Danish Ministry of the Environment
Environmental Protection Agency

Perfluoroalkylated substances: PFOA, PFOS and PFOSA

Evaluation of health hazards and proposal of a health based quality criterion for drinking water, soil and ground water

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Perfluoroalkylated substances: PFOA, PFOS and PFOSA

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Contents

Preface

This report has been prepared by Poul Bo Larsen and Estelle Giovalle, DHI.

Based on cases on PFAS (perfluoroalkylated substances) contaminated sites in Denmark the Danish EPA has requested a documentation document for health-based quality criteria for PFOA, PFOS and PFOSA in soil, drinking water and groundwater.

The Danish EPA has further pre-selected additional PFAS substances (PFHpA; PFNA; PFBS; PFHxS; PFDS; and PFHxA) for which a preliminary screening in relation to toxicity data was requested in order to assess the possibilities for further derivation of specific quality criteria for the substances.

The report has been elaborated based on existing expert assessments of for PFOA, PFOS and PFOSA as further in-depth evaluation of the extensive database of original literature was outside the scope of the project.

The report has been elaborated according to the general practice laid down in the Danish EPA guidance document for the setting of health-based quality criteria for chemical substances in relation to soil, ambient air and drinking water (Vejledning fra Miljøstyrelsen 5/2006).

The report has been subjected to review and written commenting by a steering committee with representatives from the following Danish authorities / institutions:

The National Board of Health
The Danish Nature Agency
The Danish Veterinary and Food Administration
Danish Regions
Danish Environmental Protection Agency

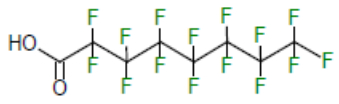
1. General description

1.1 Identity

The perfluoroalkylated substances (PFAS) consist of a large group of compounds consisting of a fully fluorinated hydrophobic alkyl chain of varying length (typically 4 to 16 carbon atoms) and a hydrophilic end group (R), $F(CF_2)_n$-R.
Within the group of perfluoroalkylated substances, the perfluoroalkyl acids (PFAAs) and their salts have been the main focal point for regulatory actions until now. The main subgroups are the perfluoroalkyl carboxylic acids and their salts (PFCAs), and the perfluoroalkane sulfonic acids (PFSAs) and their salts. This report will mainly focus on PFOA, PFOS and PFOSA as identified below.

The Danish EPA has further preselected six perfluoroalkylated acids/sulfonic acids: PFHpA; PFNA; PFBS; PFHxS; PFDS; and PFHxA. In appendix 2 of this report an initial view on the toxicological database on these substances will be made in order to examine the possibilities for further elaboration of specific quality criteria for these substances.

Among the perfluoroalkyl carboxylic acids (PFCAs), the most prominent member is perfluorooctanoic acid (PFOA) with an 8-carbon chain. Note that the substance has 7 perfluorinated carbon atoms only (Danish EPA, 2013). The PFOA derivative that is most widely used and therefore of most concern is the ammonium salt (APFO) (EFSA, 2008).



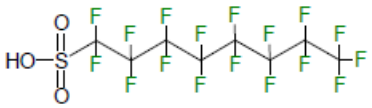
Perfluorooctanoic acid (PFOA)

Chemical name: perfluorooctanoic acid
Molecular brutto formula: C8 H F15 O2
CAS number 335-67-1

and its ammonium salt:

Chemical name: Ammoniumpentadecafluorootanoate (APFO)
Molecular formula: C8F15O2NH4
CAS number 3825-26-1

Perfluorooctane sulfonic acid (PFOS) is the most prominent of the perfluoroalkane sulfonic acids (PFSAs). PFOS has a linear perfluoroalkyl carbon chain of 8 atoms and a sulfonic acid functional group (Danish EPA, 2013).



Perfluorooctane sulfonic acid (PFOS)

Chemical name: Perfluorooctane sulfonate
Molecular formula: $C8F17SO3^-$

CAS number: 2795-39-3

Perfluorooctanesulfonamide (PFOSA) is a precursor compound to PFOS.

Structure from ChemID plus

Chemical name: Perfluorooctanesulfonamide
Molecular formula: C8-H2-F17-N-O2-S
CAS number: 754-91-6

1.2 Physical/ chemical properties

Physical and chemical properties of PFOA as free acid unless otherwise stated (EFSA, 2008).

TABLE 1-1 PFOA. PHYSICAL AND CHEMICAL PROPERTIES

Property	Value
Appearance at normal temperature and Pressure	White powder/ waxy white solid
Molecular weight	414.1 g/mol
Vapour Pressure	0.1kPa (20°C) 10mmHg (25°C) 4.2 Pa (25°C) (APFO*: 0.0081 Pa at 20°C)
Water solubility	3.4 g/L 4.1 g/L (22°C) 9.5 g/L (25°C)
Melting point	45-50 °C
Boiling point	189-192°C (736 mmHg)
Log Kow	Not measurable (APFO: 0.7)
Log Koc	2.06
Log K_D	-0.22-0.55 -0.39-0.94 soils 1.10-1.57 sludge
Air-water partition coefficient	Not available
Henry's Law Constant	Cannot be estimated
pKa	2.5, 2 to 3

*APFO: the ammonium salt of PFOA

The physical and chemical properties of the potassium salt of PFOS are listed in Table 1-2 (EFSA, 2008).

TABLE 1-2. PFOS. PHYSICAL AND CHEMICAL PROPERTIES OF PFOS, POTASSIUM SALT (TABLE EXTRACTED FROM EFSA, 2008)

Property	Value
Appearance at normal temperature and Pressure	White powder
Molecular weight	538 g/mol
Vapour Pressure	3.31 x 10^{-4} Pa (20°C)
Water solubility	519 mg/L (20 ± 0.5 °C) 680 mg/L (24-25°C)
Melting point	>400 °C
Boiling point	Not measurable
Log Kow	Not measurable
Log Koc	2.57
Log K_D	0.30-1.04 0.87-1.55
Air-water partition coefficient	<2 x 10^{-6}
Henry's Law Constant (calculated)	3.05 x 10^{-9} atm. m^3/mol pure water
pKa	-3.3 (calculated value for acid)

The physical and chemical properties of PFOSA listed in table 1-3 are taken from the HSDB database.

TABLE 1-3. PFOSA. PHYSICAL AND CHEMICAL PROPERTIES

Property	Value
Molecular weight	499.17 g/mol
Vapour Pressure	0.31 mm Hg at 25°C
Log Kow	5.8
Water solubility	8.04 X 10-3 mg/L at 25°C
Henry's Law Constant	1.8 atm-cu m/mol at 25°C

1.3 Production and use

1.3.1 Production

Perfluroalkyl and polyfluoroalkyl subtances are principally manufactured by two different processes: electrochemical fluorination (EFC) and telomerisation. The brief description below is extracted from LOUS, 2013. For more information regarding production and use, the reader should refer to the LOUS 2013-report (Danish EPA, 2013).

Electrochemical fluorination (ECF) is a technology in which an organic raw material (e.g. octane sulfonyl fluoride) undergoes electrolysis, leading to the replacement of all the H atoms by F atoms. The free-radical nature of the process leads to carbon chain rearrangement and breakage, resulting in a mixture of linear and branched perfluorinated isomers and homologues of the raw material as well as perfluorocarbons and other species. The ECF process has traditionally been used to produce

PFOS and PFOA. Today the ECF process is, among other functions, used to produce products based on perfluorobutane (Danish EPA, 2013).

Telomerisation is a technology in which a perfluoroalkyl iodide (PFAI) is reacted with tetrafluoroethylene (TFE) to yield a mixture of perfluoroalkyl iodides with longer perfluorinated chains. The starting iodide is referred to as the "telogen" and the TFE as the "taxogen." The product perfluoroalkyl iodide mixture is often then reacted further, in a second process step, where ethylene is inserted. The perfluoroalkyl commonly known as Telomer A, resulting from telomerisation, the 1st step, and the "fluorotelomer iodides", commonly known as Telomer B, formed in the 2nd step, are raw material intermediates used to produce additional building blocks that are further reacted to create a family of "fluorotelomer-based" surfactant and polymer products. When a linear telogen and taxogen are employed in the telomerisation process, the resulting perfluoroalkyl iodides have exclusively linear perfluoroalkyl chains. Mainly linear substances are produced by the process, but branched substances may be formed if a branched telogen is employed and reacted with the TFE (Danish EPA, 2013).

1.3.2 Use

The end-uses of PFOS and PFOA in articles and products are listed in Danish EPA (2013):

Hard chromium plating: PFOS is used in non-decorative hard chromium plating in Denmark which is one of the exempt applications of PFOS in connection with the REACH Annex XVII restriction of PFOS. Around the year 2010, 10-28 kg of PFOS was used annually in hard chromium plating in Denmark. In hard chromium plating, a thin layer of chromium is applied electrochemically to the surface of metals. The PFOS substances were used to limit the formation of Cr (VI) aerosols, which is considered of concern in terms of both occupational health and safety and the environment. The most commonly used PFOS substance for this purpose was tetraethylammonium perfluorooctanesulfonate (CAS no. 56773-42-3). This chemical substance is typically found in preparations with a concentration of 5-10%; e.g. Fumetrol® 140. PFOS is used in recirculating systems without wastewater drainage outlet. The substances are decomposed gradually in the baths, which are subsequently disposed of to the hazardous waste treatment plant, Kommunekemi.

Paint and lacquers: Fluorinated substances are widely used as surfactants in paint.

Impregnated clothing: The fluorinated agents are typically used for clothing for outdoor uses to make the clothing water and soil repellent. Norwegian and Swedish investigations of PFASs in all-weather jackets showed an unbound content of PFCA between <5 and 400 µg/m² of textile and an unbound content of PFOS-related compounds between <5 and 100 µg/m² of textile.
A study of PFASs in 11 textiles (mainly all-weather apparel) marketed in Norway by the Norwegian Pollution Control Authority in 2006 found the following levels of unbound PFASs in the textiles (number of samples with detectable content of the substances indicated in bracket): FASA/-FASE (FASAs: Perfluoroalkane sulphonamides; FASEs Perfluoroalkane sulfonamidoethanols): 0-23µg/m² (8), PFOS: <0.02-30 µg/m² (9), PFOA: 0.4-34 µg/m².
A recent report by Greenpeace in 2012 has found similar levels in 13 items of outdoor clothing from major brands purchased in Germany, Austria and Switzerland. The highest concentration of PFOA was 5 µg/m². PFOS was not detected in any of the samples.

Carpets
Carpets have historically been the major application area for PFOS in the EU.

Cleaning products
A survey of the chemical substances in cleaning products for ovens, cookers and ceramic hobs from 2010 did not find PFOS in the four mixtures analysed for the content of PFOS.

Fire extinguishers
PFOS-related substances have traditionally been used in firefighting foams in Denmark as well as other countries. The remaining PFOS-containing foams were allowed to be used until June 2011.

1.4 Environmental occurrence and fate

This paragraph is based on data from the Nordic Council of Ministers report from 2013 on Per- and polyfluorinated substances in the Nordic Countries, Use, occurrence and toxicology (NCM, 2013):

1.4.1 Air

In air samples, PFOA was detected in concentrations that ranged between 0.15–1.51 pg/m^3 in the Norwegian Arctic (NCM, 2013).

PFOS was detected in the Norwegian Arctic ambient air, however in very low concentrations, close to the limit of quantification (0.02 – 0.97 pg/m_3). In other air samples from Norway, concentrations of PFOS ranged between 0.03–3.32 pg/m_3 (NCM, 2013).

1.4.2 Water

PFCAs in Nordic countries have been reported in a number of papers and reports. Starting with seawater, PFCAs have been analysed in Greenland, Iceland, Faroe Islands and in Tromsø (Norway). Among PFCAs, PFOA has been the most abundant, at concentrations that reached 40 pg/L. In a study in Greenland, PFCAs were detected in snow with PFOA being again the dominant compound with concentrations up to 520 pg/L. In Denmark, PFCAs have been analysed and reported for a number of wastewater treatment plants (WWTPs), in the order of a few ng/L. It is interesting to note that there are big variations in the concentrations of PFCAs between WWTPs, but also within the same WWTPs. In particular, in two samples analysed from one WWTP, the concentration of PFOA was below 2 ng/L in the first sample and 23.5 ng/L in the second, while the concentration at the effluents was 10.1 and 16.4 ng/L, respectively. Another WWTP sample taken on the same day contained 4.5 and 6.4 ng/L of PFOS in the influent WWTP and 8.7 and 21.0 ng/L in the effluent. In Iceland and Faroe Islands, PFCAs were regularly below the limit of quantification, and when quantified, their concentrations were normally at <1 ng/g (wet weight, ww) (NCM, 2013).

In sea water and other aquatic samples, PFOS has ranged between ND and 1.18 pg/L, in the Faroe Islands. The substance was not detected in Iceland, but was found at levels as high as 90 pg/L in Tromsø, Norway. These differences underline the importance of the urban discharges. As a matter of fact, in effluent wastewaters in Denmark, PFOS was detected at concentrations up to 1,115 ng/L. Sewage sludge samples have also been analysed for PFSAs and again the prevalence of PFOS was seen. In a Danish WWTP, PFOS concentrations varied between 4.8 and 74.1 ng/g (dw), while in Norway, the range was between 1.2 and 5.16 ng/g.
Close to the training station of the company manufacturing fire-fighting foams, the concentrations in stream water was 69 µg/L for PFOS which is many orders of magnitude higher than any influent or effluent wastewater sample (NCM, 2013).
In a recent report published by the Danish EPA, the levels of PFAS (perfluoroalkyl and polyfluoroalkyl substances) were evaluated in groundwater under contaminated sites. The potential contaminated sites identified were the following five industries: fire training facilities, chromium plating industry, carpet industry, painting industry and landfills for construction waste and older municipal waste landfills. PFAS were detected in 5 out of 8 fire drill sites at levels from a few to several thousand ng/l. In two sites, PFAS levels were above or close to 100ng/l and in two others sites the levels were more than 1000 ng/l. The PFAS levels correspond to a sum of 9 PFAS compounds including PFOA, PFOS and PFOSA. A concentration of PFOS + PFOA of 1130 ng/l was found at the investigated carpet industry. The screening investigations did not find high levels of PFAS in landfills, chromium plating sites and paint manufacturers. It is noted that the most frequently occurring substance in these investigations is PFOS, which in some cases accounts for

more than half the sum of the investigated PFAS compounds. Also it should be noted that this survey was only a screening survey and that the result from this is far from being a representative picture of where the PFAS substances may be found (Danish EPA, 2014).

1.4.3 Soil

PFCAs were close to the detection limits in marine sediments in Iceland and Faroe Islands (Butt et al., 2010), and similarly not detected in background sediments in Norway (Report 2367/2008). However, in sediments close to a company that manufactures firefighting foams the concentrations of PFCAs were particularly high, reaching 101 ng/g for PFOA. The important impact of local sources such as the firefighting foam used in airports has been proven to contaminate adjacent soils, groundwater and other environmental compartments. In particular, this can be seen in the comparison between background soils close to the major Oslo airports and soils from the airport areas. For background soils, in Rygge and Gardemoen, PFCAs were not detected, whereas soils from the airports exhibited higher concentrations, particularly those from Gardemoen. In the latter, concentration of PFOA was around 4 ng/g (Klif Report TA-2444/2008) (NCM, 2013).

In abiotic environmental samples, PFOS was the only PFSA detected in marine sediments in the Faroe islands in concentrations just higher than the quantification limit (ND-0.11 ng/g ww, Butt et al., 2010) (NM, 2013).
Similarly to what was reported for PFCAs, the importance of local emission sources was assessed by analysing soils adjacent to airports and remotely from the airports, yet in the same region. The differences in concentrations were 5–10 times for PFOS (40.2 and 226.9 ng/g in soils in Rygge, and from 109.9 to 959 ng/g dw in soils close to Gardermoen) (Report 2444/2008) (NM, 2013).

1.4.4 Biodegradation and bioaccumulation

Perfluoroalkyl compounds are considered to be environmentally persistent chemicals. The carbon atoms of the perfluoroalkyl chain are protected from attack by the shielding effect of the fluorine atoms; furthermore, environmental degradation processes generally do not possess the energy needed to break apart the strong fluorine-carbon bonds. Perfluoroalkyl compounds are resistant to biodegradation, direct photolysis, atmospheric photooxidation, and hydrolysis (ATSDR, 2009).

Although transport and partitioning information indicates that air will not be a sink for perfluoroalkyl compounds in the environment, low concentrations of perfluoroalkyl carboxylic acids, sulfonic acids, and sulfonamides have been measured in air both in the vapour phase and as bound to particulates. Based on the measured absorption wave length of PFOA, PFOA is not expected to undergo direct photolysis. PFOS is not expected to undergo direct photolysis in the atmosphere. PFOSA may be photooxidised through removal of the amido or sulfonamido group (ATSDR, 2009).

PFOS and PFOA are expected to be stable to hydrolysis in the environment based on half-lives of 41 and 92 years, respectively. Available information indicates that perfluoroalkyl compounds are resistant to aerobic biodegradation. Hydrolysis data were not located for PFOSA (ATSDR, 2009).

Data are not available regarding the transformation and degradation of perfluoroalkyl compounds in sediment and soil. Based on the chemical stability of these substances and their resistance to biodegradation in screening tests, environmental degradation processes are not expected to be important removal mechanisms for perfluoroalkyl compounds in sediment and soil (ATSDR, 2009).

1.5 Human exposure

This paragraph is based on data from the Nordic Council of Ministers report from 2013 on per- and polyfluorinated substances in the Nordic Countries, Use, occurrence and toxicology (NCM, 2013).

1.5.1 Sources of exposure

In general there are two important sources of exposures of perfluorinated substances to humans namely via food and drink intake and through exposure to house dust (NCM, 2013).

1.5.1.1 Food and drinking water

PFOA

The median human intake of PFOA has, based on a duplicate diet study, been estimated to 2.9 ng/kg bw/day (Fromme et al. 2007).

However, the estimated intake of PFOA in the Norwegian population was found to be lower than what has been reported from Spain, Germany, UK, Canada and Japan. The estimated intake of PFOA from the duplicate diet study by Fromme et al. (2007) as indicated above is 5–6 times higher than the intake of 31 ng PFOA/day (corresponding to 0.5 ng/kg bw/day for an adult with a body weight of 60kg) estimated by Haug et al. (2010a) and 42 ng/day (corresponding to 0.7 ng/kg bw/day) (Haug et al., 2010b). This can be due to several parameters related to e.g. the differences in consumption pattern and the different levels of perfluorinated substances in food from the different countries as well as uncertainties in estimating the consumption of different foods. According to Norwegian data, cereals give a major contribution to the intake of PFOA. In Norway PFOA in bread was estimated to be a major source of the total intake of PFCs (Haug et al., 2010b).

Fish are assumed to be a major source of fluorinated substances. In a Norwegian study fish and shellfish were estimated to give the largest contribution of PFOA for human intake (38%). The levels of PFOA in fish found in the Norwegian study (Haug et al., 2010a) were significantly lower than the data from the UK and also lower compared to some other studies according to Haug et al. (2009). According to the author (Haug et al., 2009) this could be explained by the Nordic fish being caught in open sea rather than coastal areas and due to different fish species (NCM, 2013).

The largest intake of PFOA may occur from contaminated food, including drinking water. This is followed by the ingestion of dust and inhalation of air. The uptake of PFOA in children on a body weight basis is higher compared to adults because of a higher relative uptake from food and hand-mouth transfer from treated carpets and ingestion of dust (NCM, 2013).

Drinking water may be a significant source of PFOA exposure to humans. In drinking water produced from surface water in contaminated areas, PFOA was the main fluorinated compound found in a German study with a level of 500–640 ng/L. This is in accordance with another German study reporting high levels of PFOA (519 ng/L) in public water supplies produced from river water with bank filtration or artificial recharge. In the Netherlands the level of perfluorinated substances in drinking water resources was found to be in the range of non-detectable to 43 ng/l (study from 2007). In a recent study from 2010, three samples of tap water from different Norwegian water works in the Oslo area were analysed. The level of PFOA was 0.65–2.5 ng/L.

In a recent study, tap water from six European cities was analysed for PFCAs. The highest level of PFCA was found for PFOA (8.6 ng/l) in water samples from Amsterdam (NCM, 2013).

EFSA (2012) made a dietary exposure assessment using the overall European lower and upper bound mean occurrence of PFASs in food items. However, the low proportion of quantified results was considered to prevent calculation of a more realistic dietary exposure.

The upper bound results are highly overestimated, but still the exposure estimates in all age classes and for both mean and 95th percentile consumers were well below the TDIs for PFOA (1500 ng/kg bw per day) set by the EFSA Scientific Panel on Contaminants in the Food Chain.

For PFOA, the chronic dietary exposure in all age classes and for both average and high consumers was also far below the TDI. For adults the highest 95th percentile estimate (7.7 ng/kg bw per day) represented 0.5% of the TDI. In toddlers, the age class having the highest exposure, the highest

95th percentile estimate (32 ng/kg bw per day) represented 2.1% of the TDI. The most important contributors to PFOA exposure in all age classes were 'Fruits and fruit products' (18 to 39%) and 'Fish and other seafood' (7.6 to 27%), but high variations were observed in relation to dietary habits.

PFOS
Human intake of PFOS has been estimated to a wide range of 3.9– 530 ng/kg bw/day. Precursor compounds (as PFOSA and PFOSE) used in the production of fluorinated polymers may add to the exposure due to their degradation into PFOS.
The median intake of PFOS was found to be 1.4 ng/kg bw/day based on analysis of duplicate diet samples from various regions worldwide (n = 214) of 31 healthy individuals (age 16–45).
The estimated intake of PFOS from the duplicate diet study given by Fromme et al. (2007) is about 5 times higher than the intake estimated by Haug et al. (2010). This can be due to several parameters related e.g. to differences in consumption pattern and the level of PFOS in food from the different countries, to uncertainties in estimating the consumption of different foods and to uncertainties regarding the analytical test methods and analysis (NCM, 2013).

As for PFOA, the largest intake of PFOS seems to occur from contaminated food, including drinking water. This is followed by the ingestion of dust and inhalation of air.
A recent Norwegian study found that in general the major dietary intake of perfluorinated substances in Norway was PFOS (18 ng/day) (NCM, 2013).

Fish and shellfish were estimated to contribute with 81% of the total PFOS intake. In general the level of PFOS in fish is found to be higher than the level of PFOA. This is in accordance with a recent minor Danish study on PFOS and PFOA in fish from Danish waters, where the average level of PFOS was found to be 1.8 ng/kg (n = 9) whereas the level of PFOA was < 0.5 ng/kg (LOD). In a German study PFOS was detected in 33 wild fish (n = 112) at a concentration up to 225 µg/kg PFOS. PFOS was the perfluorinated substance (of 11 analysed) most often detected in especially fish, shellfish, liver and kidney and most often at the highest concentrations in a UK study of 252 food samples (NCM, 2013).
In tap water samples (n = 3) from the Oslo area, the level of PFOS was 0.071–0.23 ng/L. In another recent study of perfluorinated substances in tap water from six European cities the highest levels of PFOS (8.8 ng/L) were found in tap water samples from Stockholm (NCM, 2013).

EFSA (2012) made a dietary exposure assessment using the overall European lower and upper bound mean occurrence of PFASs in food items. However, the low proportion of quantified results was considered to prevent calculation of a more realistic dietary exposure. The upper bound results are highly overestimated, but still the exposure estimates in all age classes and for both mean and 95th percentile consumers were well below the TDI for PFOS (150 ng/kg bw per day) set by the EFSA Scientific Panel on Contaminants in the Food Chain. For PFOS, the highest upper bound 95th percentile estimate (7.7 ng/kg bw per day) represented 6.7% of the TDI. In toddlers, the age class having the highest exposure, the highest upper bound 95th percentile estimate (29 ng/kg bw per day) 19% of the TDI. The highest contributors to dietary PFOS exposure across all age classes were 'Fish and other seafood' (50 to 80%) followed by 'Fruits and fruit products' (8 to 27%) and 'Meat and meat products' (5 to 8%).

1.5.1.2 Indoor air exposure

PFOA
All PFCAs have been detected in indoor house dust in Norwegian houses and offices. In the latter study, PFOA was detected in houses with a median of 38.8 ng/g. In one office reported in the same study, the pattern was different, with PFOA being the most abundant chemical (694 ng/g). Finally, in a study from Sweden, PFOA was studied in houses, offices and apartments and the average concentrations were 54, 93 and 70 ng/g, respectively, thus, in the same order of magnitude as in Norwegian indoor environments (NCM, 2013).

PFOS
Exposure to PFSAs in the indoor environment occurs mainly through dust.
In the Nordic countries, PFSAs have been reported for Norwegian homes and in an office and similarly for Sweden, again for residences and offices.
PFOS is the dominant PFSA with concentrations in dust that have reached 147.7 ng/g in a Norwegian office. Very high concentrations of PFOS have been detected also in the Swedish offices. In homes/residences, PFOS ranged between 9.1 and 11 ng/g in Norway and between 39 and 85 in Sweden. The lower levels in residences demonstrate the higher relative importance of occupational exposure compared to exposure in private homes.

The uptake rate has been calculated for PFOS and based on three different scenarios, this ranged for Norwegians between 0.11 and 0.46 ng/kg bw/day, through dust, and between 0.004 and 0.36 ng/kg bw/day, through air (NCM, 2014).

1.5.1.3 Consumer products

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are used in numerous industrial and consumer products because of their special chemical properties, for instance the ability to repel both water and oil. Samples of consumer products and preparations were collected in Norway, with supplemental samples from Sweden. In 27 of the 30 analysed consumer products and preparations a number of polyfluorinated substances that were analysed were detected, but this does not exclude the occurrence of unknown PFAS. Notable was that perfluorooctanesulphonate (PFOS), which has been strictly regulated in Norway since 2007, was found in amounts close to or exceeding the EU regulatory (REACH Annex XVII) level in 4 of the 30 analysed products, all within the leather or carpet product groups.
PFOA was found in amounts close to or exceeding the EU regulatory level of PFOA in 2 of the 30 analysed products all with the coated fabrics (NCM 2013).

Consumer products like sprays, treated carpets and food contact materials may also lead to consumer exposure of PFOS, but as also for PFOA the spray sources today are probably due to the restricted use less important for most consumers/the general population. However, spray sources may contribute significantly to the exposure for those consumers frequently using e.g. PFC containing sprays and who have treated carpets in their homes (NCM 2013).

1.5.2 Biomonitoring

1.5.2.1 Levels in blood

PFOA
Few biomonitoring studies have been conducted in Denmark measuring the levels of a broad range of perfluorinated substances.
A recent study reported the levels of eight different perfluorinated substances in serum from young women planning their first pregnancy (collected during 1992–1995). Among the women who got pregnant (n = 129), the concentration of PFOA was 5.61 ng/ml.
Another study reported the levels of PFOA in 1,399 maternal blood plasma samples collected during 1996–2002 in Denmark (part of the Danish National Birth Cohort). For the first trimester the mean plasma PFOA level was 5.6 ng/ml.
A study from 2009 reported the perfluorinated substances levels in serum samples from young adult males in Denmark (n = 105) collected in 2003. The level of PFOA was 4.9 ng/ml and the remaining PFCAs (PFDA, PFNA, PFHpA, PFUnA and PFDoA) were found in much lower concentrations with medians ranging from 0.9–0.08 ng/ml. The PFCA levels detected in this study were comparable to those found in Sweden.
The current concentrations of perfluorinated substances in Denmark are unknown since the latest biomonitoring data found are from 2003 (NCM, 2013).

A recent study from Denmark indicates a mean PFOA level in plasma of children and their mothers of 3.2 ng/mL and 1.8 ng/mL, respectively, and corresponding 95-percetile levels of 5.2 ng/mL and 3.4 ng/mL, respectively (Mørck et al., 2014)

PFOS and PFOSA
For young Danish women planning their first pregnancy (serum collected during 1992–1995), the concentration of PFOS was 36.3 ng/ml. The median serum levels were similar to most levels reported for US populations during this period.
Comparable PFOS plasma concentrations of 35.1 ng/ml were reported for 1,399 pregnant women during 1996–2002 in Denmark (part of the Danish National Birth Cohort). For young adult Danish men in 2003, the median PFOS concentration was 24.5 ng/mL, and PFOSA was detected in only 56 of 105 men (median 0.06 ng/ml).
The PFSA levels detected in this study were comparable with those found in other countries such as Sweden, but lower than results from the women in Denmark (NCM, 2013).

A recent study from Denmark indicates a mean PFOS level in plasma (sum of branched PFOS isomers and linear PFOS) of children and their mothers of 9.0 ng/mL and 8.3 ng/mL, respectively, and corresponding 95-percetile levels of 16.1 ng/mL and 16.2 ng/mL, respectively (Mørck et al., 2014).

1.5.2.2 Level in cord blood

PFOA
In a Danish study PFOA was analysed in 50 cord blood plasma samples from women in the Danish National Birth Cohort (1996–2002), and the results showed mean concentrations of 3.7 ng/ml for PFOA, which corresponded to 66% of the level in maternal serum. Concentrations in cord blood and mother's blood were highly correlated.
The consistent finding of the studies is that cord blood has lower total PFOA than maternal blood; but several perfluorinated substances are able to cross the placenta barrier to foetal blood and PFOA seems to cross the placenta most easily. The concentrations of PFOA were highest in Danish and Faroes cord blood, probably because the studies are older (NCM, 2013).

PFOS
The mean concentration of PFOS in 50 cord blood plasma samples from the Danish National Birth Cohort (1996–2002) was 11 ng/ml, corresponding to 30% of the level in maternal serum (NCM, 2013).

1.5.2.3 Levels in breast milk

PFOA was detected in breast milk from women in Nordic countries and the concentrations in milk are 3–4% of what is found in the corresponding serum concentrations (NCM, 2013).

PFOS has been detected in breast milk in concentrations approximately 1–2% of the corresponding concentrations in serum (NCM, 2013).

1.5.2.4 Levels in amniotic fluids

A Danish study detected PFOA in amniotic fluids at concentrations 10–20 fold lower than in maternal blood (NM, 2013).

Few data on PFOS in amniotic fluid are reported until now. Amniotic fluid PFC concentrations are considerably lower than maternal blood (10–20 fold) and also lower than cord blood concentrations (NCM, 2013).

2. Toxicokinetics

2.1 Absorption and distribution

2.1.1 PFOA

After oral exposure PFOA is readily absorbed. In rats PFOA is mainly found in the liver, kidneys and blood with lower levels in many other organs, including the central nervous system. It can cross the blood-placenta border in a facilitated way and enter the foetus where it is mainly found in the liver (EFSA, 2008).

2.1.2 PFOS

After oral exposure PFOS is readily absorbed. In rats, PFOS also shows a tendency to accumulate when repeatedly administered (Seacat et al., 2003). In rats, PFOS is mainly found in the liver, kidneys and blood with lower levels in most other organs, including the central nervous system. It can cross the placenta and enter the foetus where it is mainly found in the liver (EFSA, 2008).

2.1.3 PFOSA

Male rats that received a single oral dose of 5 mg PFOSA/kg had liver PFOSA concentrations that were 3–5 times higher than serum concentrations, one day following the dose indicating a potential for accumulation in the liver (ATSDR, 2009).

2.2 Metabolism and elimination

2.2.1 PFOA

Metabolic elimination seems to play no relevant role. Elimination in rats occurs *via* the kidneys and to a lesser extent *via* faecal excretion. Urinary excretion is the major route of elimination in female rats, both urinary and faecal excretion the major route in male rats. Renal elimination seems to be negligible in humans. Protein-binding and expression of transporters have an important role in determining distribution and elimination. Elimination half-lives of < 24 h in female and < 9 days in male rats, of 21 – 30 days in Cynomolgus monkeys, and of about 3.8 years in humans have been estimated (EFSA, 2008).

Based on the available data, US EPA (2014a) concluded on serum elimination half-lives of 11.5 days in rats and 839.5 days in humans. These values were further used when taking into account the toxicokinetic differences between species in connection with derivation of a reference dose level (RfD) for PFOA.

Various (- also longer) half-lives have been reported; however the above estimations are based on the overall view of the database.

2.2.2 PFOS

There are no reports on PFOS metabolites formed *in vivo*. In primates metabolic elimination seems to play no relevant role as can be derived from the long elimination half-lives. Elimination in rats occurs mainly via the kidneys and to a lesser extent via faecal excretion, whilst renal elimination seems to be negligible in humans. The elimination half-lives have been estimated as > 90 days in rats, about 200 days in Cynomolgus monkeys, and about 5.4 years in humans (EFSA, 2008).

Based on the available data, US EPA (2014b) concluded on a serum elimination half-lives of 48 days in rats, 121 days in monkeys and 1971 days in humans. These values were further used when taking into account the toxicokinetic differences between species in connection with derivation of a reference dose level (RfD) for PFOS.

2.2.3 PFOSA

Perfluorooctane sulfonamide (PFOSA) is eliminated rapidly in rodents with a half-life of a few days. Branched isomers have a faster half-life than linear isomers (Danish EPA, 2013).

2.3 Mode of action

2.3.1 PFOA

Liver toxicity

The critical effects of PFOA in rodents and monkeys are on the liver (moderate grade hypertrophy, changes in liver enzyme activity, absolute or relative liver weight increases, hypolipidemia, proliferation of smooth endoplasmic reticulum and peroxisomes).

In rodents these effects may be related to the peroxisome proliferating activity of PFOA. Like many other peroxisome proliferators, PFOA has also been shown to cause hepatomegaly in rats and mice, oxidative DNA damage in livers of rats and apoptosis in HepG2 liver cells. By activating PPARα, PFOA also interferes with lipid and lipoprotein metabolism. This was also seen in studies including gene expression analysis of livers from PFOA fed rats, which showed alterations in the genes associated with lipid and fatty acid metabolism in rats treated with PFOA. The induced enzymes for fatty acid oxidation might increase the normal oxidation of fatty acids and might disrupt the normal balance of fatty acid metabolism in mammals.

In Cynomolgus monkeys dose dependent increases in liver weights associated with mitochondrial proliferation have been observed from the lowest dose tested (3 mg/kg per day during 26 weeks), the mechanism of action remains to be resolved since peroxisomal markers were not altered (EFSA, 2008).

EFSA (2008) on this basis concluded that not all of the liver toxicity could be ascribed to PPARα activity, and other possible mechanisms such as induction of genes involved in lipid metabolism and transport of lipids and drug-metabolising enzymes could be of relevance to human health.

Carcinogenicity

The negative outcome in a comprehensive series of genotoxicity tests at gene and/or chromosome level indicates an indirect (non-genotoxic) mechanism for the carcinogenicity of PFOA (or rather APFO which is the ammonium salt of PFOA). Mechanisms, attributed to a non-genotoxic mechanism involving activation of receptors and perturbations of the endocrine system, have been suggested.

APFO is a PPARα-agonist which suggests that liver carcinogenicity/toxicity could be mediated by binding to PPARα in the liver. However, taking into account that PFOA also caused liver effects in monkeys, the relevance of liver toxicity due to other mechanisms cannot be discounted. The data presently available suggest that the induction of Leydig cell tumours and mammary gland neoplasms may be due to hormonal imbalance resulting from activation of the PPARα and induction of the cytochrome P450 enzyme, aromatase. A mechanistic role for sustained increase of serum estradiol in the mechanism of induction of Leydig cell adenomas has also been hypothesised (EFSA, 2008).

Endocrine effects/disruption

As indicated under carcinogenicity, Leydig cell tumours and mammary gland neoplasms may be due to hormonal imbalance resulting from activation of the PPARα and induction of the cytochrome P450 enzyme, aromatase. The induction of hepatic aromatase activity may cause increased serum

estradiol levels that further stimulate the production of growth factors such as the transforming growth factor α which induces Leydig cell proliferation.

Also, inhibition of testosterone biosynthesis by PFOA has been observed in a mixture of *in vivo*, *ex vivo* and *in vitro* studies (Biegel et al., 1995) (EFSA, 2008).

2.3.2 PFOS

Liver toxicity

Several studies show that PFOS interferes with fatty acid metabolism and metabolism of lipids and lipoproteins. However, it is still unclear how this links to the liver toxicity that is observed in rodents and monkeys.

PFOS has been shown to activate the PPARα in *in vitro* experiments. Studies indicate that PFOS was less active than PFOA for both mouse and human PPARα and PPARβ, and neither of the substances showed significant activation of mouse or human PPARγ.

Peroxisome proliferation has been reported in some rodent studies but not in others. However, the mechanism is unlikely to be responsible for the observed liver toxicity in primates given current knowledge of relative susceptibility of primates compared with rodents to peroxisome proliferation. In primates lipid accumulation has been observed in the liver without peroxisome proliferation.

There are other pathways by which PFOS can interfere with lipid metabolism in the liver.

One of these is competition of PFOS with fatty acids and other endogenous ligands for binding to the important intracellular liver fatty acid transporter proteins, which may contribute to hepatotoxicity and lower serum cholesterol levels.

In addition, the induction of a spectrum of liver enzymes has been observed.

Further, a reduction in the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase has been observed, which may be linked to reduced levels of cholesterol and triglycerides.

Finally, PFOS has been shown to inhibit in vitro gap junction intercellular communication in rat liver cell lines and in the liver of PFOS-treated rats. This mechanism may also be involved in liver carcinogenesis (EFSA, 2008).

Carcinogenicity

Based on the complete lack of genotoxicity in a wide range of *in vitro* and *in vivo* assays at gene and/or chromosome level, the weight of evidence indicates an indirect (non-genotoxic) mechanism for the carcinogenicity of PFOS. The induction of hepatocellular tumours does not appear directly related to peroxisome proliferation; however, the increased incidences of tumours were observed at doses above those associated with non-neoplastic toxic effects. Thyroid tumours are likely to be secondary to hormonal imbalance. The thyroid and mammary gland tumours are difficult to evaluate because of the lack of dose-response relationship (EFSA, 2008).

Endocrine effects/disruption

In rats, oral administration of PFOS resulted in increased tissue availability of thyroid hormones and turnover of T4, but the pattern of changes seen was not typical of a hypothyroid state. Although reproductive and developmental toxicity have been described, the underlying mechanism for these effects remains unclear. Part of the toxicity may be related to changes in thyroid hormone levels which may affect early development. The extent to which changes in lipid metabolism, changes in transport of fatty acids or induction of liver enzymes and metabolism contribute to the changes in hormone levels is currently unknown. It is noteworthy that opposite effects have been observed in animal experimental studies (lower levels of cholesterol and estradiol after PFOS exposure in rodents and monkeys, and increased levels of estradiol in humans) (EFSA, 2008).

A study in adult female rats showed that PFOS can cross the blood brain barrier and accumulate in the hypothalamus leading to increased norepinephrine concentrations in the para ventricular nucleus of the hypothalamus. Further treatment with PFOS was shown to affect oestrous cyclicity

and lead to increased serum corticosterone levels and decreasing serum leptin concentrations (EFSA, 2008).

3. Human toxicology

This chapter is mainly based on evaluations and conclusions from the EFSA report in 2008 and the US EPA draft reports on PFOA and PFOS in 2014.

In the quotation of text from EFSA (2008) the original references are maintained in the text in order to make it possible to track the studies when discussed and presented further in the document e.g. in tables.
Note: As the current US EPA draft reports cannot be quoted or cited, the EFSA (2008) descriptions on human data will perform the basis of this section. Only if new and significant findings important for TDI derivation is mentioned in the US draft reports, these data will be included in the text.

NCM (2013) refers to a few studies where levels of PFOSA have also been included in the evaluation of the epidemiological studies; however, in none of the cases the levels of PFOSA showed any specific associations with the health outcomes.

3.1 Occupational exposure

3.1.1 PFOA

Most studies on PFOA have been carried out by 3M in the Cottage Grove plant (Minnesota, USA), where PFOA has been produced since 1947. PFOA has been manufactured since 1998 at the 3M Decatur, Alabama plant.
A cross sectional study among 191 workers engaged in PFOA production revealed an increase in mean estradiol levels among employees that had the highest levels of serum PFOA (>30 ng/mL) although this association was confounded by body mass index (Olsen et al., 1998) (EFSA, 2008).

The most recent report by Olsen and Zobel (2007) included workers from three separate 3M PFOA production sites in Antwerp (n=306), Minnesota (n=131) and Alabama (n=215). PFOA was not statistically significantly associated with total cholesterol or low-density lipoproteins (LDL). High-density lipoproteins (HDL) were negatively associated with PFOA for the three facilities combined, but not for the individual sites. Serum triglycerides were positively associated with PFOA for the three facilities combined, and individually for Antwerp but not for the other two sites. No consistent associations were found with PFOA and thyroid hormones. Overall T4 was negatively associated with PFOA and T3 was positively associated, but the trends were within normal reference ranges. The authors considered that the HDL association was likely to be explained by residual confounding, but could not rule out a biological explanation for the triglyceride observation (EFSA, 2008).

A retrospective cohort mortality study was performed on workers at the 3M Cottage Grove MN plant (Gilliland and Mandel, 1993). The cohort consisted of workers who had been employed at the plant for at least 6 months between January 1947 and December 1983. The number of months provided the cumulative exposure measurements. Of the 3537 (2788 men and 749 women) employees, 398 (348 men and 50 women) were deceased. Eleven of the 50 women and 148 of the 348 men were considered exposed to APFO. The Standardised Mortality Ratios (SMRs), adjusted for age, sex and race, and stratified for 3 latency periods (10, 15 and 20 years) and 3 periods of duration of employment (5, 10 and 20 years), were compared to US and Minnesota white death

rates for men. For women, only state rates were available. When exposure status was considered, SMRs for all causes of death and all cancers were lower than expected. When compared to Minnesota death rates, the SMR for prostate cancer was 2.03 (95% CI 0.55–4.59), based on 4 deaths (1.97 expected). There was a statistically significant (p=0.03) association with length of employment. The relative risk for a 1-year increase in employment was 1.13 (95% CI 1.01–1.27). It rose to 3.3 (95% CI 1.02– 10.6) for workers employed for 10 years (Gilliland and Mandel, 1993) (EFSA, 2008).

An update of this study was conducted to include mortality through to 1997 (Alexander, 2001a). The cohort consisted of 3992 workers, placed into 3 exposure groups based on job history information; definite exposure (n=492); probable exposure (1685) and not exposed (1815). In this new cohort, 607 deaths were identified: 46 in the exposure group, 267 in the probable exposure group, and 294 in the non-exposed group. The highest SMR reported was for bladder cancer (SMR=1.31, 95% CI=0.42–3.05). A few SMRs were elevated for employees in the definite exposure group: 2 deaths from cancer at the large intestine (SMR=1.67, 95% CI=0.02–6.02), 1 from pancreatic cancer (SMR=1.34, 95% CI=0.03–7.42), and 1 from prostate cancer (SMR=1.30, CI=0.03–7.20). In the probable exposure group, 3 SMRs were elevated: cancer of the testis and other male genital organs (SMR=2.75, 95% CI=0.07–15.3); pancreatic cancer (SMR=1.24, 95% CI=0.45–2.70); malignant melanoma of the skin (SMR=1.42, CI=0.17–5.11). These SMRs were not statistically significant at p<0.05. There were no notable excesses in SMRs in the non-exposed group, except for cancer of the bladder and other urinary organs (4 cases against 1.89 expected) (EFSA, 2008).

These studies provide little information about the relationship of PFOA to mortality or cancer incidence since no exposure information, use of other compounds, or lifestyle information was collected on the employees.

In summary, a retrospective cohort mortality study showed a statistically significant association between prostate cancer mortality and employment duration in the chemical facility of a plant that manufactures APFO. However, in an update of this study, in which more specific exposure measures were used, a significant association for prostate cancer was not observed. Other mortality studies lacked adequate exposure data which could be linked to health outcomes. A number of studies have investigated possible associations between PFOA serum levels and biochemical parameters associated with lipid metabolism. Some have shown associations with elevated cholesterol and triglycerides, or with changes in thyroid hormones, but overall there is no consistent pattern of changes. In two recent ecological studies, PFOA exposure of pregnant women, measured by maternal and/or cord serum levels was associated with reduced birth weight. EFSA noted that these observations could be due to chance, or to factors other than PFOA, but indicate a need for further research into possible developmental effects in humans (EFSA, 2008).

3.1.2 PFOS

Follow up of 2083 Decatur workers (Alabama) showed that workers in jobs involving high exposure to PFOS based materials had 13 times increased risk for bladder cancer mortality compared with the general population of Alabama (SMR= 12.77, 95% confidence limit 2.63–37.35). However, this observation was based on only 3 cases of bladder cancer and the workers were exposed to several compounds, hence it is difficult to draw definite conclusions (Alexander et al., 2003). In a follow-up study, eleven cases of bladder cancer were identified from 1400 of the workers who responded to a questionnaire, and from 185 death certificates. There were no statistically significant associations between PFOS exposure and an increased risk of bladder cancer (Alexander and Olsen, 2007) (EFSA, 2008).

3.2 Exposure to general population

3.2.1 PFOA

Apelberg et al. (2007b) investigated the association between PFOA concentrations in cord serum and gestational age, birth weight and size of 293 singleton births delivered in November 2004 to March 2005 in Baltimore, USA. PFOA was detected in all of the cord blood samples, with a median concentration of 1.6 ng/mL (range 0.3-7.1 ng/mL). PFOA was inversely associated with birth weight and head circumference, but not length or gestational duration. The concentrations of PFOA in cord serum were highly correlated with those of PFOS. In the Danish National Birth Cohort of 1400 women delivering a single child between March 1996 and November 2002, the average maternal plasma level of PFOA was 5.6 ng/mL (range < 1.0 – 41.5 ng/mL) and the cord plasma levels in a subset of 50 subjects were 3.7 + 3.4 ng/mL (mean + S.D.). Maternal plasma levels of PFOA were inversely associated with birth weight but not with risk of low birth weight (< 2500g) or small for gestational age (Fei et al., 2007) (EFSA, 2008).

US EPA (2014a) made an extensive evaluation of several, more recent epidemiological studies available since RFSA (2008). For several end-points were between serum PFOA levels in the general population and/or in workers in relation to:

- increased serum cholesterol
- increased liver enzymes
- decreased bilirubin
- increase of chronic kidney disease
- early menopause
- decreased levels of antibodies in children after vaccination
- obesity in females
- delay of puberty in girls
- parent reported ADHD

In these studies US EPA (2014a) noted a great difference in the serum levels from workers (1.02 – 4.63 µg/mL) compared to the general population (1.05 -354 ng/mL), where the highest levels in the general population were found in areas with contaminated water.
Also it was noted that co-exposure typically occurred to other fluorinated substances and that specific exposure levels for the individuals in the studies were not known.
Overall, US EPA (2014a) did not find it possible to include the epidemiological data in the further dose-response characterisation and RfD derivation of PFOA.

3.2.2 PFOS

Apelberg et al. (2007b) investigated the association between PFOS concentrations in cord serum and gestational age, birth weight and size of 293 singleton births delivered in November 2004 to March 2005 in Baltimore, USA. PFOS was detected in >99% of the cord blood samples, with a median concentration of 5 ng/mL (range <0.2-34.8 ng/mL). PFOS was significantly associated with small decreases in birth weight and size, but not newborn length or gestational age. The concentrations of PFOS in cord serum were highly correlated with those of PFOA. A study conducted in the Danish National Birth Cohort suggests that the association might be related to PFOA rather than PFOS. In this cohort of 1400 women delivering a single child between March 1996 and November 2002, the average maternal plasma level of PFOS was 35.3 ng/mL (range 6.4-106.7 ng/mL) and the cord plasma levels in a subset of 50 subjects were 11 + 4.7 ng/mL (mean +. S.D.). Maternal plasma levels of PFOS did not show a consistent association with birth weight or gestational age (Fei et al., 2007) (EFSA, 2008).

In the US EPA (2014b) evaluation, several, more recent epidemiological studies are described. Thus positive associations have been found between serum PFOS levels in the general population and increased cholesterol and increased triglycerides. However, this could not be found in occupational

studies. Furthermore, effects on thyroid hormone levels have been found; however, with inconsistent responses. US EPA (2014b) further indicated that limited and inconclusive data are available regarding PFOS levels and the immunotoxic response in children. Also data suggesting risk for low birth weight were considered as inconclusive.
Also for PFOS some general limitations for the interpretations of the epidemiological studies were noted e.g. the possible rule of concurrent exposure to other fluorinated substances, the lack of control for other co-factors and not least the lack of consistency in the findings.

Overall, USA (2014b) did not find it possible to include the epidemiological data in the further dose-response characterisation and RfD derivation of PFOS.

3.3 Conclusions, human data

Overall, the human data on PFOA and PFOS were by EFSA (2008) or US EPA (2014a+b) found to deliver some support to the findings in experimental animals; however, the data are considered far from adequate for making definitive conclusions on critical effects and dose-response relationships. Thus human data are not used further by EFSA (2008) and US EPA (2014a+b) for the derivation of TDI/RfD values.

This is somewhat in contradiction with a study by Grandjean and Budtz-Jørgensen (2013) who made a first attempt to benchmark dose levels and RfD for PFOA (and PFOS) based on human data on immunotoxicity. After vaccination of 223 children, they found a decrease in the levels of anti-bodies against tetanus which were associated to increased serum levels of perfluorinated substances. Using these data, dose–response associations were modelled and serum benchmark dose levels (BMDL5 and BMDL 10) were calculated. From this, a BMDL of 1 ng/mL was found for PFOA and PFOS and a RfD serum level of 0.1 ng/mL was suggested for the substances. This serum dose level was then translated (using pharmacokinetic modelling) to a drinking water content of 1 ng/L.
The authors emphasised that such an approach would lead to a limit value in drinking water about 300 times lower than current values indicating the need for reconsiderations of the current limit values.

4. Animal toxicity

This chapter is mainly based on evaluations and conclusions from the EFSA report in 2008 and the US EPA draft reports on PFOA and PFOS in 2014.

Note: As the current US EPA draft reports cannot be quoted or cited, the data from these reports has been extracted and included in overall summary tables of the toxicological database. Only where necessary, i.e. when newer data compared to EFSA (2008) have been evaluated or where different interpretation or important extra information compared to the EFSA evaluation is given, this has been included in the text below. In the quotation of text from EFSA (2008), the original references are maintained in the text in order to make it possible to track the studies when discussed and presented further in the document, e.g. in tables.

Swedish EPA (2012) indicated that some precursor PFASs have shown to a various extent to be transformed in rodents to their perfluorinated sulfonate, e.g. PFOSA to PFOS. As toxicity data on PFOSA are absent, it seems for the moment most appropriate to assess the toxicity of PFOSA using a read-across approach to PFOS.

4.1 Single dose toxicity

4.1.1 Inhalation

4.1.1.1 PFOA

In male CD rats the LC50 by inhalation of APFO for 4 hours was 980 mg/m³. This concentration produced an increase in liver size and corneal opacity. Repeated treatment for 10 days suppressed body weight gain (84 mg/m³) and increased liver weight. The no-observed effect level was 1 mg/m³ (Kennedy et al., 1986) (EFSA 2008).
PFOA is classified as Acute Tox. 4, H322 (Harmful if inhaled).

4.1.1.2 PFOS

Exposure of Sprague-Dawley rats, 5/sex/group, to PFOS dust in air for one hour yielded an inhalation LC50 of 5.2 mg/L with 95% confidence limits of 4.4 and 6.4 mg/L. A Wright dustfeed mechanism with dry air at a flow rate of 12 to 16 litres per minute was used to administer the PFOS dust (Rusch et al., 1979) (EFSA 2008).
PFOS is classified as Acute Tox. 4, H322 (Harmful if inhaled).

4.1.2 Oral exposure

4.1.2.1 PFOA

Oral LD50 values in rats were about 500 mg/kg bw: 680 and 430 mg/kg bw in male and female CD rats, respectively (average (540 mg/kg bw) (Dean and Jessup, 1978, reviewed in Griffith and Long, 1980). More recently, Glaza (1997) reported an oral LD50 for PFOA greater than 500 mg/kg and between 250 and 500 mg/kg in female rats (EFSA 2008).
PFOA is classified as Acute Tox. 4, H302 (Harmful if swallowed).

4.1.2.2 PFOS

A mean oral LD50 value of 251 (199-318) mg/kg bw was calculated based on a single administration of PFOS by gavage to CD rats, 5/sex/group (Dean et al., 1978) (EFSA 2008).

PFOS is classified as Acute Tox. 4, H302 (Harmful if swallowed).

4.1.3 Dermal exposure

4.1.3.1 PFOA

The dermal LC_{50} was reported to be greater than 2000 mg/kg bw in New Zealand White rabbits (Glaza, 1995). PFOA is a weak skin irritant as determined in rabbit experiments. Rats were less sensitive than rabbits (Kennedy, 1985) (EFSA 2008).

4.1.3.2 PFOS

Skin and eye irritation were not observed in albino New Zealand White rabbits (Biesemeier and Harris, 1974) (EFSA 2008).

4.2 Irritation

4.2.1 Skin irritation

4.2.1.1 PFOA

Application of as single dose of 5,000 mg/kg of an aqueous paste of APFO (ammonium salt of PFOA) to a clipped area of the skin of rats, and left in place covered for 24 hours produced mild skin irritation (Kennedy, 1985); no irritation was apparent with a dose of 3,000 mg/kg. In the 2-week study, acute necrotising dermatitis was seen in two out of five high-dose rats after the 10th treatment; doses of 200 mg/kg/day produced skin irritation. Application of 500 mg/kg (only dose tested) APFO to the intact or abraded skin of young rabbits and left covered for 24 hours was non-irritating, as scored according to the Draize procedure immediately after removal of the cover and 48 hours later (ATSDR 2009).

4.2.1.2 PFOS

A study from 1974 using rabbits treated with 0.5 grams PFOS on intact and abraded skin did not result in any irritation after 24 and 72 hours (US EPA, 2014b)

4.2.2 Eye irritation

4.2.2.1 PFOA

In a study in rabbits PFOA was found to be an eye irritant when not washed away (US EPA, 2014a). PFOA is classified as Eye Dam. 1, H318 (Causes serious eye damage).

4.2.2.2 PFOS

No adequate data seem to be available.

4.2.3 Respiratory irritation

No data retrieved in the literature.

4.3 Sensitisation

4.3.1 Skin sensitisation

No data retrieved in the literature.

4.3.2 Respiratory sensitisation

No data retrieved in the literature.

4.4 Repeated dose toxicity

Only oral studies regarding repeated exposure and subchronic/chronic toxicity have be found for PFOA and PFOS.

4.4.1 PFOA

Below the repeated dose toxicity studies as described and assessed by EFSA (2008) are given:

"In a 90 day study Crl: CDBR rats (5/sex/group) received dietary concentrations of 0, 10, 30, 100, 300 and 1000 mg/kg PFOA equivalent to doses of 0.6, 1.7, 5.6, 18 and 64 mg/kg bw per day in males and 0.7, 2.3, 7.7, 22.4 and 76 mg/kg bw per day in females. Absolute and relative liver weights were increased at the two highest doses in males and at the highest dose in females, with an increased absolute liver weight at 1.7 mg/kg bw per day in males.
Hepatocellular hypertrophy was observed in males at doses of 5.6 mg/kg bw per day and higher with hepatocellular necrosis from doses of 1.7 mg/kg bw per day and above. Based on these liver effects, the NOAEL was 0.6 mg/kg bw per day for males and 22 mg/kg bw per day for females (Goldenthal, 1978b).

A 90 day dietary toxicity study in male Crl: CDBR rats (dietary levels equivalent to 0, 0.06, 0.64, 1.94 and 6.4 mg/kg bw per day) showed reduced body weight gain in the highest dose group. Doses of 0.64 mg/kg bw per day and higher showed increased hepatic palmitoyl CoA oxidase activity, which is a marker for peroxisome proliferation, and increased relative liver weights. Histopathological changes included hepatocellular hypertrophy and necrosis of liver cells (Perkins et al., 2004).

A 90 day oral toxicity study performed in rhesus monkeys (2/sex/group) with doses of 0, 3, 10, 30 and 100 mg/kg bw per day PFOA resulted in mortality of all monkeys at week 5 at 100 mg/kg bw per day, and three monkeys from the 30 mg/kg bw per day group at week 13. In the females dosed with PFOA at 10 mg/kg bw per day, the heart and brain weights were decreased. No histopathological changes were observed. No treatment related lesions were seen in the organs of animals from the 3 and 10 mg/kg bw per day dose groups. Occasionally, marked or moderate diarrhoea was observed in the 3 mg/kg bw per day dose group (Goldenthal, 1978a).

Studies in which male Cynomolgus monkeys were given daily oral PFOA doses of 0, 3, 10 or 30 mg/kg bw for 6 months, showed dose dependent increases in liver weight associated with mitochondrial proliferation in all treatment groups. No histopathological evidence of liver injury was observed at either the 3 or 10 mg/kg bw per day group. No changes in clinical chemistry, hormones, urine composition or haematological effects were noticed. Two male animals died before termination of the study, one in the 3 mg/kg bw group and one in the 30 mg/kg bw (Butenhoff et al., 2002).

Loveless et al. (2006) compared the toxicity of linear PFOA, which is now in use, with that of the 80% linear 20% branched chain PFOA formerly used in commercial products, and a 100% branched form synthesised for the purposes of this study. Groups of rats and mice were given the different preparations by intubation at PFOA doses of 0, 0.3, 1, 3, 10 or 30 mg/kg bw per day for 14

days. In rats the LOAEL was 1 mg/kg bw per day for linear/branched PFOA and 0.3 mg/kg bw per day for linear PFOA, based on reductions in total cholesterol and triglycerides. In mice, the LOAEL was 0.3 mg/kg bw per day for all of the PFOA materials, based on liver weight, peroxisomal β-oxidation (and increased triglycerides for the linear/branched material). These LOAEL doses corresponded to serum PFOA levels of 20-51 µg/mL in rats and 10-14 µg/mL in mice. The authors concluded that the toxicity profiles were similar, but the branched form of PFOA appears to be less potent.

Sibinski (1987) conducted a 104-week chronic toxicity/carcinogenicity study in which groups of 50 male and 50 female Sprague-Dawley (Crl: CDBR) rats were fed diets containing 0, 30 or 300 mg/kg APFO for two years, equal to mean doses of 0, 1.3 and 14.2 mg/kg bw per day for males and 0, 1.6 and 16.1 mg/kg bw per day for females, respectively. There was a dose-related decrease in bodyweight gains in the male rats and to a lesser extent in the female rats compared to the controls; the decreases were statistically significant in the high-dose group of both sexes. The only clinical sign observed was a dose-related increase in ataxia in the female rats, most commonly associated with moribund animals. No significant differences were noted in survival, urinalysis or ophthalmoscopic findings. Significant non-neoplastic findings included, slightly decreased RBC, haemoglobin and haematocrit values in males (300 mg/kg), increased WBC in males (30 mg/kg), elevated serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP) and creatinine phosphokinase in males (30 mg/kg), liver masses and nodules (300 mg/kg), Leydig cell masses in males (300 mg/kg), mammary tissue masses in females (30 mg/kg), increased kidney weight in females (300 mg/kg), diffuse hepatomegalocytosis, hepatocellular necrosis, portal mononuclear cell infiltration and hepatic cystoid degeneration (300 mg/kg), tubular hyperplasia of ovarian stroma (30 mg/kg). The biological significance of the ovarian lesions was questioned by the authors on the basis of the lack of evidence of progression to tumours. Moreover, based on a re-evaluation of the slides by Mann and Frame (2004), the ovarian lesions were diagnosed and graded as gonadal stromal hyperplasia and/or adenomas, with particular emphasis placed on the proliferative effects. A NOAEL of 1.3 mg/kg bw per day was established for males on the basis of increases in liver weight and hepatic changes. In females, on the basis of reduced body weight gain and haematological changes, a NOAEL of 1.6 mg/kg bw per day was established."

US EPA (2014a) further included additional studies (preferably mouse studies) in their assessment. An overall view of the data provided by US EPA (2014a) and EFSA (2008) is given in the summary table (Table 4-1) below.

TABLE 4-1 SUMMARY OF THE REPEATED TOXICITY STUDIES ON PFOA

Animal	Study length	Dose	Target organ	N(L)OAELs	Reference (Evaluated by US EPA */ EFSA **)
Rats	1, 3 or 7 days	50 mg/kg	Liver	NOAEL not determined	Pastoor et al. (1987) (*/**)
Rats	28 days	0,5 or 20 mg/kg/day	Liver and lung	LOAEL: 5 mg/kg/day NOAEL: not established	Cui et al. (2009) (*/-)
Rats	28 days	0 or 300 pm (mean daily intake for study 1 and	Liver, lipid metabo-lism	NOAEL not determined	Elcombe et al.(2010) (*/-)

Animal	Study length	Dose	Target organ	N(L)OAELs	Reference (Evaluated by US EPA */ EFSA **)
		study 2 were 19 and 23 mg/kg/d, respectively)			
Rats	29 days	0, 0.3, 1, 10, or 30 mg/kg	Liver and lipid metabolism	NOAEL not determined	Loveless et al. (2008) (*/-)
Rats	90 days	0, 10, 30, 100, 300, and 1000 ppm	Liver	LOAEL: 30 ppm (1.72 mg/kg/day, male), 1000 ppm (76.5 mg/kg/day, female) NOAEL: 10 ppm (0.56 mg/kg/day, male), 300 ppm (22.4 mg/kg/day, female)	Goldenthal (1978a) (*/**)
Rats	13 weeks	1, 10, 30, or 100 ppm (0.06, 0.64, 1.94, and 6.50 mg/kg-day)	Liver	LOAEL: 10 ppm (0.64 mg/kg/day) NOAEL: 1 ppm (0.06 mg/kg/day)	Palazzolo (1993) and Perkins et al. (2004) (*/**)
Rats	2 years	0, 30 or 300 ppm PFOA (0, 1.3, and 14.2 mg/kg-day for males; 0, 1.6, and 16.1 mg/kg-day for females**)**	Liver, testes and lung. Hematologic effects	LOAEL male: 14.2 mg/kg bw/day LOAEL female: 16.1 mg/kg bw/day NOAEL male: 1.3 mg/kg bw/day NOAEL female: 1.6 mg/kg nw/day	Butenhoff et al. (2012) (*/-) same study as Sibinski (1987) (-/**)
Rats	2 years	0 or 300 ppm PFOA in male rats only (0, and 13.6 mg/kg/day)	Liver, testes, pancreas	LOAEL male: 13.6 mg/kg bw/day	Biegel et al. (2001) (*/**)
Mice	7 days	0, 1, (3), or 10 mg/kg	Liver	LOAEL: 1 mg/kg/day NOAEL: not established	Wolf et al. (2008a) (*/-)
Mice	2 weeks	0, 0.1, or 0.3 mg/kg/day	Liver, lipid metaboli	LOAEL: 0.3 mg/kg (mPPARα mice)	Nakamura et al. (2009) (*/-)

Animal	Study length	Dose	Target organ	N(L)OAELs	Reference (Evaluated by US EPA */ EFSA **)
			sm	NOAEL: 0.1 mg/kg (mPPARα mice), 0.3 mg/kg (PPARα-null mice and hPPARα mice)	
Mice	15 days	0, 3.75, 7.5, 15, or 30 mg/kg/day Or 0, 0.94, 1.88, 3.75, or 7.5 mg/kg/day	Liver Thymus and sleep IgM aand IgG response	Immunological LOAEL 3.75 mg/kg bw/day Immunological NOAEL 1.88 mg/kg bw/day LOAEL (increase liver weight): 0.94 mg/kg	DeWitt et al. (2008) (*/-)
Mice	21 days	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, or 30 ppm	Liver	LOAEL: 3 ppm (0.5 mg/kg/day) NOAEL: 1 ppm (0.2 mg/kg /day)	Kennedy (1987) (*/-)
Mice	21 days	0, 0.49, 2.64, 17.63, or 47.21 mg/kg	Liver	LOAEL: 0.49 mg/kg/day NOAEL: not established	Son et al. (2008) (*/-)
Mice	29 days	0, 0.3, 1, 10, or 30 mg/kg	Liver, lipid metaboli sm	NOAEL not determined	Loveless et al. (2008) (*/-)
Mice	4 weeks	, 5.4, 10.8, or 21.6 mg/kg	Liver	LOAEL: 5.4 mg/kg (male wild-type and PPARα-null mice) NOAEL: not established	Minata et al. (2010) (*/-)
Mice	6 weeks	0, 1, or 5 mg/kg/d	Testes	NOAEL not determined	Li et al. (2011) (*/-)
Monkeys	4 weeks	0, 2 or 20 m/kg day	No significan t	NOAEL: 20 mg/kg	Thomford (2001a) (*/-)
Monkeys	90 days	0, 3, 10, 30 or 100 mg/kg-day	Pituitary weight, heart and brain weight	LOAEL: 3 mg/kg day (male), 10 mg/kg day (female) NOAEL: not established (male), 3 mg/kg (female)	Goldenthal (1978b) (*/**)

Animal	Study length	Dose	Target organ	N(L)OAELs	Reference (Evaluated by US EPA */ EFSA **)
Monkeys	6 months	0, 3, 10 or 30 mg/kg b.w	Liver	LOAEL: 3 mg/kg day (male) NOAEL: not determined	Butenhoff et al. (2002) (*/**)

As seen from the table, studies with durations of 90 days or more have only been conducted with rats and monkeys, whereas the longest study duration in mice was 6 weeks.

Overall, these studies show that PFOA affects primarily the liver and the lipid metabolism. From these studies the lowest identified **LOAEL and NOAEL in rats** were 10 ppm **(0.64 mg/kg/day)** and 1 ppm (**0.06 mg/kg/day**) (Palazollo, 1993). **In mice** the lowest **LOAEL and NOAEL** were found to **0.3 mg/kg/day and 0.1 mg/kg/day** (Nakumaru et al., 2009), and in **monkeys a LOAEL of 3 mg/kg /day** was found (Goldenthal, 1978b and Butenhoff et al., 2002).

Identification of most critical studies
EFSA (2008) in their hazard characterisation concluded liver toxicity as the critical effect, and an overall **BMDL10 of 0.3 mg/kg bw/day** was set as the point of departure for the TDI derivation. The BMDL10 value was derived on the analysis made by COT (2006a) where BMDL10 levels for increased liver weight and liver toxicity were estimated from the studies by Palazzolo (1993); Perkins et al. (2004); Sibinski (1987), and from the reproduction toxicity studies by Butenhoff et al.(2004) and Lau et al. (2006)(see section 4.5.1).

Of the repeated dose toxicity studies, US EPA (2014a) identified the studies by Palazzolo (1993); Loveless (2008 rats and mice); DeWitt et al. (2008) and Thomford (2001a) as the most appropriate for RfD calculations.

4.4.2 PFOS

Below the repeated dose toxicity studies as described and assessed by EFSA (2008) are given:

"In a 90 day subchronic study groups of CD rats (5/sex/group) received PFOS at 0, 30, 100, 300, 1000 or 3000 mg/kg in the diet, equivalent to 0, 2, 6, 18, 60 and 200 mg/kg bw per day (Goldenthal et al., 1978a). At the 100 mg/kg level and above, body weight means and food consumption were lower than in controls and all rats died before the termination of the study. Slight increases in creatinine phosphokinase and alkaline phosphatase, slight to moderate increases in blood glucose and blood urea nitrogen, and slight to marked increases in plasma glutamic oxalacetic transaminase (PGOT) and glutamic pyruvic transaminase (PGPT) activities were seen after one month of the study. At the end of the study, slight to moderate decreases in haemoglobin, haematocrit and erythrocyte counts were seen in male and female rats, and slight to moderate increases in PGOT and PGPT in two out of three surviving female rats. All treated rats showed centrilobular to midzonal hypertrophy of hepatocytes and focal necrosis of the liver at the 300 mg/kg level and above, multiple changes in various organs were observed microscopically.

Seacat et al. (2003), administered PFOS in the diet of Sprague Dawley rats at doses of 0, 0.5, 2.0, 5.0, and 20 mg/kg equivalent to 0, 0.05, 0.2, 0.4 and 1.5 mg/kg b.w. per day for 4 or 14 weeks. After 4 weeks, increases in relative liver weight and blood glucose were found in males at the highest level in the diet. In females no consistent and significant changes were found after 4 weeks. After 14 weeks increases in absolute and relative liver weight, increased numbers of segmented

neutrophils in peripheral blood, decreased blood cholesterol, and increased serum alanine aminotransferase and urea nitrogen were observed in males at the highest dose level. In females, relative liver weight and blood urea nitrogen were increased at the highest dose level. Hepatic hypertrophy and cytoplasmic vacuolisation were observed after 14 weeks in the 5 and 20 mg/kg groups in males and in the 20 mg/kg group in females. The highest mean dose level of 1.5 mg/kg b.w. per day was considered as the lowest- observed-adverse-effect-level (LOAEL). The average hepatocyte proliferation index was not significantly increased and there was no effect on palmitoyl CoA oxidase, a marker of peroxisome proliferation. Serum and liver PFOS concentrations were proportional to dose and cumulative dose. Serum concentrations were generally higher in females than in males. The mean dose of 0.4 mg/kg b.w. per day was considered as the no-observed-adverse-effect (NOAEL) in this study. After 14 weeks of PFOS administration at this dose the PFOS serum concentration was 44 μg/mL in male rats and 64 μg/mL in female rats (Thomford, 2002; Seacat et al., 2003).

In a gavage study with rhesus monkeys, 2/sex/group, doses of 0, 10, 30, 100 or 300 mg/kg/day PFOS, all animals died within 20 days (Goldenthal et al., 1979).

In a subsequent study, rhesus monkeys (2/sex/group) were administered 0, 0.5, 1.5 or 4.5 mg/kg/day by gavage for 90 days (Goldenthal et al., 1978b). The animals survived in the 0.5 and 1.5 mg/kg/day group, whereas those in the top dose group died or were sacrificed in extremis between weeks 5 and 7. At 1.5 mg/kg/day, the animals occasionally exhibited signs of gastrointestinal tract toxicity such as black stools, diarrhoea, mucous in the stools and bloody stool and exhibited dehydration or general body trembling at the end of study. Furthermore, serum alkaline phosphatase activity and the concentration of inorganic phosphate in the serum were decreased. At 0.5 mg/kg/day the animals occasionally exhibited soft stools, diarrhoea, anorexia and emesis. A slight decrease in the serum alkaline phosphatase activity was noted at the end of the study.

In a study in which male and female Cynomolgus monkeys (4 to 6 animals per group) received 0, 0.03, 0.15, or 0.75 mg/kg bw per day potassium PFOS by oral intubation for 183 days, compound-related mortality occurred in 2 of 6 male monkeys in the 0.75 mg/kg bw per day dose group. The remaining animals showed decreased body weights, increased liver weights, lowered serum total cholesterol and high-density lipoproteins (HDL), increased TSH levels, lowered triiodothyronine (T3) concentrations, and lowered estradiol levels (male animals). At various time points following treatment at the lowest dose level of 0.03 mg/kg, cholesterol levels were statistically significantly decreased compared to controls in male and female monkeys, and HDL levels were decreased in male monkeys, with no clear dose or time relationship. At 0.15 mg/kg bw per day the following changes were observed: lowered levels of HDL (female animals), increased levels of TSH (male animals) and lowered triiodothyronine concentrations (male and female animals). The thyroid hormone levels of some of the serum samples taken at the end of the study were subsequently reanalysed in an independent laboratory, and were not statistically significantly different from control. Serum PFOS concentrations (mean + SD) measured at termination of the treatment, were 82.6 + 25.2 mg/L for males and 66.8 + 10.8 mg/L for females, at the dose level of 0.15 mg/kg bw per day and 15.8 + 1.4 and 13.2 + 1.4 mg/L at 0.03 mg/kg bw per day, respectively (Seacat et al., 2002). Complete reversal of clinical and hepatic effects and significant decreases in serum and liver PFOS occurred within 211 days post treatment. Seacat et al. (2002) concluded that the NOAEL in this study was 0.15 mg/kg bw per day. However, the Panel considered that the changes in thyroid hormones and in HDL observed at this dose level were treatment- related and therefore concluded that it was justified to consider 0.03 mg/kg bw per day as a NOAEL.

In summary these studies showed PFOS affected primarily the liver and biochemical parameters associated with lipid metabolism. Increased liver weight and vacuolisation and hypertrophy of hepatic cells were observed in the animal species tested (rat and monkey). PFOS also reduced body weight, serum cholesterol, serum triglycerides, and triiodothyronine levels.

Changes in thyroid hormones have been observed, although the underlying mechanisms are not understood. A steep dose response curve was observed in the Cynomolgus monkey since the dose range between no observed adverse effects and treatment related death was narrow. Monkeys died at doses of a few mg/kg per day. Rats were less sensitive than monkeys. Male rats appear to be more sensitive than female rats.

A chronic toxicity and carcinogenicity study of PFOS potassium salt was carried out in rats in compliance with Good Laboratory Practice (GLP) (Thomford, 2002). Groups of 40-70 male and female Crl:CD(SD)IGS BR rats were given PFOS at doses of 0.5, 2, 5 or 20 mg/kg in the diet, corresponding to mean achieved doses of 0.04, 0.14, 0.36 and 1.42 mg/kg bw per day in males and 0.035, 0.14, 0.37 and 1.49 mg/kg b.w. per day in females. An additional (recovery) group received the top dose of PFOS for 52 weeks followed by control diet for 52 weeks. There was a significant trend for increased survival in males, due to significant increases in the 5 mg/kg and high dose group (20 mg/kg), compared to the controls. No significant trend was noted in survival for females, although there was a statistically significant decrease at 2 mg/kg. Hepatotoxicity, characterized by significant increases in centrilobular hypertrophy, centrilobular eosinophilic hepatocytic granules, centrilobular hepatocytic pigment, or centrilobular hepatocytic vacuolation was noted in male or female rats given 5 or 20 mg/kg. A significant increase in hepatocellular centrilobular hypertrophy was also observed in the male rats receiving 2 mg/kg PFOS. Electron microscopy was conducted on livers from a subset of animals administered 0 and 20 mg/kg PFOS in the diet. PFOS treatment resulted in mild to moderate smooth endoplasmic reticulum hyperplasia and minimal to mild hepatocellular hypertrophy, but not in peroxisomal proliferation.
Based on histopathological findings in the liver, the no-observed-adverse-effect level (NOAEL) for PFOS is considered to be 2 mg/kg in the diet (0.14 mg/kg bw per day) in male and female rats."

US EPA (2014b) further included additional key studies (four 28 days studies and a 60 day study) in their assessment. An overall view of the data provided by US EPA (2014b) and EFSA (2008) is given in the summary table (Table 4.2) below.

TABLE 4-2 SUMMARY OF THE REPEATED TOXICITY STUDIES ON PFOS

Animal	Study length	Dose	Target organ	N(L)OAELs	Reference (Evaluated by US EPA */ EFSA **)
Rats	28 days	0, 2, 20, 50 or 100 mg PFOS/kg diet	Liver T4 and T3 levels decreased	Not determined	Curren et al. (2008) (*/-)
Rats	28 days	5 or 20 mg/kg/day	Liver Lungs Kidney	LOAEL: 5 mg/kg/day NOAEL: not identified	Cui et al. (2009) (*/-)
Rats	90 days	0, 2, 6, 18, 60 and 200 mg/kg/day	Liver Kidney	LOAEL: 2 mg/kg/day NOAEL: not identified	Goldenthal (1978b) (*/**)
Rats	4 or 14 weeks	0, 0.5, 2.0, 5.0 or 20 ppm	Liver	LOAEL: 20 ppm (1.33 mg/kg in males and 1.56 mg/kg in females)	Seacat et al. (2003) (*/**)

Animal	Study length	Dose	Target organ	N(L)OAELs	Reference (Evaluated by US EPA */ EFSA **)
				NOAEL: 5 ppm (0.34 mg/kg in males and 0.40 mg/kg in females)	
Rats	2 years	0.5, 2, 5 or 20 mg/kg in the diet	Liver	NOAEL: 2 ppm (0.14 mg/kg bw/day) (EFSA, 2008) LOAEL for male rats was 2 ppm (0.072 mg/kg) and for female rats was 5 ppm (0.247 mg/kg). The NOAEL for the males was 0.5 ppm (0.018 mg/kg) and 2 ppm (0.099 mg/kg) for females (US EPA, 2014b)	Thomford. (2002) (*/**)
Mice	4 weeks	3 mg/kg/day	Lipid metaboli sm in liver	Not determined	Bijand et al. (2011) (*/-)
Mice	4 weeks	0, 0.00017, 0.0017, 0.0033, 0.017, 0.033, and 0.166 mg/kg/day	IgM Suppress.	NOAEL (male): 0.00017 mg/kg/day NOAEL(female): 0.0033 mg/kg/day	Peden-Adams et al. (2008) (*/-)
Mice	60 days	0, 0.0083, 0.083, 0.42, 0.83, 2.08 Mg/kg/day	Immuno-tox Killer cell act.	NOAEL 0.008 LOAEL 0.083	Dong et al. (2009) (*/-)
Monkeys	20 days (study terminated)	0, 10, 30, 100 or 300 mg/kg/day	Liver	Not determined	Goldenthal et al., 1978a and 1979 (*/**)
Monkeys	90 days	0, 0.5, 1.5 or 4.5 mg/kg/day	Lipid metaboli sm	LOAEL: 0.5 mg/kg/day NOAEL: not identified	Goldenthal et al., 1978a and 1979 (*/**)
Monkeys	26 weeks	0, 0.03, 0.15 or 0.75 mg/kg/day	Liver Lipid metaboli	LOAEL: 0.75 mg/kg/day NOAEL: 0.15	Seacat et al. (2002) (*/**)

Animal	Study length	Dose	Target organ	N(L)OAELs	Reference (Evaluated by US EPA */ EFSA **)
		of potassium PFOS	sm	mg/kg/day (EFSA, 2008 : NOAEL of 0.03 mg/kg/day)	

From the table it can be seen that the lowest NOAELs were observed in the Pede-Adams et al. (2008) study with regard to immunotoxicity. However, US EPA (2014b) did not find support from other studies on immunotixicity for effects at these low levels, and therefore these findings would need further documentation.

When US EPA (2014b) evaluated the study by Thomford (2002), they came to a different conclusion than EFSA (2008) regarding the N(L)OEL levels.
US EPA (2014b) indicated that the doses used in the study were equivalent to approximately 0, 0.018, 0.072, 0.184 and 0.765 mg/kg/day for males and 0, 0.023, 0.099, 0.247, and 1.10 mg/kg/day for females (the reason for different conversions from ppm content in food to dose in mg/kg/day between US EPA (2014b) and EFSA (2008) is not transparent from the documents). Then, based on **non-neoplastic lesions in the liver**, a **LOAEL for male rats was found at 2 ppm (0.072 mg/kg) and for female rats at 5 ppm (0.247 mg/kg). For males the NOAEL was found at 0.5 ppm (0.018 mg/kg) and for females at 2 ppm (0.099 mg/kg)** (US EPA, 2014b). Thus, US EPA (2014b) concluded on a NOAEL 8 times lower that the NOAEL concluded by EFSA (2008).

With respect to the study by Seacat et al. (2002), US EPA (2014b) concluded on a LOAEL in male and female monkeys of 0.75 mg/kg bw/day and a NOAEL of 0.15 mg/kg bw/day. However, here EFSA (2008) used a more cautious approach as changes in plasmalipids and hornones occurred at this level and they therefore concluded on a NOAEL of 0.03 mg/kg bw/day.

Overall, the repeated dose toxicity studies showed that PFOS affects primarily the liver and biochemical parameters associated with lipid metabolism. Increased liver weight and vacuolisation and hypertrophy of hepatic cells were observed in rat and monkey. Male rats appear to be more sensitive than female rats. PFOS also resulted in reduced body weights, serum cholesterol, serum triglycerides, and triiodothyronine levels. Changes in thyroid hormones were also observed, however, the underlying mechanisms are not understood.
In rats the lowest LOAEL and NOAEL were found to 0.018 mg/kg /day and 0.018 mg/kg/day based on the Thomford et al. (2002) study and according to the interpretation by US EPA (2014). In monkeys a NOAEL of 0.03 mg/kg/day and a LOAEL of 0.15 mg/kg/day were determined by EFSA (2008) based on the data from Seacat et al. (2002).

It must also be noted that a steep dose effect curve was observed in the Cynomolgus monkey since the dose range between no observed adverse effects and treatment related deaths was narrow as monkeys died at doses of a few mg/kg per day.

Identification of most critical studies
EFSA (2008) identified the **Seacat et al. (2002)** study with a NOAEL of 0.03 mg/kg bw/day as the most critical study for derivation of a TDI value.

US EPA (2014b) considered the studies by **Seacat et al. (2002); Seacat et al. (2003); and Thomford (2002)** to be the most appropriate for RfD calculations.

4.5 Toxicity to reproduction

4.5.1 PFOA

Below, the reproduction and developmental studies as described and assessed by EFSA (2008) are given:

Rats

Developmental toxicity studies of PFOA in rats have been conducted and doses up to 100–150 mg/kg bw per day showed no significant effects (Gortner, 1981; 1982 and review Lau et al., 2004). Also no teratogenicity has been found in rats after administration of PFOA by inhalation (0, 0.1, 1, 10, and 25 mg/m^3) or in the diet (100 mg/kg bw per day) between day 6 and 15 of pregnancy (Staples and Burgess, 1984). In rats, the NOAEL for maternal toxicity and developmental toxicity were 5 and 150 mg/kg bw per day, respectively (EFSA 2008) (with no further details).

Hinderliter and co-workers (2005) showed that after oral application to rats, PFOA is transferred from the dam to the foetus via the placenta and to the pup by lactation. Concentrations in foetal plasma were half the steady-state concentrations in maternal plasma, while steady state concentrations in milk were approximately one tenth less than those in maternal plasma (EFSA, 2008).

In a two generation reproduction study, rats were given PFOA 1, 3, 10 or 30 mg/kg bw per day by oral gavage (Butenhoff et al., 2004). Male rats in the parental and F1 generations administered 3, 10 and 30 mg/kg bw per day showed decreased body weights. Liver and kidney weights increased in all treatment groups. The F1 generation at 30 mg/kg bw per day showed reduced birth weights, increased post weaning mortality and delayed pubertal onset. No effects were observed on mating or fertility parameters. From this study the NOAELs were 30 mg/kg bw for reproductive function, 10 mg/kg bw for sexual maturation, and < 1 mg/kg bw for body weight and increased liver weight (EFSA, 2008).

Mice

A study by Lau et al. (2006) revealed dose dependent growth deficits in the litters of CD-1 mice treated daily during pregnancy from day 1 until birth by oral gavage (1, 3, 5, 10, 20, 40 mg/kg bw per day). PFOA induced enlarged liver in treated dams at all dosages, but did not alter the number of implantations or malformations. The 40 mg/kg bw per day group resorbed their litters, the 20 mg/kg bw per day group had a reduced percentage of live foetuses and their weights were significantly lower. Post natal survival was significantly reduced in the 5, 10 and 20 mg/kg bw per day group. Dose dependent growth deficits were noted in all dose groups except in the 1 mg/kg bw per day dose group. Significant delays in eye opening were noted at 5 mg/kg bw per day and at higher dosages but not in the 1 mg/kg bw per day dose group. Accelerated sexual maturation was observed in male offspring but not in females (EFSA, 2008).

Wolf et al. (2007) investigated the critical windows of PFOA exposure in mice together with the relationship between lactational exposure and neonatal viability. Administration of PFOA at oral doses of 3 to 20 mg/kg bw per day resulted in increased maternal liver weight and deficits in postnatal weight gain of the pups. Pups of dams dosed on GD 7-17, and 10-17 also showed developmental delay in eye opening and hair growth. Cross-fostering studies showed that the effects were due to in utero rather than lactational exposure. A NOAEL was not identified (EFSA, 2008).

Another study by White et al. (2007) was carried out in pregnant mice to determine whether PFOA effects were linked to gestational time of exposure or to subsequent lactational changes. The study used an oral dose of 5 mg/kg bw per day PFOA at GD 1–17, 8–17, 12–17, or a vehicle on GD 1–17, Overall, mean pup bodyweights on postnatal day (PND) 1 in all PFOA-exposed groups were significantly reduced and these effects persisted until weaning. Mammary gland differentiation was also affected among dams exposed GD 1–17 or 8–17 on PND 10 and normal epithelial involution and alterations in milk protein gene expression were observed on PND 20. Overall, these findings suggest that in addition to gestational exposure, abnormal lactational development of dams may play a role in the early growth retardation of developmentally exposed offspring (EFSA, 2008).

Abbott et al., (2007) investigated the involvement of PPARα in PFOA-induced developmental toxicity using wild type (WT) and PPARα knock out (KO) pregnant mice dosed orally with PFOA at 0.1, 0.3, 0.6, 1, 3, 5, 10 or 20 mg/kg bw per day on GD 1-17. PFOA did not affect maternal weight, embryonic implantation, or number or weight of pups at birth. At 5 mg/kg bw, the incidence of full litter resorptions increased in both WT and KO mic. At 1 mg/kg bw per day, pup weights were significantly lower than control at some time points in WT, but not in KO mice. In WT, but not KO, a reduction in neonatal survival was observed at 0.6 mg/kg bw per day giving a NOAEL of 0.3 mg/kg bw per day. Eye opening was delayed at 1 mg/kg bw per day in WT mice. The authors concluded that early pregnancy loss was independent of PPARα expression whereas PPARα appeared to have a role in delayed eye opening and deficits in postnatal weight gain, although other mechanisms may also contribute (EFSA, 2008).

Rabbit
In rabbit doses up to 50 mg/kg bw per day for rabbits showed no significant effects in a teratoplogical study (review by Lau et al., 2004; EFSA, 2008) (no further details given.

Overview
US EPA (2014b) further included additional studies (preferably developmental mice studies). An overall view of the data provided by US EPA (2014a) and EFSA (2008) is given in the summary table (Table 4-3) below.

TABLE 4-3. OVERVIEW OF REPRODUCTION TOXICITY STUDIES, PFOA

Animal	Study length	Dose and route	N(L)OAELs	Reference (Evaluated by US EPA */ EFSA **)
Rats	10 weeks for F0 and F1	0, 1, 3, 10, and 30 mg/kg-day	LOAEL for F0 parental males: 1 mg/kg NOAEL for F0 parent females: 30 mg/kg LOAEL for developmental/reproductive toxicity F1 males: 10 mg/kg, NOAEL: 3 mg/kg LOAEL for adult systemic toxicity in the F1 males is 1 mg/kg-day (liver and kidney) LOAEL for developmental/reproductive toxicity was considered to be 30 mg/kg-day, NOAEL was 10 mg/kg-day. NOAEL and LOAEL for adult systemic	York (2002) (*/-) Butenhoff et al.(2004a) (*/**) York et al. (2010) (*/-)

Animal	Study length	Dose and route	N(L)OAELs	Reference (Evaluated by US EPA */ EFSA **)
			toxicity in F1 females are 10 and 30 mg/kg-day, respectively NOAEL for developmental/reproductive toxicity in the F2 offspring was 30 mg/kg-day	
Rats	GD 4-10, 4-15 or 4-21, or from gestation day 4 to lactation day 21	0, 3, 10 or 30 mg/kg-day Oral gavage	Maternal LOAEL was 30 mg/kg-day, the NOAEL was 10 mg/kg-day. Developmental NOAEL was 30 mg/kg-day	Hinderliter et al. (2005) (*/**) Mylchreest (2003) (*/-)
Rats	GD 6-15	0, 0.1, 1, or 10 (or 25)mg/m3	NOAEL and LOAEL for maternal toxicity of 1 and 10 mg/m3, respectively, NOAEL and LOAEL for developmental toxicity of 10 and 25 mg/m3	Staples et al. (1984) (*/**)
Mice	Day 1 until birth	1, 3, 5, 10, 20, 40 mg/kg b.w. per day oral gavage	Developmental LOAEL: 1 mg/kg (skeletal defects) Developmental female: LOAEL: 3 mg/kg, NOAEL was 1 mg/kg BMD5 and BMDL5 for maternal liver weight at term: 0.20 mg/kg and 0.17 mg/kg, respectively	Lau et al. (2006) (*/**)
Mice	GD7-17, 10-17, 13-17, 15-17.	0 or 5 mg/kg, or 20 mg/kg (only on GD 15-17) Oral	No NOAEL established	Wolf et al. (2007) (*/**)
Mice	GD1-17	0,3, or 5 mg/kg Oral	LOAEL for dams: 3 mg/kg LOAEL for pups: 5 mg/kg No NOAEL was established	Wolf et al. (2007) (*/**) White et al. (2009) (*/-)
Mice	GD1-17	0.1, 0.3, 0.6, 1, 3, 5, 10 or 20 mg/kg b.w oral	maternal/reproductive LOAEL for WT mice was 0.6 mg/kg/day NOAEL was 0.3 mg/kg/day Developmental LOAEL for WT offspring	Abbott et al. (2007) (*/**)

Animal	Study length	Dose and route	N(L)OAELs	Reference (Evaluated by US EPA */ EFSA **)
			was 0.1 mg/kg/day, NOAEL was not established Maternal LOAEL for PPARα-null mice was 3 mg/kg/day, NOAEL was 1 mg/kg/day Developmental LOAEL for PPARα-null offspring was 3 mg/kg/day, NOAEL was 1 mg/kg/day	
Mice	GD1-17	0, or 3 mg/kg Oral gavage	No NOAEL established	Albrecht et al.(2013) (*/-)
Mice	GD0-17 or 18	0, 1, 5, or 10 mg/kg Oral gavage	maternal LOAEL was 1 mg/kg developmental LOAEL was 5 mg/kg, NOAEL was 1 mg/kg	Yahia et al. (2010) (*/-)
Mice	GD11 to GD16	0, 2, 10, or 25 mg/kg	No NOAEL established	Suh et al. (2011) (*/-)
Mice	GD10-17	0, 0.01, 0.1, or 1.0 mg/kg gavage	LOAEL was 0.01 mg PFOA/kg based on delayed mammary gland development	Macon et al. (2011) (*/-)
Mice	GD1-17	0, 1, or 5 mg PFOA/kg 0 or 1 mg PFOA/kg received drinking water containing 5 ppb PFOA beginning on GD7	No NOAEL was established.	White et al. (2011) (*/-)
Mice	4 weeks Peripubertal exposure	0, 1, 5, or 10 mg/kg gavage	LOAEL was 1 mg/kg/day	Yang et al. (2009) (*/-)

Overall, from the developmental and reproductive toxicity studies described above, it can be concluded that rats are less sensitive than mice to PFOA. The observed effects in the studies presented above are increased liver and kidney weight in dams, growth deficits in the litter including delays in eye opening, delayed in growth on body hair, increased skeletal defect, delayed phalange ossification, delayed eruption of incisors, increased incidence of cleft sternum, delayed

vaginal opening and decreased uterine weight, and altered mammary gland development The lowest LOAEL reported regarding maternal toxicity is the LOAEL of 0.01 mg PFOA/kg based on delayed mammary gland development in mice from the Macon et al. (2011) study, however the relevance of this finding to humans is still unclear. The lowest LOAEL reported regarding developmental toxicity is the LOAEL for wild type offspring of 0.1 mg/kg/day based on increased liver weight from the Abbott et al, (2007) study, whereas a NOAEL of 1 mg/kg/day was found for PPARα-null mice.
In rats a LOAEL for parental males of 1 mg/kg/d was observed for increased liver and kidney weight and for developmental effects a NOAEL of 3 mg/kg/d and a LOAEL of 10 mg/kg/day was observed for decreased body weight of males during the lactation period.

Based on these findings PFOA has been classified as Repr. 1B, H360D (May damage the unborn child) and Lact., H362 (May cause harm to breast-fed children).

Identification of most critical studies
As indicated in section 3.4.1 on repeated dose toxicity, the liver effects are also considered as the most susceptible effect parameter in the reproductive toxicity studies by EFSA (2008). The data from the studies by Butenhoff et al. (2004) and Lau et al. (2006) were by EFSA (2008) together with the repeated dose toxicity studies by Palazzolo (1993); Perkins et al. (2004) and Sibinski (1987) used for deriving an overall **BMDL10 of 0.3 mg/kg bw/day** in relation to liver toxicity.

As indicated in section 3.4.1 on repeated dose toxicity, the liver effects are also considered the most consistent and susceptible effect parameter in the reproductive toxicity studies by US EPA (2014a). However, US EPA (2014a) noted that in the studies by York et al. (2002) (referring to the same data as Butenhoff et al. (2004)) and Lau et al. (2006), parental kidney toxicity and developmental toxicity, respectively, were seen at the LOAEL for liver toxicity. Thus US EPA included these studies together with the repeated dose toxicity studies by Palazzolo (1993); Loveless (2008 rats and mice); DeWitt et al. (2008) and Thomford (2001a) as the most appropriate for RfD calculations.

4.5.2 PFOS
Below the reproduction and developmental studies as described and assessed by EFSA (2008) are given:

Rats
Administration of PFOS by gavage to groups of 22 pregnant rats during GD 6-15 at doses of 0, 1, 5 and 10 mg/kg bw per day (Gortner, 1980) resulted in maternal toxicity (decreased body weight) with a NOAEL of 5 mg/kg bw per day and a LOAEL of 10 mg/kg bw per day while in all dose groups the most notable signs of developmental toxicity were abnormalities of the lens of the eye, the incidence of which was significantly greater than control only in the top dose group (10 mg/kg bw per day. In a similar study, gavage administration of PFOS to pregnant rats between GD 6 and 15 resulted in maternal weight loss and developmental toxicity in the 5 and 10 mg/kg bw per day dose groups. Reduced birth weight as well as visceral anomalies, delayed ossification and skeletal variations were observed. A NOAEL of 1 mg/kg bw per day and a LOAEL of 5 mg/kg bw per day for maternal and developmental toxicity were indicated (Wetzel, 1983).

Studies with Sprague-Dawley rats in which PFOS was administered by gavage during pregnancy indicated that in utero exposure to PFOS severely compromised postnatal survival of neonatal rats, and caused delays in growth and development that were accompanied by hypothyroxinemia in the surviving rat pups. The rats received by gavage PFOS doses of 1, 2, 3, 5 and 10 mg/kg bw per day during GD2 to 21. Maternal weight gain was reduced in a dose-dependent manner, which was statistically significant compared to control at 2 mg/kg bw per day and above. There was a marked reduction in maternal serum T4 and T3 in all dose groups from GD7. At 10 mg/kg bw per day, there was a reduction in foetal body weight and an increase in cleft palate and anasarca and all pups died

within 4-6 hours after birth. In the 5 mg/kg bw per day group, 95% of the pups died within 24 hours, approximately 50% of the offspring died after 3 mg/kg bw per day in rats. The maternal dose corresponding to the BMDL5 (lower limit of the 95% confidence interval on the benchmark dose for a 5% increase in response above background incidence) for survival of rat pups at postnatal day 8 was estimated at 0.58 mg/kg (Lau et al., 2003).

Post-natal growth rate and the average age at eye opening were significantly delayed at 2 mg/kg bw per day and above. PFOS-exposed neonates showed reductions of T4 at all dose groups, but not T3 or TSH. Cross-fostering the PFOS-exposed rat neonates (5 mg/kg) to control nursing dams failed to improve survival (Thibodeaux et al., 2003; Lau et al., 2003). Changes in thyroid hormones, observed after exposure of pregnant rats to PFOS may influence brain development and hence affect behaviour in the offspring. The ontogeny of neurochemical and neurobehavioral markers was evaluated after prenatal PFOS exposure (Lau et al., 2003). Prenatal exposure to PFOS did not affect learning and memory behaviours determined by T- maze delayed alternation. However marginal but statistically significant deficits in the developmental patterns of choline acetyltransferase activity (an enzyme marker sensitive to thyroid hormone status) were observed in rats with a LOAEL of 1 mg/kg bw per day.

Grasty et al., (2003) investigated the critical window for prenatal exposure to PFOS, by administering PFOS potassium salt to pregnant rats by gavage at 25 mg/kg bw on GD 2-5, 6-9, 10-13, 14-17 or 17-20, or at 25 or 50 mg/kg bw on GD 19-20. Neonatal rat mortality occurred after dosing in all time periods, but the incidence of neonatal death increased as the exposure period occurred later during gestation, reaching 100% in the treatment group of GD 17–20). Considering that PFOS-induced organ toxicity is incompatible with postnatal survival, the authors suggested that maturation of the lung and pulmonary function is a plausible target for PFOS. In a subsequent study, Grasty et al. (2005) found that the alveolar walls were thicker in PFOS-exposed newborn mice compared to controls, but the failure of rescue agents and the normal pulmonary surfactant profile indicated that this was not likely to be due to lung immaturity.

Luebker et al. (2005b) administered PFOS by gavage to female rats for 6 weeks prior to mating and through gestation to day 4 of lactation at doses of 0, 0.4, 0.8, 1.0, 1.2, 1.6 and 2.0 mg/kg bw per day. Statistically significant decreases in gestation length and pup viability were observed at 0.8 mg/kg bw per day and above. A range of BMDL5 values of 0.27 to 0.89 mg/kg bw per day was calculated for these effects.

A two generation study in rats (Christian et al., 1999), showed high sensitivity for PFOS. PFOS was administered by gavage at doses of 0, 0.1, 0.4, 1.6 and 3.2 mg/kg bw per day for 42 days before mating and in females also during pregnancy and lactation. Gestation length was significantly reduced in the high-dose group and there also was a significant reduction in the number of implantation sites followed by a concomitant reduction in litter size. Reduced survival was observed in F1 offspring at the highest doses of 1.6 and 3.2 mg/ kg per day (26% of the offspring died within 4 days after birth in the 1.6 mg/kg bw per day dose group). In the 3.2 mg/kg bw per day dose group 45% of the pups died within one day after birth and 100% died thereafter). Pup body weights were significantly reduced at the two highest dose groups. Transient delays in reflex and physical development were observed in the F1 generation offspring which raises concerns about possible neurotoxicity of PFOS. At post weaning days 1-8 animals showed significant reductions in absolute food consumption at the 0.1 and 0.4 mg/kg bw per day dose levels.

In the F2 generation of the group treated with 0.4 mg/kg bw per day birth weight was reduced (LOAEL). No other toxicological signs were reported in the F2 mice. Serum concentration in the group treated with 0.4 mg/kg bw per day (F0) at gestation day 21 was 26.2 mg/kg, in the foetuses it was 34.3 mg/kg (pooled liver and serum). The NOAEL was 0.1 mg/kg bw per day (Christian et al., 1999). A study in which neonates of treated mothers were suckled by untreated mothers showed

that in utero exposure was responsible for some of the effects in the offspring (Luebker et al., 2005a).

Mice
Mice received doses of 1, 5, 10, 15 and 20 mg/kg bw per day during GD1 to GD18. The survival of the lower dose groups (1 and 5 mg/kg) was not different from that of controls. A statistically significant trend in growth lags was detected in surviving mouse pups exposed to PFOS prenatally. Slight delays in eye opening were statistically significant at all doses and liver weight was significantly increased at 5 mg/kg bw per day and above. (Lau et al., 2003; Thibodeaux et al., 2003).

Rabbits
Case et al. (2001) administered PFOS to pregnant New Zealand white rabbits by gavage at 0, 0.1, 1.0, 2.5 and 3.75 mg/kg bw per day from GD 6-20. Reduced birth weight and delayed ossification of the offspring were reported at the two higher doses. The LOAELs and NOAELs were respectively 1 and 0.1 mg/kg bw per day for maternal toxicity (decreased weight gain); 2.5 and 1.0 mg/kg bw per day for foetal toxicity.

Endocrine effects/disruption
EFSA (2008) made the following considerations regarding mode of action in relation to reproductive and developmental toxicity:

In rats, oral administration of PFOS resulted in increased tissue availability of thyroid hormones and turnover of T4, but the pattern of changes seen was not typical of a hypothyroid state (Chang et al., 2007). Although reproductive and developmental toxicity have been described, the underlying mechanism remains unclear. Part of the toxicity may be related to changes in thyroid hormone levels which may affect early development. The extent to which changes in lipid metabolism, changes in transport of fatty acids or induction of metabolising liver enzymes contribute to the changes in hormone levels is currently unknown. It is noteworthy that opposite effects have been observed in experimental studies (lower levels of cholesterol and estradiol after PFOS exposure in rodents and monkeys, increased levels of estradiol in humans (increased levels of cholesterol and estradiol in male workers reported in OECD (2002). There is also evidence that PFOS has effects on membrane permeability (Jernbro et al., 2007) (EFSA, 2008).

A study in adult female rats that were injected intraperitoneally with 0, 1, or 10 mg PFOS/kg bw for 2 weeks showed that PFOS can cross the blood brain barrier and accumulated in the hypothalamus at the higher dose level (Austin et al., 2003). It increased norepinephrine concentrations in the para ventricular nucleus of the hypothalamus. Treatment with PFOS affected oestrous cyclicity and increased serum corticosterone levels while decreasing serum leptin concentrations (Austin et al., 2003). PFOS was shown to activate the stress axis while inhibiting the reproductive axis. Hypothalamic nor-epinephrine levels could play a role (EFSA, 2008).

Overview
US EPA (2014b) further included additional studies (rat and mice studies). An overall view of the data provided by US EPA (2014a) and EFSA (2008) is given in the summary table (Table 4-4) below.

TABLE 4-4 OVERVIEW OF REPRODUCTION TOXICITY STUDIES, PFOS

Animal	Exposure/ Study length	Dose and route	N(L)OAELs	Reference (Evaluated by US EPA */ EFSA **)
Rats	GD6-15	0, 1, 5 and 10 mg/kg bw per day gavage	NOAEL of 5 mg/kg bw per day LOAEL of 10 mg/kg bw per day	Gortner (1980) (*/**)
Rats	GD6-15	0, 1, 5 and 10 mg/kg bw per day gavage	NOAEL of 1 mg/kg bw per day and a LOAEL of 5 mg/kg bw per day for maternal and developmental toxicity	Wetzel (1983) (*/**)
Rats	GD 2-20	0, 1, 2, 3, 5 or 10 mg/kg gavage	Maternal weight reduction (polynomial model) BMD_5 = 0.22 mg/kg and $BMDL_5$ = 0.15 mg/kg T_4 effects on GD7 (Hill model) BMD_5 = 0.23 mg/kg and $BMDL_5$ = 0.05 mg/kg Developmental effects: LOAEL of 2 mg/kg NOAEL of 1 mg/kg. Foetal sternal defects (logistic model) BMD_5 = 0.31 mg/kg and $BMDL_5$ = 0.12 mg/kg Foetal cleft palate (logistic model) BMD_5 = 8.85 mg/kg and $BMDL_5$ = 3.33 mg/kg	Thibodeaux et al. (2003) (*/**)
Rats	GD2-21	0, 1, 2, 3, 5 or 10 mg/kg gavage	developmental LOAEL is 2 mg/kg PFOS and the NOAEL is 1 mg/kg survival of the neonates on PND 8 (NCTR model): BMD_5 = 1.07 mg/kg and $BMDL_5$ = 0.58 mg/kg	Lau et al. (2003) (*/**)
Rats	GD 2-5, 6-9, 10-13, 14-17, 17-20, or GD 19-20	25 mg/kg bw or at 25 or 50 mg/kg bw on GD 19-20	No NOAEL was established	Grasty et al. (2003) (*/**)
Rats	GD 0 – PND 20	0, 0.1. 0.3, or 1.0 mg/kg bw gavage	Neurobehavioural development: LOAEL males was 1 mg/kg bw NOAEL males was 0.3 mg/kg bw NOAEL femates was 1 mg/kg bw	Butenhoff et al. (2009) (*/-)
Rats	Gestation + lactation	0, 3.2 ppm in diet	LOAEL of 3.2 ppm Liver, decrease in T_4 levels	Yu et al. (2009) (*/-)
Rats	6 weeks	0, 0.4, 0.8,	gestation length and pup viability	Luebker et al.

Animal	Exposure/ Study length	Dose and route	N(L)OAELs	Reference (Evaluated by US EPA */ EFSA **)
	prior to mating and through gestation to day 4 of lactation	1.0, 1.2, 1.6 and 2.0 mg/kg bw per day gavage	BMDL5 values of 0.27 to 0.89 mg/kg bw per day LOAEL for the F0 dams was 0.8 mg/kg/day and the NOAEL was 0.4 mg/kg/day. LOAEL for the F1 generation was 0.4 mg/kg/day	(2005b) (*/**)
Rats	Two-generation study	0, 0.1, 0.4, 1.6 or 3.2 mg/kg/day	LOAEL for both the F0 male and female parents was 0.4 mg/kg/day and the NOAEL was 0.1 mg/kg/day. F1 parents, the NOAEL was 0.4 mg/kg/day and the LOAEL was not identified. F1 offspring, the LOAEL was 1.6 mg/kg/day based on the significant decrease in the pup viability, pup weight and survival; the NOAEL was 0.4 mg/kg/day. F2 generation offspring, the LOAEL was 0.4 mg/kg/day, based on the significant decreases in mean pup body weight; the NOAEL was 0.1 mg/kg/day.	Luebker et al. (2005a) (*/**)
Rats	Two-generation study	0, 0.1, 0.4, 1.6 and 3.2 mg/kg bw per day gavage	F2 generation LOAEL: 0.4 mg/kg bw per day NOAEL was 0.1 mg/kg bw per day	Christian et al.(1999) (*/**)
Rats	GD1-21	0, 0.1 or 2.0 mg/kg/day gavage	NOAEL of 0.1 mg/kg/day	Chen et al. (2012) (*/-)
Rats	GD12-18	0, 5, or 20 mg PFOS/kg/day	no NOAEL was established	Ye et al. (2012) (*/-)
Mice	GD1 to 17 or 18	0, 1, 10 or 20 mg/kg gavage	maternal LOAEL was 10 mg/kg/day and the NOAEL was 1 mg/kg/day developmental LOAEL was 10 mg/kg/day and the NOAEL was 1 mg/kg/day	Yahia et al.(2008) (*/-)
Mice	GD 1-17	0, 0.1, 1, or 5 mg/kg	Immunotoxicity development: LOAEL males was 1 mg/kg bw NOAEL males was 0.1 mg/kg bw LOAEL females was 5 mg/kg bw NOAEL females was 1 mg/kg bw	Keil et al. (2008) (*/-)

Animal	Exposure/ Study length	Dose and route	N(L)OAELs	Reference (Evaluated by US EPA */ EFSA **)
Mice	GD1-17	0, 1, 5, 10, 15 or 20 mg/kg	maternal weight reduction (polynomial model) BMD_5 = 15.15 mg/kg and $BMDL_5$ = 3.14 mg/kg maternal T_4 effects on GD6 (Hill model) BMD_5 = 0.51 mg/kg and $BMDL_5$ = 0.35 mg/kg foetal sternal defects (logistic model) BMD_5 = 0.06 mg/kg and $BMDL_5$ = 0.02 mg/kg foetal cleft palate (logistic model) BMD_5 = 7.03 mg/kg and $BMDL_5$ = 3.53 mg/kg.	Thibodeaux et al. (2003) (*/**)
Mice	GD1-17	0, 1, 5, 10, 15 or 20 mg/kg	LOAEL for mouse pups was 5 mg/kg and the NOAEL was 1 mg/kg survival of the neonates on PND 6 (NCTR model): BMD_5 = 7.02 mg/kg and $BMDL_5$ = 3.88 mg/kg	Lau et al. (2003) (*/**)
Rabbits	GD7-20	0, 0.1, 1.0, 2.5 or 3.75 mg/kg/day	maternal rabbit LOAEL was 1.0 mg/kg/day and the NOAEL was 0.1 mg/kg/day. developmental LOAEL was 2.5 mg/kg/day, The developmental NOAEL was 1.0 mg/kg/day	Christian et al. (1999) (*/**)
Rabbits	GD6-20	0, 0.1, 1.0, 2.5 and 3.75 mg/kg bw per day gavage	LOAELs and NOAELs were respectively 1 and 0.1 mg/kg bw per day	Case et al. (2001) (*/**)

Overall, the maternal effects were decreased body weight, increased liver weight, T_4 effects on GD6-7, and reduction of implementation sites. Developmental effects were: increased mortality in pups, delayed ossification, skeletal variation, visceral anomalies, foetal sternal defects and cleft palate, maturation of lung and pulmonary function. Delays in reflex and physical development were observed in the pups which could be link to possibile neurotoxicity of PFOS.
The lowest NOAEL/$BDML_5$ reported regarding maternal toxicity was the NOAEL of 0.1 mg/kg/day in rats from the Luebker et al.(2005b) study and $BDML_5$ of 0.05 mg/kg in rats for T_4 effects on GD7 (Hill model) from the Thibodeaux et al. (2003) study.
The lowest NOAEL/$BDML_5$ reported regarding developmental toxicity was the NOAEL of 0.1 mg/kg/day in rats based on the significant decreases in mean pup body weight from the Luebker et al. (2005b) study, the $BDML_5$ of 0.02 mg/kg in mice for foetal sternal defects (logistic model) from the Thibodeaux et al. (2003) study.

PFOS has been classified as Repr. 1B, H360D (May damage the unborn child) and Lact., H362 (May cause harm to breast-fed children).

Identification of most critical studies
EFSA (2008) concluded that foetal toxicity and neonatal effects have been observed at doses similar to or below those resulting in maternal toxicity. Observed developmental effects included reduction of foetal weight, cleft palate, anasarca (oedema), delayed ossification of bones (sternebrae and phalanges) and cardiac abnormalities (ventricular septal defects and enlargement of the right atrium). Dose response curves were generally steep, with high mortality observed early after birth. Late gestational age seems to be a very vulnerable period. Two-generation reproduction studies have revealed effects in the F1 and F2 generation, with a LOAEL of 0.4 mg/kg bw per day and NOAEL of 0.1 mg/kg bw per day (Christian et al., 1999 and Luebker et al., 2005a).

US EPA (2014b) identified the NOAELs from the rat studies by Thibodeaux et al. (2003); Lau et al. (2003); Butenhoff et al. (2009); Luebker et al. (2005a); and Luebker et al. (2005b) as critical studies for RfD derivation (together with the repeated dose toxicity studies by Seacat et al. (2002); Seacat et al. (2003); and Thomford (2002), see section 3.4.2 and section 5.5.2).

4.6 Mutagenic and genotoxic effects

4.6.1 PFOA
EFSA (2008) referred to an US EPA (2006) evaluation regarding the genotoxicity of PFOA. From the database on both *in vitro* and *in vivo* mutagenicity testing on the ammonium salt of PFOA, APFO, it was concluded that PFOA should not be considered as a genotoxic substance.
This is in line with the evaluation and conclusion by US EPA (2014a).

4.6.2 PFOS

The genotoxicity of PFOS and its salts was reviewed by OECD (2002), Health Canada (2004), the US EPA (2006) and the UK Committee on toxicity of chemicals in food, consumer products and the environment (COT, 2006a+b).

EFSA (2008) concluded based on these assessments and "the negativity in a large series of *in vitro* and/or *in vivo* short-term tests at gene and/or chromosome or DNA repair levels genotoxicity does not appear to be a property of PFOS, its salts".
This is in line with the evaluation and conclusion by US EPA (2014b)

4.7 Carcinogenic effects

4.7.1 PFOA

EFSA (2008) in their evaluation of the carcinogenic potential of PFOA referred to the Evaluation by US EPA (2006).

Two dietary studies have been carried out in rats. The first was a 104-week chronic toxicity/ carcinogenicity study (Sibinski, 1987) in which groups of 50 male and 50 female Sprague-Dawley (Crl: CDBR) rats were fed diets containing 0, 30 or 300 mg/kg APFO for two years, equal to mean doses of 0, 1.3 and 14.2 mg/kg bw per day for males and 0, 1.6 and 16.1 mg/kg bw per day for females, respectively. Concerning carcinogenicity, there was a significant increase in the incidence of testicular Leydig cell adenomas (0/50, 2/50 and 7/50 at 0, 30 and 300 mg/kg, respectively). There was also a significant increase in the incidence of mammary fibroadenomas in both groups of females (10/46, 19/45 and 21/44 at 0, 30 and 300 mg/kg, respectively). The tumour incidences were comparable to historical controls, and therefore not considered to be biologically significant (EFSA, 2008).

In a follow-up 2-year mechanistic study, male CD rats (153 treated animals and 80 animals in the control group) were administered APFO at a dietary level of 300 mg/kg, equal to 14 mg/kg bw per day (Cook et al., 1994; Biegel et al., 2001). In the treated group, relative liver weights and hepatic β-oxidation activity were statistically significantly increased at all the sampling time points, while absolute testis weights were increased only at 24 months. There were no significant differences in serum testosterone, FSH, LH, or prolactin in the treated rats compared to the controls. There was a significant increase in the incidence of Leydig cell adenomas in the treated rats (8/76; 11%) compared to the controls (0/80, 0%). In addition, the treated group had a significant increase in the incidence of liver adenomas (10/76, 13% vs. 2/80, 3%) and pancreatic acinar cell tumours (7/76, 9% vs. 0/80, 0%). This observation prompted a re-examination of the pancreas sections from the Sibinski and Biegel studies: it was then reported that APFO increased the incidences of proliferative pancreatic acinar cell lesions in both studies at 14.2 mg/kg bw per day, but not adenomas/ carcinomas (Frame and McConnell, 2003). There was a greater tendency of progression to adenomas in the study by Biegel et al. (2001) than in the Sibinski study (EFSA, 2008).

EFSA (2008) concluded that the two carcinogenicity dietary studies of PFOA (APFO) had shown that the compound induced hepatocellular adenomas, Leydig cell adenomas and pancreatic acinar cell hyperplasia in male rats.

US EPA (2014a) concluded that the carcinogenic mode(s) of action of PFOA are not clearly understood. However, the weight of evidence suggests that non-genotoxic mechanisms involving binding to receptors and disturbance of the endocrine system may be key events.
For liver tumours in rats, it was concluded that this most probably was due to peroxisome proliferation and not relevant for humans. In relation to Leydig cell tumours it was found that mechanisms resulting in inhibition of testosterone and/or increase in estradiol levels may result in

the induction of Leydig cell tumours, whereas no mechanistic mode of action could be suggested for pancreatic acinar cell tumours.
Further, it was stated that the tumour dose-response data do not indicate high potency of the substance and that protection for other non-cancer end-points e.g. liver toxicity, immunotoxicity, developmental effects and delays, would also protect against tumorigenic effects (US EPA, 2014a).

PFOA has recently been evaluated by the Risk Assessment Committee at ECHA, and according to this conclusion the substance is in *EU classified as Carc. 2, H351 (Suspected of causing cancer)* (ECHA/RAC, 2011).

WHO/IARC (The international Agency on Research on Cancer, WHO) has recently evaluated PFOA and based on limited evidence for the carcinogenicity in animals together with limited evidence for the carcinogenicity in humans PFOA was classified as *possibly carcinogenic to humans (group 2B)* (Rusyn et al., 2014). No evaluation was made on PFOS/PFOSA.

4.7.2 PFOS

EFSA (2008) in their evaluation of the carcinogenic potential of PFOS referred to the evaluation by OECD (2002), Health Canada (2004) and US EPA (2006).

A chronic toxicity and carcinogenicity study of PFOS potassium salt was carried out in rats in compliance with Good Laboratory Practice (GLP) (Thomford, 2002). Groups of 40-70 male and female Crl:CD(SD)IGS BR rats were given PFOS at doses of **0.5, 2, 5 or 20 mg/kg in the diet**, corresponding to mean achieved doses of **0.04, 0.14, 0.36 and 1.42 mg/kg bw per day in males and 0.035, 0.14, 0.37 and 1.49 mg/kg bw per day in females**.
For neoplastic effects, a significant increase in the incidence of hepatocellular adenomas was noted in male rats in the high-dose group (7/60) compared to the control (0/60). A significantly increased incidence was observed for thyroid follicular cell adenomas in the recovery group (9/39) compared to the controls (3/60) and high dose group (4/59). No other neoplastic effects were seen in the males. In the females, significant increase in the incidences of hepatocellular adenomas (5/60) and combined hepatocellular adenomas and carcinomas (6/60) were observed in the high-dose group (20 mg/kg). A significant increase in combined thyroid follicular cell adenomas and carcinomas was observed in the 5 mg/kg group (3/50) compared to the controls (0/60). Increased incidences of mammary fibroadenomas/adenomas were observed in all treated female groups, apart from the high-dose group which showed a significant decrease. The incidences of combined mammary fibroadenomas/adenomas/carcinomas were significantly increased in the low-dose (0.5 mg/kg) and 2 mg/kg dose groups, (36/50 and 31/48, respectively), but not in the 5 mg/kg or 20 mg/kg dose groups (29/50 and 24/60, respectively), compared to the controls (29/60).

EFSA (2008) concluded that PFOS is hepatotoxic and carcinogenic, inducing tumours of the liver. The evidence for induction of thyroid and mammary tumours was considered limited.
For **non-neoplastic effects**, based on histopathological findings in the liver, the no-observed adverse-effect level **(NOAEL) for PFOS is considered to be 2 mg/kg in the diet (0.14 mg/kg bw per day)** in male and female rats.

US EPA (2014b) in their evaluation of the Thomford (2002) study concluded that the evidence of carcinogenicity was suggestive but not definitive, as the tumour incidence did not indicate a dose response. With respect to mode of action, data were considered inadequate for suggesting peroxisome proliferation as mode of action for the liver and thyroid adenomas.

PFOS is EU-classified as Carc. 2, H351 (Suspected of causing cancer).

5. Regulations

As indicated in the Danish EPA (2013) report: "Survey of PFOS, PFOA and other perfluoroalkyl and polyfluoroalkyl substances", PFOS is subject to the strict registration as a persistent organic pollutant (POP) substance, as it was added to Annex B of the Stockholm Convention on Persistent Organic Pollutants in May 2009, and also subjected to restriction under REACH (Annex XVII entry 53).
PFOA (and its ammonium salt APFO) in 2013 were included on the candidate list for authorisation under REACH. For more detailed description of the regulation, see Danish EPA (2013).

This chapter, however, covers selected regulatory areas such as hazard classification of the substances and guidance values/limit values for drinking water. Further, in recent national and international reports regarding hazard characterisation of PFOA and PFOS and the derivation of tolerable daily intake values, TDIs are included.

5.1 Classification

5.1.1 PFOA, EU-classification

PFOA has obtained according to the CLP regulation harmonized classification as follows:

Acute Tox. 4	H302	Harmful if swallowed
Eye Dam. 1	H318	Causes serious eye damage
Acute Tox. 4	H332	Harmful if inhaled
Carc. 2	H351	Suspected of causing cancer
Repr. 1B	H360D	May damage the unborn child
Lact.	H362	May cause harm to breast-fed children
STOT RE 1	H372	Causes damage to the liver through prolonged or repeated exposure

5.1.2 PFOS/ PFOSA , EU-classification

PFOS has obtained according to the CLP regulation harmonized classification as follows:

Acute Tox. 4	H302	Harmful if swallowed
Acute Tox. 4	H332	Harmful if inhaled
Carc. 2	H351	Suspected of causing cancer
Repr. 1B	H360D	May damage the unborn child
Lact.	H362	May cause harm to breast-fed children
STOT RE 1	H372	Causes damage to the liver through prolonged or repeated exposure
Aquatic Chronic 2	H411	Toxic to aquatic life with long lasting effects

No harmonised classification has been made for PFOSA.

5.1.3 WHO/ IARC

WHO/IARC (The international Agency on Research on Cancer, WHO) has recently evaluated PFOA and based on limited evidence for the carcinogenicity in animals together with limited evidence for the carcinogenicity in humans PFOA was classified as *possibly carcinogenic to humans (group 2B)* (Rusyn et al., 2014). No evaluation was made on PFOS/PFOSA.

5.2 Drinking water values

Below drinking water values for only PFOA and PFOS are given as no values have been found for PFOSA.

Overall the following values have been found, Table 5-1

TABLE 5-1. OVERVIEW OF DRINKING WATER GUIDELINES FOR PFOA/PFOS

	Health based values µg/L		Administrative values µg/L	
	PFOA	PFOS	PFOA	PFOS
Germany, 2006	0.3	0.3	0.1 PFOA+PFOS combined	
UK, 2009	10	0.3	Level 1 : 0.3 Level 2: 10 Level 3 : 90	Level 1 : 0.3 Level 2: 1.0 Level 3 : 9
US EPA, 2009	0.4	0.2	-	-
Netherlands, 2011	-	0.53		0.0053
Sweden, 2014		0.09	0.09*	0.09*

* the sum of seven PFAS substances found in contaminated drinking water: Perfluoroctane sulfonate (PFOS); Perfluorhexane sulfonate (PFHxS);Perfluorobutane sulfonate (PFBS); Perfluoroctanoic acid (PFOA); Perfluoroheptanoic acid (PFHpA); Perfluorohexanoic acid (PFHxA); and Perfluoropentanoic acid (PFPeA).

(As mentioned in chapter 7, this report proposes a health based quality criterion on PFOA and PFOS of 0.3 µg/L and 0.1 µg/L, respectively.)

The derivation of the values in table 5-1 are described in more detail below.

5.2.1 Germany, 2006 (GMH 2006)

5.2.1.1 PFOA health based level: 0.3 µg/L

In Germany ***a composite precautionary guidance value*** for PFOA and PFOS of ***0.1 µg/L*** in drinking water has been set for long term exposure. A strict health based level was set at **0.3 µg/L** (Drinking Water Commission, 2006). For PFOA the lowest NOAEL was found to be in the range of 0.1-1.0 mg/kg bw/day and a tolerable daily intake of 0.1 µg/kg/bw/day was calculated using an assessment factor of 100 and an extra factor of 10 due to the extremely long half-life of PFOA.

5.2.1.2 PFOS health based level: 0.3 µg/L

Also for PFOS a tolerable daily intake of 0.1 µg/kg/bw/day was calculated based on a NOAEL of 0.025 mg/kg/day from a two-year study in rats (specific assessment factors not further explained). From these tolerable levels a composite health based guide value of **0.3 µg/L** was calculated as 10% of the tolerable daily intake was allocated to the intake of 2 liter drinking water per day for a person weighing 70 kg.

5.2.2 UK, 2007

In 2007 the Drinking Water Inspectorate announced three tiered guidance levels for PFOA and PFOS in drinking (DEFRA, 2008):

5.2.2.1 PFOA, maximum acceptable concentration in drinking water: 10 µg/L

Tier 1 level > 0.3 µg/L: at this level the local health professionals should be consulted and increased monitoring established.
Tier 2 level > 10 µg/L: further to put in place measures to reduce concentrations to below 10 µg/l as soon as is practicable.
Tier 3 level >90 µg/L: ensure consultation with local health professionals takes place as soon as possible; take action to reduce exposure from drinking water within 7 days (DEFRA, 2008).

The health based Tier 2 level of 10 µg/L was based on a tolerable daily intake of 3 µg/kg bw/d derived by the Committee of Toxicity (COT, 2006a). For deriving a tolerable daily intake (TDI) a dose level of 0.3 mg/kg bw/day was selected as a suitable point of departure expected to be without adverse effect on the basis of a number of end-points of PFOA toxicity.
An uncertainty factor of 100 was applied to allow for inter and intra-species variation. Therefore, the TDI indicated for PFOA is 3 µg/kg bw/day.
For deriving the limit value of 10 µg/L in drinking water 50% of the TDI was allocated to 0.75 l of drinking water consumed daily by a bottle-fed baby weighing 5 kg.

5.2.2.2 PFOS, maximum acceptable concentration in drinking water: 0.3 µg/L

Tier 1 level > 0.3 µg/L: at this level the local health professionals should be consulted and increased monitoring established.
Tier 2 level > 1.0 µg/L: further to put in place measures to reduce concentrations to below 1.0 µg/l as soon as is practicable.
Tier 3 level >9 µg/L: ensure consultation with local health professionals takes place as soon as possible; take action to reduce exposure from drinking water within 7 days.

The health based tier 1 levels for PFOS was based on a provisional tolerable intake of 0.3 µg/kg bw/day for PFOS derived by the Committee of Toxicity, (COT, 2006b). Allocation of 10% of the provisional TDI to 1 litre of drinking water consumed daily by a one year-old child weighing 10 kg yields a 'maximum acceptable' concentration of 0.3 µg/l of PFOS.

The COT found a NOAEL of 0.03 mg/kg bw/day for decreased serum T3 levels in a 26-week study with cynomolgus monkey as the most suitable basis for deriving a tolerable daily intake (TDI) for PFOS. COT noted that, on the basis of the pharmacokinetic data indicating an elimination half-life of between 110 and 180 days, the Cynomolgus monkeys would be at approximately half steady state at the end of this study. Taking into account that this was a primate study and the effects were mild, the COT concluded that it was not necessary to apply an additional uncertainty factor to allow for the incomplete attainment of steady state. The Committee applied an uncertainty factor of 100 to allow for inter- and intra-species variation to the NOAEL of 0.03 mg/kg bw/day from the Cynomolgus monkey study and concluded on a provisional TDI for PFOS of 0.3 µg/kg bw/day. Because of the accumulative properties of PFOS, exposure should be averaged over prolonged periods for comparison with the TDI.

5.2.3 US EPA (2009)

5.2.3.1 PFOA, Provisional Health Advisory value drinking water: 0.4 µg/L.

As critical study for derivation of the drinking water value, US EPA identified the study by Lau et al. (2006). The study included a number of developmental end-points: neonatal eye opening, neonatal survival and body weight at weaning, reduced phalangeal ossification at term, live foetus weight at term, maternal liver weight at term, and maternal weight gains during pregnancy. The most

sensitive end-point was for increased maternal liver weight at term. It was noted that this end-point for liver effects was identified in a number of other studies described in EFSA (2008). Benchmark dose (BMD10) and the 95% lower bound on the BMD (BMDL10) were calculated for these toxicity end-points by the EFSA on the basis of raw data provided by the principal author Lau (personal communication). The lowest BMDL10 in the Lau et al. (2006) study was 0.46 mg/kg/day for increase in maternal liver weight at term. This value was used as the point of departure for the derivation of the Provisional Health Advisory value for PFOA. It should be noted that liver effects were also reported in studies in rats and monkeys. BMDL10 values for increased liver weight in studies in mice and rats ranged from 0.29 to 0.74 mg/kg/day (EFSA, 2008). The BMDL10 for Lau et al. (2006) was in the middle of this range.

The general equation for the derivation of a Provisional Health Advisory is:

$$\text{PHA} = \frac{(\text{NOAEL or BMDL10}) \times \text{BW} \times \text{RSC}}{\text{UF} \times \text{Extrapolation Factor} \times \text{Water intake}}$$

Where BW = body weight; RSC = relative source contribution; UF = uncertainty

An exposure scenario of a 10-kg child consuming 1 L/day of drinking water is used to calculate the Provisional Health Advisories is used for PFOA and PFOS. This population subgroup was used because children, who consume more drinking water on a body weight basis than adults, have a higher exposure on a body weight basis than adults. The selection of children's exposure parameters will help to ensure that this Provisional Health Advisory is protective of sensitive populations potentially exposed. A default relative source contribution (RSC) of 20% was used to allow for exposure from other sources such as food, dust and soil. The relevant period of exposure for the Health Advisory is a short-term exposure. This time period is consistent with the toxicity data used for PFOA and PFOS, both of which rely upon subchronic data. The value should be protective of all population subgroup and life stages.

Thus:

$$\text{PFOA Provisional Health Advisory} = \frac{0.46 \times 1000 \times 10 \times 0.2}{10 \times 3 \times 81 \times 1} = 0.4\ \mu\text{g/L}$$

An uncertainty factor of 10 was used to account for variability among humans, a factor of 3 to account for possible differences in toxicodynamics between mouse and humans and a factor 81 was used as the data derived ratio between the PFOA clearance in mouse compared to humans (US EPA, 2009).

5.2.3.2 **PFOS Provisional Health Advisory: 0.2 µ g/L**

The subchronic toxicity study in Cynomolgus monkeys (Seacat et al., 2002) was selected as the critical study for the derivation of the Provisional Health Advisory value for PFOS. In the study by Seacat et al. (2002), groups of male and female monkeys orally received potassium PFOS at doses of 0, 0.03, 0.15 or 0.75 mg/kg/day for 183 days. Compound-related mortality in 2 of 6 male monkeys, decreased body weights, increased liver weights, lowered serum total cholesterol, lowered triiodothyronine (T3) concentration, and lowered estradiol levels were seen at the highest dose tested. At 0.15 mg/kg/day, increased levels of thyroid-stimulating hormone (TSH) in males, reduced total T3 levels in males and females, and reduced levels of high-density lipoproteins (HDL) in females were seen. A NOAEL of 0.03 mg/kg/day was identified in this study.

From this the following Provisional Health Advisory is obtained:

$$\text{PFOS Provisional Health Advisory} = \frac{0.03 \times 1000 \times 10 \times 0.2}{10 \times 3 \times 13 \times 1} = 0.2\ \mu g/L$$

An uncertainty factor of 10 was used to account for variability among humans, a factor of 3 to account for possible differences in toxicodynamics between mouse and humans and a factor 13 was used as the data derived ratio between the PFOS clearance in monkeys compared to humans. (US EPA, 2009)

5.2.4 Netherlands (RIVM 2011)

A maximal tolerable level of PFOS in drinking water of 0.53 µg/L was derived on the basis of the EFSA (2008) TDI 0.15 µg /kg bw/day (the calculation was not further specified). A negligible level of 0.0053 µg/L was derived by the further use of a factor of 100 (RIVM, 2011).

5.2.5 Sweden (Livsmedelsverket 2014)

A maximal tolerable level of 0.09 µg/L for PFOS was derived for drinking water based on the TDI of 0.15 µg/kg bw/d derived by EFSA (2008) and considering an exposure scenario where 10% of this value was allocated to the consumption of infant formula based on drinking water.

As a precautionary measure, the limit value of 0.09 µg/L was further applied for the sum of seven PFAS substances found in contaminated drinking water: Perfluoroctane sulfonate (PFOS); Perfluorhexane sulfonate (PFHxS); Perfluorobutane sulfonate (PFBS); Perfluoroctanoic acid (PFOA); Perfluoroheptanoic acid (PFHpA); Perfluorohexanoic acid (PFHxA); and Perfluoropentanoic acid (PFPeA). PFOS was considered to be the most toxic of these substances.

As risk management measures, Livsmedelverket (2014) recommended that when the total level of PFAS exceeded 0.09 µg, the actions should be issued in order to reduce the levels to below 0.09 µg (however, the water could still be used if the levels did not exceed 0.9 µg/L).
When exceeding 0.9 µg/L, pregnant women or women trying to get pregnant and infants should not consume the water.

5.3 Soil and ground water

5.3.1 Norway: 0.1 mg PFOS /kg soil

Norwegian Pollution Control Authority has proposed a guideline value for PFOS in soils of 100 ng/g (0.1 mg/kg) based upon effect studies on earthworms (Stubberud, 2006).

5.3.2 The Netherlands: 2.3 µg PFOS/kg soil and 23 ng PFOS /L groundwater

RIVM has made proposals for the maximum permissible concentration (MPC), the serious risk concentration (SRC) and the negligible concentration (NC). The derivation is based on a limited amount of information. Environmental risk limits focus on different protection goals and exposure routes: (ground)water/soil organisms, predators (food chain) and man. In general the lowest value then determines the overall risk limit. The exposure of (ground)water organisms is expected to be the most critical route for PFOS in groundwater, resulting in an overall maximum permissible concentration (MPC) of 23 ng/L. For soil, the accumulation via the food chain is most critical and a maximum permissible concentration (MPC) of 2.3 µg/kg soil was derived.
With the available data, no soil risk limits could be derived covering the consumption of vegetables, milk and meat by man. Potential human risks from the use of groundwater abstracted for drinking water are taken into account (RIVM, 2011).

5.4 Tolerable daily intake/ RfD[1]

The most recent and updated derivations of tolerable daily intake levels of PFOA and PFOS have been conducted by EFSA (2008) and US EPA (2014). The derivations of the values will be described in more detail below, as this information will be further used in Chapter 7 of this report where a proposal for TDI values will be given.

5.4.1 EU (EFSA, 2008)

5.4.1.1 PFOA. TDI= 1500 ng/kg bw per day

The Scientific Panel on Contaminants in the Food Chain (CONTAM) under EFSA noted that the 95% lower confidence limit of the benchmark dose for a 10% increase in effects on the liver (BMDL10) values from a number of studies in mice and male rats were in the region of 0.3 - 0.7 mg/kg bw per day. Therefore, the panel concluded that the lowest BMDL10 of 0.3 mg/kg bw per day was an appropriate point of departure for deriving a TDI. The CONTAM Panel established a TDI for PFOA of 1.5 µg/kg bw per day by applying an overall UF of 200 to the BMDL10. An UF of 100 was used for inter and intra-species differences and an additional UF of 2 to compensate for uncertainties relating to the internal dose kinetics.

The BMDL10 value was derived on the analysis made by COT (2006a) where BMDL10 levels for increased liver weight and liver toxicity were estimated from the studies by Lau et al. (2006); Palazzolo (1993); Perkins et al. (2004); Sibinski (1987); and Butenhoff et al. (2004). From these studies BMDL10 levels in the range of 0.29-0.74 mg/kg bw/day were estimated and based on this a BMDL10 level of 0.3 mg/kg bw/day was chosen as point of departure for the TDI estimation. The value of 0.2 mg/kg bw/day was both derived from the Palazzolo, 1993 study and from the Butenhoof et al. (2004) study.

5.4.1.2 PFOS, TDI=150 ng/kg bw per day

The CONTAM panel identified 7 relevant studies as relevant for the hazard characterisation:
-Two subchronic studies by Seacat et al. (2002 and 2003) with monkeys and rats, respectively.
-One chronic study by Thomford et al. (2002) in rats.
-Four developmental studies reported by Case at al. (2001) using rats; by Lau et al. (2003) and Thibodeaux et al. (2003) using rats and mice; and by Christian et al. (1999) using rats.

From the NOAELs and LOAELs from these studies the CONTAM panel identified the lowest no-observed adverse-effect level (NOAEL) of 0.03 mg/kg bw/day from the study by Seacat et al. (2002) with Cynomolgus monkeys showing changes in lipids and thyroid hormones at the next higher dose of 0.15 mg/kg bw per day. The panel concluded this as a treatment related adverse effect even though Seacat et al. (2002) concluded on a NOAEL of 0.15 mg/kg bw/day based on more pronounced changes on hormones and lipids, increased liver weight and increased mortality at 0.75 mg/kg bw/day.
The NOAEL of 0.03 mg/kg bw/day was in females associated with a plasma concentration of 13.2 µg/mL PFOS at the end of the exposure period (day 183). However, as the estimated half-life of PFOS in monkeys is about 200 days, this internal dose does not represent steady state.

The CONTAM Panel established a TDI for PFOS of 150 ng/kg bw/day by applying an overall uncertainty factor (UF) of 200 to the NOAEL. An UF of 100 was used for inter and intra-species differences and an additional UF of 2 to compensate for uncertainties in connection to the relatively short duration of the key study and the internal dose kinetics.

[1] RfD: Reference dose. Is a term used by US EPA that is comparable to a tolerable daily intake, TDI.

5.4.2 Sweden 2011

For PFOA provisional TDIs in the range of 0.30-4.3 µg/kg bw/day were estimated.
For PFOS provisional TDIs in the range of 0.15 - 1.0 µg/kg bw/day were estimated.
(Livsmeddelverket, 2011)
See further details in a table in appendix 1.

5.4.3 US-EPA (2014)

5.4.3.1 PFOA; RfD[2] = 0.02 µg/kg/day

US EPA derived 0.00002 mg/kg/day as the RfD for PFOA based on the consistency of the response and with recognition of the use of liver weight as a common denominator for loss of homeostasis and protection against co-occurring adverse effects. This value is the outcome for modeled serum values from three rat studies and one mouse study. In two of the rat studies, liver effects were accompanied by developmental effects and kidney weight increases. A pharmacokinetic model was used to predict a serum area under the curve (AUC) concentration for each LOAEL and NOAEL for liver weight effects. From each AUC, the average serum concentration was calculated which was then used to calculate a human equivalent dose (HED) Pharmacokinetic modelling is used to estimate the human oral dose (the HED) that result in the same serum levels in humans as in the experimental animals. Thus when estimating a HED no use of an interspecies pharmacokinetic factor is necessary when calculating the RfD. Thus, the total uncertainty factor (UF) applied to the HED-NOAEL from rat study was 30 which included a UF of 10 for intrahuman variability and a UF of 3 to account for toxicodynamic differences between animals and humans. For the critical studies that lacked an HED-NOAEL, and additional 10-fold UF was added to adjust for the use of a HED based on a LOAEL. Four of six candidate studies resulted in the same RfD. All of the studies supporting the RfD, except one, are studies with exposures to PFOA for ≥ 84 days meeting the duration requirements for determination of a lifetime exposure value; the exception is a 17-day developmental toxicity study in the mouse.

Details
US EPA identified effects *on liver weight and hepatocellular necrosis* as the most susceptible end-point in the toxicity studies and identified the studies by Palazolo (1993) (rat); Loveless et al. (2008) (rat and mouse), York (2002) (rat); Lau et al. (2006) (mouse); Dewitt (2008) (mouse); and Thomford (2001) (monkey) as the most relevant studies for further examining the critical dose-levels for the RfD derivation.

The RfD calculations were based on the identified, NOAEL, LOAEL transferred to a human equivalent dose levels (HEDs) using pharmacokinetic modelling or from an estimated BMDL10 level if the specific data set allowed for this estimation (done for the studies by Loveless et al., 2008; Palazzolo, 1993; and York, 2002).

Thus the human equivalent dose (HED) was calculated for the identified NOAEL and LOAEL values but not for the BMDL10-levels, however no explanation was given for omitting this. This was done by determining the time integrated serum concentration (the AUC in mg/L x h) and estimation of the average serum concentration (mg/l) for each of the studies. This predicted serum concentration was then converted to an oral daily dose (mg/kg bw/day) at steady state. This dose was then scaled to a human dose HED (mg/kg bw/day) taking account of pharmokinetic differences affecting the clearance. Thus the HED is the dose that in human will result in an equivalent average serum concentrations as found in the experimental animals. In the calculations for PFOA a human half-life in serum of 2.3 years was used based on human data.

[2] RfD: Reference dose. Is a term used by US EPA that is comparable to a tolerable daily intake, TDI

RfD was then calculated both from NOAELs and LOAELs; the derived HEDs; and from the derived BMDL10 levels by using the following relevant uncertainty factors, Table 5-2:

TABLE 5-2 USE OF UNCERTAINTY FACTORS BY US EPA (2014A)

Uncertainty factors used for RfD derivation						
POD	UFH	UFA	UFL	UFS	UFD	UFtotal
NOAEL	10	219/150*	1	1 or 10	1	up to 21900/15000*
LOAEL	10	219/150*	10	1 or 10	1	up to 219000/15000*
BMDL10	10	219/150*	1	1 or 10	1	up to 21900/15000*
HED-NOAEL	10	3	1	1	1	30
HED-LOAEL	10	3	10	1	1	300

POD: Point of departure
UFH: intraspecies (human) variability
UFA: interspecies extrapolation
UFL: LOAEL to NOAEL extrapolation
UFS: Duration of the study factor
UFD: Strength of the database
*consists of a subfactor of 3 accounting for interspecies pharmacodynamics differences and a subfactor 73/ 50 for rat/mouse based on the ratio of clearance for animal/human extrapolation. (see details for derivation of these values in section 7.1.2).

After identification of the point of departures the following RfDs were estimated, Table 5-3:

TABLE 5-3. RFD ESTIMATIONS FOR PFOA BY US EPA (2014A)

RfD estimations by US EPA (2014a)			
POD ref, species, duration	POD-value mg/kg bw/d	UF total	RfD µg/ kg bw/d
NOAEL Palazzolo, rat, 90D	0.06	21400	0.003
LOAEL Palazzolo, rat, 90D	0.64	214000	0.003
BMDL Palazzolo, rat, 90D	0.456	21400	0.02
BMDL York, rat, 70-127D	0.274	21900	0.01
BMDL Loveless, rat, 29D	0.152	21900	0.007
BMDL Loveless, mouse, 29D	0.0681	15000	0.005
HED-NOAEL Palazzolo, rat, 90D	0.00047	30	0.02
HED-LOAEL Palazzolo, rat, 90D	0.0045	300	0.02
HED-LOAEL Dewitt, mouse, 15D	0.0028	300	0.009
HED-LOAEL Lau, mouse, 17-18D	0.0057	300	0.02
HED-LOAEL York, rat, 70-127D	0.0065	300	0.02
HED-LOAEL Thomford, monkey, 182D	0.0124	300	0.04

When concluding on the RfD value, US EPA put most emphasis on the *HED-derived values* and found the most consistent picture for a value of 0.02 µg/kg bw/day. Especially emphasis was put on

the Palazzolo et al. (1993) study, as data from this study permitted both BMDL and HED calculations. It was however noted that the studies by Lau et al. (2006) and York et al. (2002) at the LOAEL levels also found developmental effects and effects on kidneys, respectively.

5.4.3.2 PFOS; RfD = 0.030 µg/kg/day

US EPA derived 0.00003 mg/kg/day as the RfD for PFOS based on the consistency of the response and with recognition of the use of developmental toxicity and liver weight as the most sensitive end-points for protection against co-occurring adverse effects. This value is the outcome for modeled rat serum values for developmental neurotoxicity. In the standard developmental neurotoxicity study, male offspring showed increased motor activity and decreased habituation on PND 17 following a maternal dose of 1 mg/kg/day in the absence of effects on pup body weight. The human equivalent dose (HED) used as the basis for the RfD was calculated from an average serum concentration of 10.87 mg/L derived from the NOAEL of 0.3 mg/kg/day for developmental neurotoxicity. A pharmacokinetic model was used to predict an area under the curve (AUC) for the NOAEL and used to calculate an HED-NOAEL. The total uncertainty factor (UF) applied to the HED-NOAEL from the rat study was 30 which included a UF of 10 for intrahuman variability, and a UF of 3 to account for toxicodynamic differences between animals and humans. Comparable values derived from the HED for liver effects in rats and developmental effects in mice are slightly higher than the RfD indicating that it will be protective.

Details

US EPA identified effects on liver and developmental effects as the most susceptible end-points and identified the studies by Seacat et al. (2002, monkey); Seacat et al. (2003, rat); Thomford (2002, rat); Thibodeaux et al. (2003, rat); Lau et al. (2003, rat); Butenhoff et al. (2009, rat); Luebker et al. (2005b, rat); and Luebker et al. (2005a, rat) as the most relevant studies for RfD derivation.

The RfD calculations were based on the identified NOAELs from the studies and from BMDL10 or BMDL05 (from six of the studies that allowed for benchmark dose estimations). Further HED doses based on the NOAEL values was established for six of the studies where data was sufficient to derive these HED-NOAEL values and these values were used for further RfD derivation.

When using these starting points the following uncertainty factors were used for the RfD derivation, table 5-4:

TABLE 5-4. UNCERTAINTY FACTORS USED BY US EPA (2014B)

Uncertainty factors used for RfD derivation						
POD	UFH	UFA	UFL	UFS	UFD	UFtotal
NOAEL	10	48/123*	1	1 or 10	1	up to 4800/12300*
BMDL10	10	48/123*	1	1 or 10	1	up to 4800/12300*
HED-NOAEL	10	3	1	1	1	30

POD: Point of departure
UFH: intraspecies (human) variability
UFA: interspecies extrapolation
UFL: LOAEL to NOAEL extrapolation
UFS: Duration of the study factor
UFD: Strength of the database
*consists of a subfactor of 3 accounting for interspecies pharmacodynamics differences and a subfactor 16/ 41 for monkey/rat based on the ratio of clearance for animal/human extrapolation, (see details for derivation of these values in section 7.1.2).

The following RfD-values were obtained:

TABLE 5-5. RFD ESTIMATIONS FOR PFOS BY US EPA (2014A)

Study	NOAEL	RfD NOAEL	BMDL	RfD BMDL	HED-NOAEL	RfD HED-NOAEL
	----------mg/kg bw/day----------					
Seacat et al., 2002 monkey	0.15	0.00003	0.015	0.000003	0.0019	*0.00006*
Seacat et al., 2003 rat	0.34	0.00003	0.059	0.000005	0.0014	0.00005
Thomford, 2002 rat	0.018	0.00001	0.033	*0.00003*	-	-
Thibodeaux et al., 2003 rat	1.0	0.00008	0.12	0.00001	-	-
Lau et al., 2003 rat	1.0	0.00008	0.58	0.00005	0.0014	0.00005
Butenhoff et al., 2009 rat	0.3	0.00002	-	-	0.00088	***0.00003***
Luebker et al., 2005b rat	0.1	0.000008	-	-	0.00037	0.00001
Luebker et al., 2005a rat	0.4	0.00003	0.27	0.00002	0.0019	0.00006

Overall, US EPA found the HED-NOAEL starting point as the most relevant as these estimates specifically considered toxicokinetic differences between experimental animals and humans by using pharmacokinetic modelling.

From these results a HED- RfD level of 0.00003 mg/kg bw/day or 0.03 µg/ kg bw/day was concluded based on the HRD-RfD value derived from the Butenhoff et al. (2009) study. It was in this connection noted that the lower HED-RfD based on the study by Luebker et al. (2005) was based on transient changes in body weights as differences compared to controls were only monitored at 2 out of five time points.

6. Summary and evaluation

The Chapters 1-5 have been elaborated based on expert evaluations and review reports regarding PFOA, PFOS and PFOSA. Thus, most data have been compiled from EFSA (2008), US EPA (2014a+b), NCM (2013) and Danish EPA (2013).

6.1 Chemical identification and use

Perfluroalkylated substances are divided in subgroups where the main members are the perfluoroalkyl carboxylic acids and their salts (PFCAs), and the perfluoroalkane sulfonic acids (PFSAs) and their salts. Perfluorooctanoic acid (PFOA) is the main member of the PFCA subgroup and Perfluorooctane sulfonic acid (PFOS) is the main member of the PFSA subgroup. Perfluorooctanesulfonamide (PFOSA) is a precursor of PFOS and is a member of the PFSA subgroup. Among the perfluoroalkyl carboxylic acids (PFCAs), the most prominent member is perfluorooctanoic acid (PFOA) with an 8-carbon chain. The substance has 7 perfluorinated carbon atoms. The PFOA derivative that is most widely used and therefore of most concern is the ammonium salt (APFO). Perfluorooctane sulfonic acid (PFOS) is the most prominent of the perfluoroalkane sulfonic acids (PFSAs). PFOS has a linear perfluoroalkyl carbon chain of 8 atoms and a sulfonic acid functional group.

These substances together with other perfluoroalkylated substances are used in industrial and consumer products such as hard chromium plating, paint and lacquers, impregnated clothing, carpets, packaging, cleaning products and fire extinguishers due to their special chemical properties giving the ability to repel water and oil.

6.2 Environmental fate and levels

PFOS and PFOA are stable to hydrolysis in the environment based on half-lives of 41 and 92 years, respectively. Available information indicates that perfluoroalkyl compounds are resistant to aerobic biodegradation. Hydrolysis data were not located for PFOSA.

The perfluoroalkylated substances are present in the environment. In air, the concentration of PFOA and PFOS measured in Norway is up to 1.5 pg/m3 for PFOA and 3.3 pg/m3 for PFOS. In water, PFOA has been detected at concentrations up to 520 pg/L in snow in Greenland. In Denmark, PFCAs have been analyzed and reported for a number of wastewater treatment plants (WWTPs), in the order of a few ng/L, whereas in effluent waste water levels up to 1 µg/L has been detected. PFOS was detected at levels up to 90 pg/L in Tromsø, Norway. In a Danish WWTP, PFOS concentrations varied between 4.8 and 74.1 ng/g, while in Norway, the range was between 1.2 and 5.16 ng/g. In groundwater under contaminated sites in Denmark, the sum of 9 PFAS compounds including PFOA, PFOS and PFOSA detected in fire drill sites is above 1000 ng/l in fire drill sites, and a concentration of PFOS + PFOA of 1130 ng/l was found at the investigated carpet industry. In soil, data from Norway indicate that in background soil PFCAs including PFOA, PFOS and PFOSA were not detected, whereas near airports contamination from fire fighter foam have resulted in increased levels. Thus PFOS concentrations of 109.9 to 959 ng/g dw in soils have been found close to Gardermoen airport.

6.3 Human exposure

In general there are two important sources of exposure of perfluorinated substances to humans namely via food and drink intake and through exposure to house dust. The median human intake of PFOA in several regions studied worldwide is estimated to 2.9 ng/kg bw/day. Cereals and fish are major sources of the PFOA intake. The largest intake of PFOA may occur from contaminated food, including drinking water. The median intake of PFOS was found to be 1.4 ng/kg bw/day. Fish and shellfish are a major source to the PFOS intake. In general the level of PFOS in fish is found to be higher than the level of PFOA. As for PFOA, the largest intake of PFOS seems to occur from contaminated food, including drinking water.

EFSA (2012) estimated mean exposure for adults of 4.3 ng/kg/day representing 0.3% of the TDI while the highest 95th percentile estimate (7.7 ng/kg bw per day) represented 0.5% of the TDI. In toddlers, the age class having the highest exposure, the highest 95th percentile estimate of 32 ng/kg bw per day represented 2.1% of the TDI. For PFOS, the mean exposure estimate for the adult population (5.2 ng/kg bw per day) represented 3.5% of the TDI while the highest 95th percentile estimate (10 ng/kg bw per day) represented 6.7% of the TDI. In toddlers, the age class having the highest exposure, the highest 95th percentile estimate of 29 ng/kg bw per day represented 19% of the TDI.

Further, PFOA and PFOS have been detected in indoor house dust at concentrations up to 694 ng/g and 147.7 mg/g, respectively. Humans are also exposed via consumer products. PFOA was found in coated fabrics and PFOS was found in leather and carpet products at concentrations higher than the EU regulatory level for PFOS. PFOS is included in REACH Annex XVII covering restricted use of substances.

Biomonitoring study data show that PFOA was detected in blood of pregnant women at a concentration of 5.6 ng/ml and in men at a concentration of 4.9 ng/ml. PFOS was detected in blood of pregnant women at a concentration of 36.3 ng/ml. PFOS and PFOSA was detected in men blood at concentrations of 24.5 mg/ml and 0.06 ng/ml, respectively. PFOA was detected in cord blood and PFOA seems to cross the placenta most easily. PFOS was also detected in cord blood. PFOA and PFOS were detected in breast milk and in amniotic fluids at concentrations lower than maternal blood and cord blood concentrations.

6.4 Absorption, distribution, metabolism and elimination

Regarding the toxicokinetics, PFOA and PFOS are readily absorbed after oral exposure, and are found in the liver, kidneys and blood with lower levels in many other organs, including the central nervous system. Also, the substances can cross the placenta barrier. Metabolic elimination seems to play no relevant role for both PFOA and PFOS.

For PFOA elimination half-lives of < 24 h in female and < 9 days in male rats, of 21 – 30 days in Cynomolgus monkeys, and of about 3.8 years in humans have been estimated.

For PFOS elimination half-lives have been estimated to > 90 days in rats, about 200 days in Cynomolgus monkeys, and about 5.4 years in humans.

6.5 Human data

Regarding occupational exposure, a retrospective cohort mortality study showed a statistically significant association between prostate cancer mortality and employment duration in the chemical facility of a plant that manufactures APFO. However, in an update of this study, in which more specific exposure measures were used, a significant association for prostate cancer was not observed. A number of studies have investigated possible associations between PFOA serum levels

and biochemical parameters associated with lipid metabolism. Some have shown associations with elevated cholesterol and triglycerides or with changes in thyroid hormones, but overall there is no consistent pattern of changes. There were no statistically significant associations between PFOS exposure and an increased risk of bladder cancer.
With respect to the general population, it was shown that PFOA was inversely associated with birth weight and head circumference, but not length or gestational duration. PFOS was significantly associated with small decreases in birth weight and size, but not newborn length or gestational age. The concentrations of PFOS in cord serum were highly correlated with those of PFOA.

Although some epidemiological studies find associations between levels of PFOA/PFOS in humans and adverse health outcomes, e.g. in relation to increased levels of cholesterol, immunotoxicity (antibody formation), neurotoxic effects and effects that may be the cause of endocrine disruption, neither EFSA (2008) nor US EPA (2014a+b) find that the human data provide a basis from which a TDI or RfD (reference dose) can be derived. US EPA (2014a+b) found a lack in consistency among the findings and considered it impossible to find robust numerical values that could form the basis for RfD estimations.

A first attempt has been made to use human data on immunotoxicity for calculation of RfD levels by Grandjean and Budtz-Jørgensen (2013); however, this was done on a single data-set and further support and human data for the dose-response associations used in this study seem warranted. Also for regulatory purposes the study suffers from limitations in transparency and descriptions of the methodology used.

6.6 Experimental animal data

6.6.1 Single exposure

Acute toxicity studies in male rats resulted in the LC_{50} upon inhalation of APFO for 4 hours of 980 mg/m^3. For PFOS dust in air for one hour yielded an inhalation LC_{50} of 5.2 mg/L. Oral LD_{50} values in rats for PFOA and PFOS were about 500 mg/kg bw and 251 mg/kg bw, respectively. These LD_{50} values suggest that PFOA and PFOS can be classified as moderately toxic after acute oral exposures. The dermal LC_{50} was reported to be greater than 2000 mg/kg bw for PFOA.
PFOA is a weak skin irritant as determined in rabbit experiments. PFOA was found to be an eye irritant in rabbits. For PFOS, skin and eye irritation were not observed in rabbits.

6.6.2 Repeated exposure

Regarding repeated dose toxicity several studies are available and show that *PFOA* affects primarily the liver and the lipid metabolism. From these studies the lowest identified **LOAEL and NOAEL in rats** were 10 ppm **(0.64 mg/kg/day)** and 1 ppm (**0.06 mg/kg/day**) (Palazollo, 1993). **In mice** the lowest **LOAEL and NOAEL** were found to **0.3 mg/kg /day and 0.1 mg/kg/day**, and in **monkeys a LOAEL of 3 mg/kg /day** was found. Mice and rats seem to be more susceptible than monkeys.

Studies performed with *PFOS* also showed that PFOS primarily affected the liver and biochemical parameters associated with lipid metabolism. Increased liver weight and vacuolisation and hypertrophy of hepatic cells were observed in the animal species tested (rat and monkey). PFOS also reduced body weight, serum cholesterol, serum triglycerides, and triiodothyronine levels. Changes in thyroid hormones were observed, although the underlying mechanisms are not understood. Male rats appear to be more sensitive than female rats.
A steep dose response curve was observed in the Cynomolgus monkey since the dose range between no observed adverse effects and treatment related deaths was narrow. Monkeys died at doses of a few mg/kg per day.

In rats the lowest LOAEL and NOAEL were found in male rats to 0.072 mg/kg /day and 0.018 mg/kg/day based on the Thomford et al. (2002) study (according to the interpretation of US EPA, 2014). In monkeys a NOAEL of 0.03 mg/kg/day and a LOAEL of 0.15 mg/kg/day were determined by EFSA (2008) based on the data from Seacat et al. (2002).

6.6.3 Reproductive toxicity

The observed effects in the studies on *PFOA* presented above are increased liver and kidney weights in dams, growth deficits in the litter, including delays in eye opening, delayed in growth of body hair, increased skeletal defect, delayed phalange ossification, delayed eruption of incisors, increased incidence of cleft sternum, delayed vaginal opening and decreased uterine weight, and altered mammary gland development. It was evaluated that the PPARα is involved in delayed eye opening and deficits in postnatal weight. Further, foetal growth retardation was noted.
The lowest LOAEL reported regarding maternal toxicity is the LOAEL of 0.01 mg PFOA/kg based on delayed mammary gland development in mice from the Macon et al. (2011) study, however the relevance of this finding to humans is still unclear as the CD1-mouse strain used in this study is very sensitive to this end-point. The lowest LOAEL reported regarding developmental toxicity is the LOAEL for wild type offspring of 0.1 mg/kg/day based on increased liver weight from the Abbott et al. (2007) study, whereas a NOAEL of 1 mg/kg/day was found for PPARα-null mice.
In rats a LOAEL for parental males of 1 mg/kg/day was observed for increased liver and kidney weight and for developmental effects a NOAEL of 3 mg/kg/day and a LOAEL of 10 mg/kg/day were observed for decreased body weights of males during the lactation period.

With regard to *PFOS* the observed maternal toxicity effects are decreased body weights, increased liver weights, T_4 effects on GD6-7, and reduction of implementation sites. Developmental toxicity was also observed with increased mortality in pups, delayed ossification, skeletal variation, visceral anomalies, foetal sternal defects and cleft palate, maturation of lung and pulmonary function. Delays in reflex and physical development were observed in the pups which could be link to possible neurotoxicity of PFOS.
The lowest NOAEL/ BMDL5 reported regarding maternal toxicity was the NOAEL of 0.1 mg PFOS/kg/day in rats from the Luebker et al. (2005b) study and BDML5 of 0.05 mg/kg in rats for T_4 effects on GD7 (Hill model) from the Thibodeaux et al. (2003) study.
The lowest NOAEL/ BDML5 reported regarding developmental toxicity was the NOAEL of 0.1 mg/kg/day in rats based on the significant decreases in mean pup body weight from the Luebker et al. (2005b) study, the BDML5 of 0.02 mg/kg in mice for foetal sternal defects (logistic model) from the Thibodeaux et al. (2003) study.

6.6.4 Mutagenicity and carcinogenicity

PFOA and PFOS were evaluated in *in vitro* and *in vivo* genotoxicity tests, and the conclusion was that PFOA and PFOS are not mutagenic and genotoxic.

In carcinogenicity studies with rats *PFOA* has shown to induce increased incidences of Leydig cell adenomas and pancreatic acinar hyperplasia/adenomas, primarily at the highest dose level of 300 ppm (14-16 mg/kg/day) in diet. US EPA (2014a) concluded that the carcinogenic mode(s) of action of PFOA are not clearly understood. However, the weight of evidence suggests that non-genotoxic mechanisms involving binding to receptors and disturbance of the endocrine system may be key events

For *PFOS*, EFSA (2008) concluded that PFOS is hepatotoxic and carcinogenic, inducing tumours of the liver. The evidence for induction of thyroid and mammary tumours was considered limited. US EPA (2014b) in their evaluation of the Thomford (2002) study concluded that the evidence of carcinogenicity was suggestive but not definitive as the tumour incidence did not indicate a dose response. With respect to mode of action data was considered inadequate for suggesting peroxisome proliferation as mode of action for the liver and thyroid adenomas.

6.6.5 Modes of action

The critical effects of PFOA in rodents and monkeys are on the liver. In rodents these effects may be related to the peroxisome proliferating activity of PFOA. Rats showed PPAR activity at exposure levels of 0.64 mg/kg bw/day and more showing that PFOA acts as a PPARα-agonist. By activating PPARα, PFOA also interferes with lipid and lipoprotein metabolism. The EFSA Panel concluded that not all of the liver toxicity could be ascribed to PPARα activity and other possible mechanisms such as induction of genes involved in lipid metabolism and transport of lipids and drug-metabolising enzymes could be of relevance to human health. The negative outcome of a series of genotoxicity tests indicated an indirect mechanism for the carcinogenicity of PFOA. The mechanisms underlying its carcinogenicity activity in rats were attributed to a non-genotoxic mechanism, involving activation of receptors and perturbations of the endocrine systems. The data presently available suggest that the induction of Leydig cell tumours and mammary gland neoplasms may be due to hormonal imbalance resulting from activation of the PPARα and induction of the cytochrome P450 enzyme, aromatase. Endocrine disruption effects were observed, including inhibition of testosterone biosynthesis and increase serum estradiol.

Several studies show that PFOS interferes with fatty acid metabolism and metabolism of lipids and lipoproteins. The link to the liver toxicity that is observed in rodents and monkeys is not well understood. It was reported that PFOS was less active than PFOA for both mouse and human PPARα and PPARβ. There are other pathways by which PFOS can interfere with lipid metabolism in the liver. One of these is competition of PFOS with fatty acids and other endogenous ligands for binding to the important intracellular liver fatty acid transporter proteins which may contribute to hepatotoxicity and lower serum cholesterol levels. In addition the induction of a spectrum of liver enzymes has been observed. PFOS has been shown to inhibit *in vitro* gap junction intercellular communication in rat liver cell lines and in the liver of PFOS-treated rats. This mechanism may also be involved in liver carcinogenesis. Based on the complete lack of genotoxicity in a wide range of *in vitro* and *in vivo* assays, the weight of evidence indicates an indirect (non-genotoxic) mechanism for the carcinogenicity of PFOS. Regarding endocrine disruption effect, oral administration of PFOS resulted in increased tissue availability of thyroid hormones and turnover of T4, but the pattern of changes seen was not typical of a hypothyroid state. Although reproductive and developmental toxicity have been described, the underlying mechanism remains unclear. Besides, PFOS can cross the blood brain barrier and accumulated in the hypothalamus and it increased norepinephrine concentrations in the para ventricular nucleus of the hypothalamus. Treatment with PFOS affected oestrous cyclicity and increased serum corticosterone levels while decreasing serum leptin concentrations.

6.7 TDI-derivation

6.7.1 PFOA, identification of critical studies for TDI derivation

EFSA (2008)

For PFOA, EFSA (2008) identified increased liver weight and liver toxicity as the critical end-point and established a TDI for PFOA of 1.5 µg/kg bw per day by applying an overall UF of 200 to a BMDL10 level for this effect. An UF of 100 was used for inter- and intra-species differences and an additional UF of 2 to compensate for uncertainties relating to the internal dose kinetics.

The BMDL10 value was derived on the analysis made by COT (2006a) where BMDL10 levels for increased liver weight and liver toxicity were estimated from the studies by Lau et al. (2006); Palazzolo (1993); Perkins et al. (2004); Sibinski (1987); and Butenhoff et al. (2004). From these studies BMDL10 levels in the range of 0.29-0.74 mg/kg bw/day were estimated and based on this a BMDL10 level of 0.3 mg/kg bw/day was chosen as point of departure for the TDI estimation. A specific BMDL10 level of 0.2 mg/kg bw/day was both derived from the Palazzolo (1993) study and from the Butenhoof et al. (2004) study.

US EPA (2014a)
US EPA (2014a) also identified the liver effects as the most critical effects. And made RfD calculations on BMDL10 estimates from the Palazollo (1993), the York (2002) study (these data also reported by Butenhoff et al. (2004), and the newer Loveless et al. (2008) study). However, even though very low RfD levels of 0.005-0.007 µg/kg/day were found from the Loveless et al. (2008) BMDL10 levels on mouse and rat data, these data did not allow derivation of a human equivalent dose level (HED) as did the data from the Palazollo (1993) and York (2002) studies. In the end US EPA put most emphasis on the RfD derived from studies, where data allowed for a HED derivation from the NOAEL and LOAEL dose levels (i.e. the studies that contain data on serum levels at these dose levels from which to extrapolate to humans). RfD values obtained from the HED values from the studies by Palazzolo (1993); Lau et al. (2006) and York (2002) all resulted in a value of 0.02 µg/kg/day which was then considered by the US EPA as the most robust value for the Rfd.

Thus, these evaluations and the studies referred to will, together with the methods for TDI/RfD derivation as described in Chapter 5, be the starting point for the TDI derivation for PFOA in Chapter 7.

6.7.2 PFOS, identification of critical studies for TDI derivation

EFSA (2008)
EFSA (2008) identified the lowest no-observed adverse-effect level (NOAEL) of 0.03 mg/kg bw/day from the study by Seacat et al. (2002) with Cynomolgus monkeys showing changes in lipids and thyroid hormones at the second highest dose of 0.15 mg/kg bw per day. The NOAEL of 0.03 mg/kg bw/day was concluded, even though Seacat et al. (2002) concluded the next dose level of 0.15 mg/kg bw/day as a NOAEL, as adverse effects in relation to changes on hormones and lipids, increased liver weights and increased mortality were found to occur at the dose level of 0.75 mg/kg bw/day.

EFSA (2008) then established a TDI for PFOS of 150 ng/kg bw/day by applying an overall uncertainty factor (UF) of 200 to the NOAEL. An UF of 100 was used for inter and intra-species differences and an additional UF of 2 to compensate for uncertainties in connection to the relatively short duration of the key study and the internal dose kinetics.

US EPA (2014b)
US EPA (2014) analysed the same data base as EFSA (2008) but derived 0.03 µg/kg/day as the RfD for PFOS from the newer study by Butenhoff et al. (2009) based on developmental toxicity and liver weight as the most sensitive end-points. This value is the outcome for modeled rat serum values for developmental neurotoxicity. In the standard developmental neurotoxicity study, male offspring showed increased motor activity and decreased habituation on PND 17 following a maternal dose of 1 mg/kg/day in the absence of effects on pup body weight. The human equivalent dose (HED) used as the basis for the RfD, was calculated from an average serum concentration of 10.87 mg/L derived from the NOAEL of 0.3 mg/kg/day for developmental neurotoxicity. A pharmacokinetic model was used to predict an area under the curve (AUC) for the NOAEL and used to calculate an HED-NOAEL. The total uncertainty factor (UF) applied to the HED-NOAEL from the rat study was 30 which included a UF of 10 for intrahuman variability, and a UF of 3 to account for toxicodynamic differences between animals and humans. Comparable values derived from the HED for liver effects in rats and developmental effects in mice are slightly higher than the RfD indicating that it will be protective.

Thus, these evaluations and the studies referred to will, together with the methods for TDI/RfD derivation as described in Chapter 5, be the starting point for the TDI derivation for PFOS in Chapter 7.

7. TDI and quality criteria

Below the evaluations and TDI/RfD derivations for PFOA and PFOS made by EFSA (2008) and US EPA (2014a+b) will form the basis for the discussion and derivation of TDI values for PFOA and PFOS and PFOSA.

7.1 TDI

7.1.1 PFOA

Compared to the EFSA (2008), the evaluation by US EPA (2014) further included two newer studies reported by Loveless et al. (2008) and Dewitt (2008) in their analysis and for the RfD estimations as given in Table 5-3.

TABLE 5-3. RFD ESTIMATIONS FOR PFOA BY US EPA (2014A)

RfD estimations by US EPA (2014a)			
POD ref, species, d uration	POD-value mg/kg bw/d	UF total	RfD µg/ kg bw/d
NOAEL Palazzolo, rat, 90D	0.06	21400	0.003
LOAEL Palazzolo, rat, 90D	**0.64**	214000	0.003
BMDL Palazzolo, rat, 90D	**0.456**	21400	0.02
BMDL York, rat, 70-127D	0.274	21900	0.01
BMDL Loveless, rat, 29D	0.152	21900	0.007
BMDL Loveless, mouse, 29D	0.0681	15000	0.005
HED-NOAEL Palazzolo, rat, 90D	0.00047	30	0.02
HED-LOAEL Palazzolo, rat, 90D	**0.0045**	300	0.02
HED-LOAEL Dewitt, mouse, 15D	0.0028	300	0.009
HED-LOAEL Lau, mouse, 17-18D	0.0057	300	0.02
HED-LOAEL York, rat, 70-127D	0.0065	300	0.02
HED-LOAEL Thomford, monkey, 182D	0.0124	300	0.04

BMDL: Bench Mark Dose Level (lower 5% confidence value)
HED: Human Equivalent Dose level (obtained from pharmacokinetic modelling to achieve equivalent serum concentrations as in experimental animals)

Although lower RfD values were obtained from these studies they did not, however, influence the choice of the RfD value. So overall, it was the same studies that were the basis for the TDI derivation by EFSA (2008) and for RfD derivation by the US EPA (2014).

It should be noted that immunotoxicity has also been found as a sensitive end-point and most clearly in the Dewitt et al. (2008) study that observed a NOAEL of 1.88 mg/kg bw/day, however the

HED-LOAEL in the above table is based at the even lower LOAEL of 0.94 mg/kg/bw/day in relation to liver toxicity.

It may be noted that the lowest uncertainty factor of 30 was used in connection with a HED-NOAEL from the Palazzolo 90D rats study, whereas an uncertainty factor of 300 (an extra factor of 10) was used for the HED values as these were based on LOAEL levels. For 5 of the 6 RfD-values obtained from the HED values as point of departure were obtained from a HED-LOAELs using an extra assessment factor UFL of 10. Thus only one RfD value obtained from HED values was based on a HED-NOAEL. However, the NOAEL of 0.06 mg/kg bw/day in rats used for estimation of the HED-NOAEL was a dose level 10 below the LOAEL of 0.64 mg/kg bw/day in the study by Palazzolo (1993). The data from this 90D rat study also allowed for a *benchmark dose approach* leading to a BMDL10 level in rats of 0.456 mg/kg bw/day that is rather close to the LOAEL value of 0.64 mg/kg bw/day.

When available, it is generally preferred to use a BMDL as point of departure for TDI/RfD derivation compared to the NOAEL/LOAEL where the spacing and selection of the specific dose levels determine the value rather than the actual dose-response relationship. So overall, it may be more relevant to use a benchmark dose as point of departure (as also used by EFSA, 2008), in order to avoid additional use of assessment factors for LOAEL to NOAEL extrapolation and the influence of the actual dose-levels selected for the studies.

In relation to interspecies extrapolation, EFSA (2008) used the traditional factor of 100 to account for inter and intraspecies differences and an extra factor of 2 to account for additional uncertainties relating to the internal dose kinetics.

To overcome this aspect regarding differences in toxicokinetic factors, including serum half-lives among species, great efforts have been made in the US EPA (2014) for using pharmacokinetic modelling in order to take account of this. In that sense the use of HED values as starting points for RfD derivation is to be considered more reliable than the use of an experimental animal NOAEL level and applying standard assessment factors.

Based on these considerations, it therefore seems most relevant to use the BMDL10 values derived by EFSA and US EPA for the Palazzolo (1993) study as a starting point and then use this dose as a starting point for derivation of a HED value.
EFSA (2008) derived a BMDL10 of 0.29 mg/kg bw/day and *0.44 mg/kg bw/day* from the Palazzolo (1993) study in connection with study durations of 49 days and 90 days, respectively. US EPA derived a BMDL10 $_{\text{Palazzolo, rat, 90D}}$ of *0.456 mg/kg bw/day* for the 90 days exposure duration. Further, US EPA made their HED derivation for the NOAEL and LOAEL obtained from the 90 days of exposure. However, they did not make a HED derivation based in the BMDL10 value.

An estimate for this may be done by transforming the BMDL10 level to a HED level by dividing the BMDL10 level with the LOAEL/ HED-LOAL ratio , i.e.:

HED-BMDL10 = BMDL10 $_{\text{Palazzolo, rat, 90D}}$ / (LOAEL $_{\text{Palazzolo, rat, 90D}}$ / HED-LOAEL $_{\text{Palazzolo, rat, 90D}}$)
HED-BMDL10 = 0.456 mg/kg bw/d* / (0.64 mg/kgbw/d */ 0.0045 mg/kg bw/d*)
HED-BMDL10= 0.003 mg/kg bw/d

(*The **bolded** figures in table 7.1.)

From this HED-BMDL10 the TDI can be derived:

TDI = HED-BMDL10 / UFI x UFII…UFn

TDI = 0.003 mg/kg bw/day / 10 x 3 = 0.0001 mg/kg bw/day = 0.1 µg/ kg bw/day

Here an intra-species uncertainty factor of 10 (default value) for the human population is used. An interspecies uncertainty factor of 3 is further used for possible differences in toxicodynamics (according to Danish EPA, 2006).

No uncertainty factors for intrapecies differences for toxicokinetics and study duration are used, as these aspects are covered when deriving the pharmacokinetic modelled HED-value where modelling takes into account that steady state serum levels is achieved in humans.
Further, the BMDL10 is considered to correspond to a very low effect level generally corresponding to a NOAEL value.

7.1.2 PFOS

As mentioned in Chapter 5 there are some different interpretations among EFSA (2008) and US EPA (2014) of the identification of the N(L)OAEL values for liver toxicity in the studies by Seacat et al. (2002) and Thomford (2002) which may have implication on the derivation of the TDI values.

As indicated in Section 5.3.2, EFSA (2008) concluded on a TDI of 0.0015 mg/kg bw/day based on a NOAEL of 0.03 mg/kg bw/day from the Seacat et al. (2002) studies in monkeys and using an overall assessment factor of 200, where an extra factor of 2 in addition to the standard factor of 100 was used based on uncertainties regarding the kinetics.

As indicted in the data from the US EPA (2014b) assessment (see data below) US EPA (2014b) used a NOAEL of 0.15 mg/kg bw/day for the Seacat et al. (2002) study and calculated a RfD of 0.00006 mg/kg bw/day, see Table 5-5. Thus this value would have been about a factor 5 lower if the starting point was a NOAEL of 0.03 mg/kg bw/day as concluded by EFSA (2008).

TABLE 5-5. RFD ESTIMATIONS FOR PFOS BY US EPA (2014A)

Study	NOAEL	RfD NOAEL	BMDL	RfD BMDL	HED-NOAEL	RfD HED-NOAEL
	----------mg/kg bw/day----------					
Seacat et al., 2002 monkey	0.15	0.00003	0.015	0.000003	0.0019	*0.00006*
Seacat et al., 2003 rat	0.34	0.00003	0.059	0.000005	0.0014	0.00005
Thomford, 2002 rat	0.018	0.00001	0.033	*0.00003*	-	-
Thibodeaux et al., 2003 rat	1.0	0.00008	0.12	0.00001	-	-
Lau et al., 2003 rat	1.0	0.00008	0.58	0.00005	0.0014	0.00005
Butenhoff et al., 2009 rat	0.3	0.00002	-	-	0.00088	***0.00003***
Luebker et al., 2005b rat	0.1	0.000008	-	-	0.00037	0.00001
Luebker et al., 2005a rat	0.4	0.00003	0.27	0.00002	0.0019	0.00006

Overall, US EPA (2014) concluded on an overall RfD of 0.0003 mg/kg/day based on the calculated HED-RfD value calculated from the NOAEL of 0.3 mg/kg bw/day (based on changes in acidity/ motility in pups) from Butenhoff et al. (2009).

It might, however, have been interesting to derive a HED-RfD based on the Thomford (2002) study, as this study in fact provides the lowest NOAEL of 0.018 mg/kg bw/day based on liver finding after chronic exposure to rats. It should be noted that this NOAEL is rather comparable to the NOAEL of 0.03 mg/kg bw/day in relation to effects on the liver in monkeys concluded by EFSA (2008).

Overall, a valid NOAEL should be considered to be identified with a greater accuracy from a chronic study using 40-70 animals of each gender at each dose levels (Thomford et al., 2002) compared to a subchronic/subacute study in monkeys using 6 animals of each gender at each dose level.

There is, however, some discrepancy between EFSA (2008) and US EPA (2014b) about the identification of the NOAEL from the Thomford et al. (2002) study. But US EPA (2014) also made a benchmark dose analysis of this dataset and derived a BMDL10 level of 0.033 mg/kg bw/day. Thus the discrepancy defining the NOAEL may be overcome by using the BMDL10 as calculated by US EPA (2014b) as a starting point for the TDI estimation for PFOS.

Therefore, the most adequate starting point for TDI derivation is to be considered a BMDL10 **(**Thomford, 2002 rat level) of 0.033 mg/kg/day (indicated in **bold** in Table 7.2).

It should be noted that no HED estimations were made from the Thomford et al. (2002) study, and therefore an intraspecies factor for toxicokinetic differences has to be accounted for when using the BMDL10 of 0.033 mg/kg bw/day for the TDI calculation:

$TDI = BMDL10_{\text{Thomford, 2002 rat}} / UFI \times UFII \times ...UFn$

TDI = 0.033 mg/kg bw/day / 41 x 3 x 10 = 0.00003 mg/ kg bw/day

Where the intraspecies uncertainty factor UF1 consists of a data derived subfactor of 41* for pharmacokinetic differences (in relation to clearance rate) and a subfactor of 3 for possible differences in pharmacodynamics.

Factor UFII is the default factor of 10 for intraspecies (human) variability in kinetics and dynamics.

No further uncertainty factors are used as the use of a BMDL10 level is considered to correspond to a NOAEL value. Also the basis for the BMDL10 levels is data from a chronic study so no duration factor is needed.

* the factor of 41 can according to US EPA (2014) by derived by using the following assumptions and method:

> Pharmacokinetic studies with PFOS have measured a serum half-life of approximately 48 days in rats. The equation also utilizes a volume of distribution component, which for humans has been calibrated as 230 mL/kg. This volume of distribution is similar to those reported for monkeys, female rats, and mice in pharmacokinetic studies (Chang et al., 2012).
>
> The equation that describes first order kinetics:
>
> $CL = Vd \times (\ln 2 / t1/2)$
>
> Where:
>
> Vd = 0.23 L/kg
>
> Ln 2 = 0.693

Half-life = 48 days for rats and 1971 days for humans

CLrat = 0.23 L/kg x (0.693 / 48 days) = 0.0033 L/kg/day

CLhuman = 0.23 L/kg x (0.693 /1971 days) = 0.000081 L/kg/day

The ratio between CLrat and CLhuman can be calculated to 41 (0.0033 L/kg/day divided by 0.000081 L/kg/day = 41) and is used as the pharmacokinetic adjustment for clearance differences between these species.

7.1.3 Derived TDI values for PFOA, PFOS and PFOSA

The following TDI values have been derived:

TDI_{PFOA} = 0.1 µg/ kg bw/day
TDI_{PFOS} = 0.03 µg/ kg bw/day

PFOSA. Sufficient data was not available for derivation of a specific TDI value for PFOSA. However, as the chemical structure of PFOSA is very comparable to PFOS (the amide derivate of PFOS) and as PFOSA is used as a precursor for PFOS formation it seems justifiable to apply the TDI for PFOS on PFOSA as well
TDI_{PFOSA} = 0.03 µg/ kg bw/day

7.2 Health-based quality criteria in drinking water

The health based quality criteria in drinking water can according to the method described by the Danish EPA (2006) guidance be calculated as follows:

7.2.1 PFOA (and salts thereof e.g. APFO)

$$QC_{dw} = \frac{TDI \times f}{Ingestion_{dw}\ (L/kg\ w/day)}$$

Where f is the percent of the TDI allocated to exposure from drinking water.

$$QC_{dw} = \frac{0.1\ \mu g/\ kg\ bw/d \times 0.1}{0.03\ L\ /kg\ bw/day} = 0.3\ \mu g/L$$

An allocation f = 0.1 for exposure through drinking water is used as already the population is exposed via food, dust and from (old) consumer products. EFSA (2012) assessed the existing 95th percentile estimate for dietary exposure in toddlers to 32 ng/kg bw/day, which is nearly 33% of the TDI value used. Thus only a fraction of the TDI can be used for the drinking water and an allocation of 10% is chosen as indicated as the default value by Danish EPA (2006).

A drinking water ingestion rate of 0.03 L/kg bw/day is used as the average water intake of children (Danish EPA 2006).

7.2.2 PFOS

$$QC_{dw} = \frac{TDI \times f}{Ingestion_{dw}\ (L/kg\ w/day)}$$

Where f is the percent of the TDI allocated to exposure from drinking water.

$$QC_{dw} = \frac{0.03\ \mu g/\ kg\ bw/d \times 0.1}{0.03\ L\ /kg\ bw/day} = 0.1\ \mu g/L$$

An allocation f = 0.1 for exposure through drinking water is used as already the population is exposed via food, dust and from (old) consumer products.
EFSA (2012) assessed the existing upper bound 95th percentile estimate for dietary exposure in toddlers to 29 ng/kg bw/day, which is nearly 100% of the TDI value used. Thus only a fraction of the TDI can be used for the drinking water and an allocation of 10% is chosen as indicated as the default value by Danish EPA (2006).

A drinking water ingestion rate of 0.03 L/kg bw /day is used as the average water intake of children (Danish EPA, 2006).

7.2.3 PFOSA

As for the TDI value, the health based quality criteria of 0.1 µg/L for PFOS may be applied for PFOSA as well, as PFOSA as a precursor for PFOS may be transformed to PFOS.

7.2.4 Composite drinking water quality criteria on PFOA, PFOS and PFOSA

As the toxicological profiles and the toxicological potency of PFOA and PFOS are very similar it seems justified to use an additive approach for these substances when evaluating situations where PFOA, PFOS and PFOSA occur in the drinking water at the same time.
Thus for complying with a composite drinking water quality criteria the addition of the *concentration/limit value* ratios for PFOA, PFOS and PFOSA should be kept below the value of 1. This concentration addition approach also termed the hazard index approach (DG Health and Consumers, 2011) can be written as:

$$PFOA\ (conc. / QC_{dw}) + PFOS\ (conc. / QC_{dw}) + PFOSA\ (conc. / QC_{dw}) < 1$$

or

$$PFOA\ (conc.\ \mu g/L) / 0.3\ \mu g/L + PFOS\ (conc.\ \mu g/L) / 0.1\ \mu g/L + PFOSA\ (conc.\ \mu g/L) / 0.1\ \mu g/L < 1$$

In Sweden, Livsmedelsverket (2014) has adopted an approach where the limit value for PFOS as the most toxic substance also is used for the total content (the sum) of other perfluorinated alkyl acids. Using such an approach for PFOA and the PFAS as listed in appendix 2 is considered as a conservative and protective approach (based on the preliminary assessment of these substances in Appendix 2).

7.3 Ground water quality criteria

In cases where the ground water is used directly for drinking water consumption the same health based quality criteria for PFOA, PFOS and PFOSA should apply for the ground water.

In cases where contaminated grounds affects the ground water the same health based drinking water quality criteria can be applied for the ground water affected by the contamination.

7.4 Health based soil quality criteria

The health based quality criteria in soil can according to the method described by the Danish EPA (2006) guidance be calculated as follows:

7.4.1 PFOA (and salts thereof, e.g. APFO)

$$QC_{soil} = \frac{TDI \times V \times f}{E_{soil}\ (kg/day)}$$

Where f is the percent of the TDI allocated to exposure from soil; V is the bodyweight of a child (13 kg); Esoil is the daily soil ingestion rate of the child (0.0001 kg/d) according to the methods provided by Danish EPA (2006).

$$QC_{soil} = \frac{0.1\ \mu g/\ kg\ bw/day \times 13 \times 0.1}{0.0001\ kg\ /day} = 1300\ \mu g/kg \quad or \quad 1.3\ mg/kg\ soil$$

An allocation f = 0.1 for exposure through soil is used as already the population is exposed via food, dust and from (old) consumer products. EFSA (2012) assessed the existing 95th percentile estimate for dietary exposure in toddlers to 32 ng/kg bw/day, which is nearly 33% of the TDI value used. Thus only a fraction of the TDI can be used for the contribution from soil and an allocation of 10% is chosen as indicated as the default value by Danish EPA (2006).

7.4.2 PFOS

$$QC_{soil} = \frac{TDI \times V \times f}{E_{soil}\ (kg/day)}$$

Where f is the percent of the TDI allocated to exposure from soil; V is the bodyweight of a child (13 kg); Esoil is the daily soil ingestion rate of the child (0.0001 kg/d) according to the methods provided by Danish EPA (2006).

$$QC_{soil} = \frac{0.03\ \mu g/\ kg\ bw/day \times 13 \times 0.1}{0.0001\ kg\ /day} = 390\ \mu g/kg \quad or \quad 0.39\ mg/kg\ soil$$

An allocation f = 0.1 for exposure through soil is used as already the population is exposed via food, dust and from (old) consumer products.
EFSA (2012) assessed the existing upper bound 95th percentile estimate for dietary exposure in toddlers to 29 ng/kg bw/day, which is nearly 100% of the TDI value used. Thus only a fraction of the TDI can be used for the contribution from soil and an allocation of 10% is chosen as indicated as the default value by Danish EPA (2006).

7.4.3 PFOSA

As for the TDI value, the health based soil quality criteria of 0.39 mg/kg soil for PFOS may be applied for PFOSA as well, as PFOSA as a precursor for PFOS may be transformed to PFOS.

7.4.4 Composite soil quality criteria for PFOA, PFOS and PFOSA

As the toxicological profiles and the toxicological potency of PFOA and PFOS are very similar it seems justified to use an additive approach for these substances when evaluating situations where PFOA, PFOS and PFOSA occur in the soil at the same time.
Thus for complying to a composite soil quality criteria the addition of the *concentration / limit value* ratios for PFOA, PFOS and PFOSA should be kept below the value of 1. This concentration addition approach also termed the hazard index approach (DG Health and Consumers, 2011) can be written as:

PFOA (conc. / QC_{soil}) + PFOS (conc. / QC_{soil}) + PFOSA (conc. / QC_{soil}) < 1

or

PFOA (conc. mg/kg) / 1.3 mg/kg + PFOS (conc. mg/kg) / 0.39 mg/kg
+ PFOSA (conc. mg/kg)/0.39 mg/kg < 1

In Sweden, Livsmedelsverket (2014) has adopted an approach where the limit value for PFOS as the most toxic substance also is used for the total content (the sum) of other perfluorinated alkyl acids. Using such an approach for PFOA and the PFAS as listed in appendix 2 is considered as a conservative and protective approach (based on the preliminary assessment of these substances in Appendix 2).

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Appendix 1: Provisional TDI values derived by Livsmedelsverket, 2013

Table with provisional TDI values derived by Livsmedelsverket, 2013

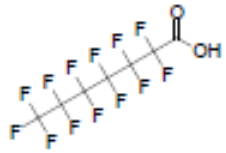
Substans	Djurslag, exponering	Kritisk effekt	NOAEL/LOAEL/BMDL[1]	Blodhalt (ng/ml)	Faktor	Tolerabelt intag (ng/kg/dag)
PFHxS[a]	Råtta, 42 dagar	Lever (♂)	NOAEL: 1 mg/kg/d	89 000	200	5 000
PFHxS[a]	Råtta, 14 d före/under dräktighet	Reproduktion (♂♀ inga effekter)	NOAEL: 10 mg/kg/d	60 000	200	100 000
PFHxS[a]	Råtta, 42 dagar	Kolesterol (♂)	LOAEL: 0,3 mg/kg/d	44 000		
PFOS[b]	Apa, 182 dagar	Kolesterol, sköldkörtelhormoner (♂♀)	NOAEL: 0,03 mg/kg/d	13 000	200	150
PFOS[a]	Råtta, 2 år	Lever	NOAEL: 0,025 mg/kg/d	4 040	100	250
PFOS[a]	Råtta, 2 generationer	Reproduktion (♀)	NOAEL: 0,1 mg/kg/d	4 900	100	1 000
PFOS[a]	Mus, 28 dagar	Immunförsvaret (♂♀)	NOAEL: 0,00016 mg/kg/d	18		
PFHxA[a]	Råtta, 90 dagar	Lever (♂)	NOAEL: 20 mg/kg/d		200	100 000
PFHxA[a]	Råtta, 49 d före/under dräktighet	Reproduktion (♂♀ avkomma)	NOAEL: 100 mg/kg/d		100	1 000 000
PFOA[b]	Råtta, under dräktighet	Lever (♂ avkomma)	BMDL10: 0,3 mg/kg/d		200	1 500
PFOA[b]	Råtta, 13 veckor	Lever (♂)	NOAEL: 0,06 mg/kg/d	7 100	200	300
PFOA[a]	Mus, dräktighet	Reproduktion (♀)	BMDL05: 0,86 mg/kg/d	15 700	200	4 300
PFOA[a]	Mus, under dräktighet	Bröstkörtelutveckling (♀ avkomma)	LOAEL: 0,01 mg/kg/d	150		
PFNA[a]	Mus, 14 dagar	Lever (♂♀)	LOAEL: 0,5 mg/kg/d		1200	420
PFNA[a]	Mus, under dräktighet	Reproduktion (♂♀ avkomma)	NOAEL: 0,83 mg/kg/d	8 900	100	8 300
PFDA[a]	Mus, under dräktighet 10 d	Lever (♀)	NOAEL: 0,3 mg/kg/d		600	500
PFDA[a]	Mus, under dräktighet	Reproduktion (♂♀ avkomma)	NOAEL: 0,03 mg/kg/d		100	300
PFUnDA	Inga studier					
PFDoDA[a]	Råtta, 110 d	Lever (♂)	LOAEL: 0,02 mg/kg/d		400	50
PFTrDA	Inga studier					

a: Borg D, Håkansson H. Environmental and Health Risk Assessment of Perfluoroalkylated and Polyfluoroalkylated Substances (PFASs) in Sweden. Stockholm, Sverige: Naturvårdsverket 2012.

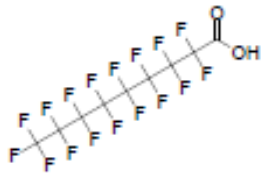
b: EFSA. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and thier salts. Scientific opinion of the Panel on Contaminants in the Food Chain. The EFSA Journal. 2008;653:1-131.

Appendix 2: Information on selected PFAS substances: PFHpA, PFNA, PFBS, PFHxS, PFDS, PFHxA

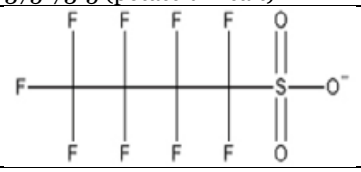
The reports from ATSDR (2009), Swedish EPA (2012); NCM (2013); and Livsmedelsverket (2013) were found to the best sources for collecting an updated overview of the available data on the substances PFHpA, PFNA, PFBS, PFHxS, PFDS, PFHxA.
Data are given in the six tables below and for each substance an *initial assessment* is given whether data indicate that read-across to PFOA and PFOS can be made and whether it may be considered to apply the TDIs and quality criteria for PFOA/PFOS.

Substance name	**Perfluoroheptanoic acid**
Abbreviation	PFHpA
CAS	375-85-9
Molecular formula	$C_7HF_{13}O_2$
Molecular structure	
Molecular weight	364.06
Physic-chemical properties	pKa: -0.15 (estimated) (ATSDR 2011) Solubility: 118 g/l (24 °C) (Danish EPA, 2014) Vapour pressure: 128 Pa (Danish EPA, 2014)
Toxicological properties	Hepatocytic hypertrophy effect in laboratory animals (NCM, 2013). Toxicity data for PFHpA was not considered sufficient for use in the risk characterization. Therefore toxicity data on PFOA were used. (Swedish EPA, 2012) PFOA point of departure for hepatotoxicity and reproductive toxicity, and other end-points: • Hepatotoxicity (rat, subchronic exposure, NOAEL, hepatocellular hypertrophy): 0.06 mg/kg bw/day, 7.1 µg/ml serum. No hepatic levels were available. However, a conservative approach is to use a liver:serum ratio of 2:1. • Reproductive toxicity (mouse, reduced F1 body weight, BMDL): 0.86 mg/kg bw/day, 15.7 µg/ml serum. No hepatic levels were available. However, a conservative approach is to assume a liver:serum ratio of 2:1. • Other end-points (mouse, mammary gland development, increased body weight, LOAEL): 0.01 mg/kg bw/day, 0.15 µg/ml serum (Swedish EPA, 2012).

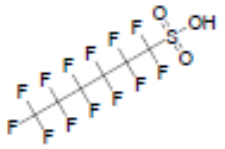
Initial assessment: Based on the conclusion by Swedish EPA (2012) and the structural similarity to PFOA it may be considered to apply the quality criteria on PFOA on PFHpA as well and using an additive concentration approach.

Substance name	**Perfluorononanoic acid**
Abbreviation	PFNA
CAS	375-95-1
Molecular formula	C9HF17O2
Molecular structure	
Molecular weight	464.08
Physic-chemical properties	pKa: -0.17 (estimated) (ATSDR, 2009)
Toxicological properties	Studies suggest that PFNA exposures of rodents are related to liver toxicity including disrupting glucose metabolism; PPAR dependent effects on development and survival upon in utero exposure and PFNA induced immune toxicity. Associations of serum PFNA with thyroid hormone levels was observed (NCM, 2013) Points of departure for hepatotoxicity and reproductive toxicity: Hepatotoxicity (mouse, subacute exposure, LOAEL, increased liver weight): 0.83 mg/kg bw/day, 28.5 µg/ml serum. No hepatic levels were available. However, a conservative approach is to use a liver:serum ratio of 2:1. (Swedish EPA, 2012) Reproductive toxicity (mouse, reduced F1 survival, NOAEL): 0.83 mg/kg bw/day, 8.9 µg/ml serum. No hepatic levels were available. However, a conservative approach is to assume a liver/serum ratio of 2:1 (Swedish EPA, 2012) Derived-No-Effect-Levels (DNELs) (ng/ml serum) for hepatotoxicity and reprotoxicity in individuals exposed indirectly via the environment is 190 ng/ml serum and 356 ng/ml serum respectively and in occupationally exposed individuals is 380 ng/ml serum and 712 ng/ml serum respectively (Swedish EPA, 2012). A provisional TDI value of 0.42 µg/kg/day has been derived based on a LOAEL of 0.5 mg/kg/day for liver effects (Livsmedelsverket 2013).

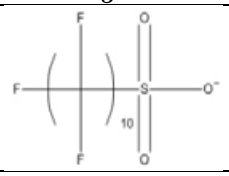
Initial assessment: Based on the data by Swedish EPA (2012) and Livsmedelsverket (2013) it may be further investigated whether to derive a specific quality criteria for PFNA, or whether to apply the quality criteria on PFOA on PFNA based on the structural similarity of the substances.

Substance name	**Perfluorobutane sulfonate**
Abbreviation	PFBS
CAS	45187-15-3 75-22-4 (acid) 375-73-5 (potassium salt)
Molecular formula	
Molecular structure	C4F9O3S
Molecular weight	300.0969 (acid)
Physic-chemical properties	Solubility: 0,51 g/l (Danish EPA, 2014)
Toxicological properties	Mild effects at the liver, kidney and blood parameters have been observed in rat studies upon exposure to PFBS (C4) at relatively high doses (NCM, 2013). No human studies (NCM, 2013). Low to moderate acute toxicity following oral or inhalatory exposure (Swedish EPA, 2012) Points of departure: Hepatotoxicity (rat, subchronic exposure, NOAEL, increased liver weight,): 100 mg/kg bw/day. Reproductive toxicity (rat, NOAEL, reduced F1 bodyweight): 300 mg/kg bw/day. Other end-point (rat, subchronic exposure): Hematological effects: 60 mg/kg bw/day (Swedish EPA, 2012). (Swedish EPA, 2012).

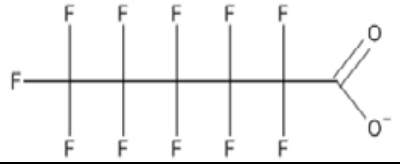
Initial assessment:
Based on the findings by the Swedish EPA, 2012 it may be considered whether it seems possible to derive a specific quality criteria for the substance.

Substance name	**Perfluorohexane sulfonic acid**
Abbreviation	PFHxS
CAS	355-46-4
Molecular formula	
Molecular structure	C6HF13O3S
Molecular weight	400.111
Physic-chemical properties	No data
Toxicological properties	A reproductive and developmental toxicity study of PFHxS was conducted in rats. No treatment-related effect was reported on the fertility and reproductive outcomes or on viability and growth of the offspring at doses as high as 10 mg/kg/day. A NOAEL of 10 mg/kg/day was therefore estimated for the developmental effects of PFHxS (NCM, 2013). Point of departure: Hepatotoxicity (rat, subacute exposure, NOAEL, hepatocellular hypertrophy/increased liver weight): 1 mg/kg bw/day, 89 µg/ml serum, 150 µg/g liver. • Reproductive toxicity (rat, no effects): > 10 mg/kg bw/day, > 60 µg/ml serum, > 17 µg/g liver. • Other end-point (rat, subacute exposure, LOAEL): Hematological effects at 0.3 mg/kg bw/day, 44 µg/ml serum, 44 µg/g liver (Swedish EPA, 2012). A provisional TDI value of 5 µg/kg/day has been derived based on a NOAEL of 1 mg/kg/day for liver effects (Livsmedelsverket 2013).

Initial assessment:
Based on the findings by the Swedish EPA (2012) and Livsmedelsverket (2013) it may be considered whether it seems possible to derive a specific quality criteria for the substance.

Substance name	**Perfluorodecane sulfonate**
Abbreviation	PFDS
CAS	126105-34-8 335-77-3 (acid) 2806-16-8 (potassium salt) 67906-42-7 (ammonium salt)
Molecular formula	C10F21O3S
Molecular structure	
Molecular weight	600.139 (acid)
Physic-chemical properties	No data
Toxicological properties	No toxicology studies were found in animals or human (NCM, 2013). No assessments or relevant scientific publications on the toxicity of PFDS were found. Due to the lack of toxicity data on PFDS, data from PFOS was used (Swedish EPA, 2012).

Initial assessment: Based on the conclusion by Swedish EPA (2012) and the structural similarity to PFOS it may be considered to apply the quality criteria on PFOS on PFDS as well, and using an additive concentration approach.

Substance name	**Perfluorohexanoate**
Abbreviation	PFHxA
CAS	92612-52-7 307-24-4 (acid) 2923-26-4 (sodium salt) 21615-47-4 (ammonium salt)
Molecular formula	C6F11O2
Molecular structure	
Molecular weight	314.05 (acid)
Physic-chemical properties	No data
Toxicological properties	A 90D study suggest that PFHxA exposures of rats affect body and liver weight, changes in liver and hematologic parameters, and that PFHxA is a poor peroxisomal inducer (NCM, 2013). No human studies found (NCM, 2013). Points of departure: • Hepatotoxicity (rat, subchronic exposure, NOAEL, hepatocellular hypertrophy/increased liver weight): 20 mg/kg bw/day. • Reproductive toxicity (rat, reduced F1 body weights, NOAEL): 100 mg/kg bw/day. Due to lack of internal dose measurements, data from PFOA was used for the risk characterization (Swedish EPA, 2012). A provisional TDI value of 100 µg/kg/day has been derived based on a NOAEL of 20 mg/kg/day for liver effects (Livsmedelsverket, 2013).

Initial assessment: It may be further investigated whether the specific data allow for derivation of a TDI and a qualtity criteria for PFHxA as done by Livsmedelsverket (2013).

Overall conclusion on preliminary assessment of PFHpA, PFNA, PFBS, PFHxS, PFDS, PFHxA.

As indicated above data is sparse on these substances. For some of the closest analogue substances Livsmedelverket (2013) has suggested to read across to PFOA or PFOS and their respective TDI values. For the remaining substances data are sparse but indicate lower degree of toxicity than PFOA and PFOS. However, further assessment would be needed in order to derive specific TDI values and limit values for the substances.

Recently EFSA (2014) has published an external report regarding "Extensive literature search and provision of summaries of studies related to the oral toxicity of perfluoroalkylated substances (PFASs)…" which have to be further considered in more in-depth evaluation of the substances. (http://www.efsa.europa.eu/en/search/doc/572e.pdf)

An alternative approach would be the approach used by Livsmedelsverket (2014) where the limit value for PFOS as the most toxic substance also is used for the total content (the sum) of other perfluorinated alkyl acids (se section 5.2.5.).

Using such an approach for PFOA and the PFAS listed in this appendix 2 is considered as a conservative and protective approach (based on the preliminary assessment of these substances as given here).

Perfluoroalkylated substances: PFOA, PFOS and PFOSA

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to the perfluoroalkylated substances, PFOA, PFOS and PFOSA. Additional PFAS substances have furthermore been selected for a preliminary screening in relation to toxicity in order to assess the possibilities for derivation of specific quality criteria for the substances.

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