995-1007.
- 9002 Zeng XW, Qian Z, Emo B, Vaughn M, Bao J, Qin XD, Zhu Y, Li J, Lee YL and Dong GH, 2015.
9003 Association of polyfluoroalkyl chemical exposure with serum lipids in children. Science of Total
9004 Environment, 512-513, 364-370.
- 9005 Zeng XW, Bloom MS, Dharmage SC, Lodge CJ, Chen D, Li S, Guo Y, Roponen M, Jalava P, Hirvonen MR,
9006 Ma H, Hao YT, Chen W, Yang M, Chu C, Li QQ, Hu LW, Liu KK, Yang BY, Liu S, Fu C and Dong GH,
9007 2019 Prenatal exposure to perfluoroalkyl substances is associated with lower hand, foot and mouth
9008 disease viruses antibody response in infancy: Findings from the Guangzhou Birth Cohort Study.
9009 Science of the Total Environment, 663, 60-67. doi: 10.1016/j.scitotenv.2019.01.325

- 9010 Zhang T and Qin X, 2014. Assessment of fetal exposure and maternal elimination of perfluoroalkyl
9011 substances. *Environmental Sciences: Processes & Impacts*, 16, 1878-1881.
9012 <https://doi.org/10.1039/C4EM00129J>
- 9013 Zhang H, Shi Z, Liu Y, Wei Y and Dai J, 2008. Lipid homeostasis and oxidative stress in the liver of male
9014 rats exposed to perfluorododecanoic acid. *Toxicology and Applied Pharmacology*, 227(1), 16-25.
- 9015 Zhang H, Ding L, Fang X, Shi Z, Zhang Y, Chen H, Yan X and Dai J, 2011. Biological responses to
9016 perfluorododecanoic acid exposure in rat kidneys as determined by integrated proteomic and
9017 metabolomic studies. *PLoS One*, 6, e20862-e20862. doi:10.1371/journal.pone.0020862
- 9018 Zhang Y, Beesoon S, Zhu L and Martin JW, 2013a. Biomonitoring of perfluoroalkyl acids in human urine
9019 and estimates of biological half-life. *Environmental Science & Technology*, 47, 10619-10627.
- 9020 Zhang LY, Ren XM and Guo LH, 2013b. Structure-Based Investigation on the Interaction of
9021 Perfluorinated Compounds with Human Liver Fatty Acid Binding Protein. *Environmental Science &
9022 Technology*, 47, 11293-11301. DOI: 10.1021/es4026722
- 9023 Zhang T, Sun H, Lin Y, Qin X, Zhang Y, Geng X and Kannan K, 2013c. Distribution of poly- and
9024 perfluoroalkyl substances in matched samples from pregnant women and carbon chain length related
9025 maternal transfer. *Environmental Science & Technology*, 47, 7974-7981.
- 9026 Zhang H, Hou J, Cui R, Guo X, Shi Z, Yang F and Dai J, 2013d. Phosphoproteome analysis reveals an
9027 important role for glycogen synthase kinase-3 in perfluorododecanoic acid-induced rat liver toxicity.
9028 *Toxicology Letters*, 218, 61-69. doi:10.1016/j.toxlet.2013.01.012
- 9029 Zhang Y, Jiang W, Fang S, Zhu L and Deng J, 2014. Perfluoroalkyl acids and the isomers of
9030 perfluorooctanesulfonate and perfluorooctanoate in the sera of 50 new couples in Tianjin, China.
9031 *Environment International*, 68, 185-191.
- 9032 Zhang J, Kamstra JH, Ghorbani Zadeh M, Weiss JM, Hamers T and Andersson PL, 2015. *In Silico* Approach
9033 to Identify Potential Thyroid Hormone Disruptors among Currently Known Dust Contaminants and
9034 their Metabolites. *Environmental Science & Technology*, 49, 10099-10107.
- 9035 Zhang L, Krishnan P, Ehresman DJ, Smith PB, Dutta M, Bagley BD, Chang SC, Butenhoff JL, Patterson
9036 AD and Peters JM, 2016. Perfluorooctane Sulfonate-Choline Ion Pair Formation: A Potential
9037 Mechanism Modulating Hepatic Steatosis and Oxidative Stress in Mice. *Toxicological Sciences*, 153,
9038 186-197.
- 9039 Zhang H, Vestergren R, Wang T, Yu J, Jiang G and Herzke D, 2017. Geographical Differences in Dietary
9040 Exposure to Perfluoroalkyl Acids between Manufacturing and Application Regions in China.
9041 *Environmental Science & Technology*, 51, 5747-5755. doi: 10.1021/acs.est.7b00246
- 9042 Zhang S, Tan R, Pan R, Xiong J, Tian Y, Wu J and Chen L, 2018. Association of Perfluoroalkyl and
9043 Polyfluoroalkyl Substances With Premature Ovarian Insufficiency in Chinese Women. *Clin Endocrinol
9044 Metab* 103, 2543-2551. doi: 10.1210/jc.2017-02783
- 9045 Zhao Y, Tan YS, Haslam SZ and Yang C, 2010. Perfluorooctanoic acid effects on steroid hormone and
9046 growth factor levels mediate stimulation of peripubertal mammary gland development in C57BL/6
9047 mice. *Toxicological Sciences*, 115, 214-224. <https://doi.org/10.1093/toxsci/kfq030>
- 9048 Zhao B, Lian Q, Chu Y, Hardy DO, Li X-K and Ge R-S, 2011. The inhibition of human and rat 11 β -
9049 hydroxysteroid dehydrogenase 2 by perfluoroalkylated substances. *Journal of Steroid Biochemistry
9050 and Molecular Biology*, 125, 143-147.
- 9051 Zhao Y, Tan YS, Strynar MJ, Perez G, Haslam SZ and Yang C, 2012. Perfluorooctanoic acid effects on
9052 ovaries mediate its inhibition of peripubertal mammary gland development in Balb/c and C57BL/6
9053 mice. *Reproductive Toxicology*, 33, 563-576. <https://doi.org/10.1016/j.reprotox.2012.02.004>

- 9054 Zhao W, Zitzow JD, Ehresman DJ, Chang SC, Butenhoff JL, Forster J and Hagenbuch B, 2015.
 9055 Na⁺/Taurocholate Cotransporting Polypeptide and Apical Sodium-Dependent Bile Acid Transporter
 9056 Are Involved in the Disposition of Perfluoroalkyl Sulfonates in Humans and Rats. *Toxicological*
 9057 *Sciences*, 146, 363-373. doi:10.1093/toxsci/kfv102
- 9058 Zhao W, Zitzow JD, Weaver Y, Ehresman DJ, Chang SC, Butenhoff JL and Hagenbuch B, 2017. Organic
 9059 Anion Transporting Polypeptides Contribute to the Disposition of Perfluoroalkyl Acids in Humans and
 9060 Rats. *Toxicological Sciences*, 156, 84-95. doi:10.1093/toxsci/kfw236
- 9061 Zheng L, Dong GH, Jin YH and He QC, 2009. Immunotoxic changes associated with a 7-day oral
 9062 exposure to perfluorooctanesulfonate (PFOS) in adult male C57BL/6 mice. *Archives of Toxicology*,
 9063 83, 679–689.
- 9064 Zheng L, Dong GH, Zhang YH, Liang ZF, Jin YH and He QC, 2011. Type 1 and Type 2 cytokines imbalance
 9065 in adult male C57BL/6 mice following a 7-day oral exposure to perfluorooctanesulfonate (PFOS).
 9066 *Journal of Immunotoxicology*, 8, 30–38.
- 9067 Zheng F, Sheng N, Zhang H, Yan S, Zhang J and Wang J, 2017. Perfluorooctanoic acid exposure disturbs
 9068 glucose metabolism in mouse liver. *Toxicology and Applied Pharmacology*, 335, 41-48.
 9069 doi:10.1016/j.taap.2017.09.019
- 9070 Zhou Z, Shi Y, Vestergren R, Wang T, Liang Y and Cai Y, 2014. Highly elevated serum concentrations
 9071 of perfluoroalkyl substances in fishery employees from Tangxun lake, China. *Environmental Science*
 9072 *& Technology*, 48, 3864-3874.
- 9073 Zhou X, Dong T, Fan Z, Peng Y, Zhou R, Wang X, Song N, Han M, Fan B, Jia J and Liu S, 2017.
 9074 Perfluorodecanoic acid stimulates NLRP3 inflammasome assembly in gastric cells. *Scientific Reports*,
 9075 7, 45468. doi:10.1038/srep45468
- 9076
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Abbreviations

ACOX	acyl-CoA oxidase
AD	Alzheimer's disease
ADHD	attention deficit hyperactivity disorder
ADME	absorption, distribution, metabolism and excretion
AFB1	aflatoxin B1
AFFF	Aqueous Film Forming Foam
AhR	aryl hydrocarbon receptor
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
APFN	ammonium perfluorononanoate
APFO	ammonium perfluorooctanoate
AST	aspartate aminotransferase
AT	Austria
ATSDR	Agency for Toxic Substances and Disease Registry
BAF	Bioaccumulation factor
BE	Belgium
BfR	Federal Institute for Risk Assessment in Germany
BMD	Benchmark dose
BMDL10	Benchmark dose for a 10% increase
BMI	Body mass index
BMDU	Benchmark dose upper confidence limit
BMF	Biomagnification factor
BMR	Benchmark response
br-PFOA/PFOS	Branched PFOA/PFOS
BSA	bovine serum albumin
bw	Body weight
CA	Canada
CAR	constitutive activated/androstane receptor
CCK	cholecystokinin
CHMS	Canadian Health Measures Survey
CHO	Chinese hamster ovary
CI	Confidence interval

CIMT	carotid artery intima media thickness
CKD	chronic kidney disease
CLRTAP	Convention on Long-range Transboundary Air Pollution
CN	China
CS	Cross-sectional study
CSF	cerebrospinal fluid
CY	Cyprus
CZ	The Czech Republic
DATA Unit	EFSA former EFSA Dietary and Chemical Monitoring Unit
DCFDA	dichlorofluorescein diacetate
DCFH-DA	dichlorodihydrofluorescein diacetate
DCH	Diet Cancer and Health
DE	Germany
DK	Denmark
DRD	dopamine receptor
dw	Dry weight
DXA	dual-energy x-ray absorptiometry
ECF	Electro-Chemical Fluorination
EFSA	European Food Safety Authority
eGFR	estimated glomerular filtration rate
EPA	Environmental Protection Agency
ES	Spain
ESI	electrospray ionization
EtFASAs	N-ethyl perfluoroalkane sulfonamides
EtFASEs	N-ethylperfluoroalkane sulfonamidoethanols
f	female
FASAs	perfluoroalkane sulfonamides
FAT	fatty acid translocase
FEP	fluorinated ethylene-propene
Fi	Finland
FR	France
FSANZ	Food Standards Australia New Zealand
FTOHs	fluoroteleomer alcohols
GC	gas chromatography

GDM	gestational diabetes
GFR	glomerular filtration rate
GGT	gamma-glutamyl transferase
GL	Greenland
GM	Geometric mean
GR	Greece or HE
GS	Gestation day
HA	Health Advisory
HBGv	health-based guidance value
HBM	Human Biomonitoring
HC	High consumer
HDL	high-density lipoproteins
HED	human equivalent dose
HOMA-IR	Homeostasis Model Assessment – Insulin resistance
IARC	International Agency for Research on Cancer
IDL	intermediate-density lipoprotein
IF5	iodine pentafluoride
IFN	interferon
IHD	ischemic heart disease
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IGT	impaired glucose tolerance
IL	interleukin
i.p.	intraperitoneal
IPE	ion-pair extraction
IQR	Interquartile range
IR	Ireland
IT	Italy
i.v.	intravenous
JP	Japan
Ka	association constant
Kd	dissociation constant
KLH	keyhole limpet haemocyanin
KR	Korea (South)

L	Longitudinal study
LB	Lower bound
LBWLC	Low birth weight
LC	Left-censored
LC-MS/MS	LC coupled to quadrupole tandem mass spectrometry
LDH	Lactate dehydrogenase
L-FABP	liver fatty acids binding protein
Li	labelling index
LOAEL	Lowest-observed-adverse-effect-level
LOAL	lowest-observed-adverse level
LOD	limit of detection
LOQ	limit of quantification
Lpl	lipoprotein lipase
LPS	Lipopolysaccharide
m	male
MAC	maximum acceptable concentration
mMCD	marginal methionine/choline-deficient
MNNG	N-methyl-N-nitro-N-nitrosoguanidine
MoA	Mode of action
MRI	magnetic resonance imaging
MS	mass spectrometry
MT	Malta
N/A	Not applicable
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NC	Non consumer
NCEG1	neutral cholesterol ester hydrolase 1
NK	natural killer (cell)
NO	Norway
NOAEL	no-observed-adverse-effect-level
n-PFOA/PFOS	Linear PFOA/PFOS
NR	Not reported
Nrf2	nuclear factor erythroid 2-related factor 2

NTP	National Toxicology Program
OAT	organic anion transport protein
Oatp	organic anion-transporting polypeptide
Occup	Occupational study
OR	Odds ratio
PAD	peripheral artery disease
PAPs	polyfluoroalkyl phosphoric acid esters
PBDEs	polybrominated diphenylethers
PBL	peripheral blood leukocyte
PBMC	peripheral blood mononuclear cell
PBPK	Physiologically-based pharmacokinetic (model)
PCBs	polychlorinated biphenyls
PD	postnatal day
PFBS	perfluorobutane sulfonic acid
PFCAs	perfluoroalkyl carboxylic acids
PFDA	perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonic acid
PFNA	perfluorononanoic acid
PFOAs	perfluorooctanoic acids
PFOSs	perfluooctane sulfonates
PFOSF	perfluorooctane sulfonyl fluoride
PFSAs	perfluoroalkyl sulfonic acids
PFUnDA	perfluoroundecanoic acid
PHA	Phytohemagglutinin
PIGE	particle-induced γ -ray emission
PK	pharmacokinetic
PO	Poland
POD	point of departure
POP	persistent organic pollutant
POSF	perfluorooctane sulfonyl fluoride
PPAR	peroxisome proliferator-activated receptors

PTFE	Polytetrafluoroetane
Q	quartile
RCR	risk characterization ratio
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RfD	reference dose
ROS	Reactive oxygen species
SCF	Scientific Committee on Food
SD	Standard deviation
SE	Sweden
SGA	small for gestational age
SHE	Syrian hamster embryo
SI	Slovenia
SLE	solid–liquid extraction
SOD	superoxide dismutase
SOP	standard operational procedure
SRBC	sheep red blood cells
SREBP	sterol regulatory element-binding protein 1 and 2
S-UA	serum uric acid
SVHC	Substances of Very High Concern
Syn	synapsin
Syp	synaptophysin
T	tertile
T3	triiodothyronine
T4	thyroxine
TAD	total administered dose
TBG	thyroxin binding globulin
TC	Total cholesterol
TDI	tolerable daily intake
TDS	Total Diet Study
TEB	terminal end buds
TG	triglycerides
TH	tyrosine hydroxylase
TL	telomerisation
TNF	Tumour necrosis factor

TNP	tri-nitrophenyl
TPOab	thyroid peroxidase antibodies
TrxR	thioredoxin reductase
TSH	thyroid stimulating hormone
TTR	transthyretin
TW	Taiwan
UA	Ukraine
UB	Upper bound
UCP1	uncoupling protein 1
UF	uncertainty factor
UGT	Uridine 5'-Diphospho-Glucuronosyl Transferase
UK	The United Kingdom
URAT	urate transporter
US	United States
VLDL	very low density lipoprotein
WG	working group
WHO	World Health Organization
ww	Wet weight

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