Literature review to guide the PFAS field survey in the Westerschelde in 2023

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Summary

A literature review was performed to create an overview of the available knowledge on the potential ecological impact of PFAS compounds. This will be used to guide the specific field survey for PFAS in the Westerschelde estuary that is planned for spring and autumn 2023, and to facilitate interpretation of the data that will be collected.

The results of this review are summarised in overviews of relevant European Quality Standards, and effect concentrations of PFAS compounds in water and tissue of invertebrates, fish, birds and mammals. This can help interpretation of results of PFAS analyses in environmental samples. Further it was listed what PFAS have been detected in biota from the Westerschelde during previous research.

Advice for the Westerschelde field campaign
The WMR standard list of PFAS scheduled for analysis covers the most relevant PFAS compounds, based on the information from this review. It should be noted that since the field campaign in 2023 is restricted to analyses in biota it is, based on previous campaigns in the Westerschelde, likely that compounds PFBS, PFTeDA, PFDS, PFPeA, PFHxA and PFHpA will not be detected in the samples above limits of quantification. However, since these compounds are part of the standard list they can be analysed without additional costs.

The additional measurement of biochemical biomarkers may be of interest for the Westerschelde field campaign, as they can be induced by or be a consequence of exposure to PFAS. However, other environmental parameters and contaminants can also play a role in these cases and clear relationships between PFAS and marker could be hard to detect. Nonetheless, we advise to perform a selection of biomarkers in blood samples from seals and harbour porpoises. This concerns biomarkers that are routinely applied in medical research to check functioning of the liver, kidneys, thyroid and the lipid metabolism that in humans can be disrupted by PFAS exposure.

1 Introduction

The abbreviation PFASs is used to address a group of more than 4700 per- and polyfluoroalkyl substances (Panieri et al., 2022). Each of these molecules combine a hydrophilic head with a hydrophobic tail, which makes them surface active and water repellent, while the carbon-fluor connections ensures a high chemical-physical stability. Given these characteristics PFAS are being used in a wide array of products, for instance in heat resistant coatings, in rain clothing and as firefighting foam. An general overview of PFASs (taken from (Nakayama et al., 2019) is included in Annex 1.
The stability of PFASs is also reflected by a high persistency of the substances in the environment. The persistency, indications of bioaccumulation potential, and increasing concern about toxic effects were the reason that in 2009 the production and use of one of the PFASs, perfluorooctane sulfonic acid (PFOS), and its precursor, perfluorooctane sulfonyl fluoride (PFOSF), were globally restricted. In 2015, perfluorooctanoic acid (PFOA), another broadly used PFAS with a carbon chain length of C=8, was listed in the annex B of the Stockholm Convention as substances that should be restricted due to their high persistence and bioaccumulative potential (Panieri et al., 2022) (Ahrens and Bundschuh, 2014). The major fluorochemical producers also agreed to strongly reduce the production of other PFAS substances with longer carbon chains. In search of alternatives for the long chained PFOS and PFOA (C=8), that are now often referred to as the 'legacy PFAS', the industry shifted to shorter chain molecules, such as PFHxA, PFHxS (both C=6), or PFBS (C=4)(Table 1), that were initially expected to have a lower environmental and health impact, although new information does not always support this (De Toni et al., 2022; Dharpure et al., 2023).

Table 1 lists the PFAS compounds with relevance for this report, because 1) European Quality Criteria are available (see paragraph 2), 2) historic data for the Westerschelde are available (see paragraph 3), and 3) there is some insight in effects and effect concentrations (see paragraphs 4, 5, and 6). A more extended list of PFAS compound names and acronyms, taken from Nakayama *et al.,* 2019*,* is included in Annex 1 of this report.

Table 1 List of PFASs and the acronyms, that are relevant for this literature research, based on the availability of 1) European quality criteria, 2) historic data for Westerschelde estuary, and 3) internal threshold concentrations. Ordered by chemical composition and length of the C-chain.

Compound name	C-chain length	Acronym
Perfluorotetradecanoic acid	14	PFTeDA
Perfluorotridecanoic acid	13	PFTrDA
Perfluorododecanoic acid	12	PFDoDA
Perfluoroundecanoic acid	11	PFUnDA
Perfluorodecanoic acid	10	PFDA
Perfluorononanoic acid	9	PFNA
Perfluorooctanoic acid	8	PFOA
Perfluoroheptanoic acid	7	PFHpA
Perfluorohexanoic acid	6	PFHxA
Perfluoropentanoic acid	5	PFPeA
Perfluorobutanoic acid	4	PFBA
Hexafluoropropylene oxide dimer acid	3	HFPO-DA (trade: GenX)
Perfluorodecane sulphonic acid	10	PFDS
Perfluorooctane sulphonic acid	8	PFOS
Perfluoroheptane sulphonic acid	7	PFHpS
Perfluorohexane sulphonic acid	6	PFHxS
Perfluorobutane sulphonic acid	4	PFBS

1.1 Aim of this review

The aim of this review is to create an overview of the available knowledge on the potential ecological impact of PFAS compounds. This will be used to guide the specific field survey for PFAS in the Westerschelde estuary in spring and autumn 2023, and to facilitate interpretation of the data that will be collected on PFAS concentrations in biota. The data can also be used to interpret data on PFAS concentrations in water that is produced in regular monitoring activities.

Therefore, the following research questions were formulated:

- What are the relevant current quality standards for PFAS in biota?
- Which type of effects have been associated with PFAS exposure?
- At what exposure (water) concentrations have effects been reported?
- At what internal (biota) concentrations have effects been reported?

2 European quality criteria

The European Water Framework Directive mentions 'Perfluorooctanesulfonic acid and its derivatives (PFOS)' as priority substance, for which environmental quality standards (EQS) for water and/or biota have to be determined that form the threshold for a good chemical status of the water body. For water, maximum acceptable (peak) concentrations, as well as maximum allowable annual average concentrations have been determined (EU, 2014) (Table 2). For the marine environment EQS for PFOS in water are five times lower than for freshwater. For biota the Water Framework Directive does not differentiate the EQS for marine and freshwater species. The EQS-biota is set at 9.1 µg/kg based on fresh weight in fish, which is standardised to a dry weight percentage of 26%. Since this EQS-biota is related to the risk of secondary poisoning, it is based on the analysis of the whole fish (Foekema et al., 2016).

On 9 July 2020, the European Union adopted the scientific opinion from the European Food and Safety Authority (EFSA, 2020) that concluded that PFOS, PFOA, PFNA and PFHxS can cause developmental effects in humans and may have adverse effects on serum cholesterol, the liver and the immune system and birth weight (EU, 2022). To avoid potential effects on the immune system of young children, a tolerable weekly intake (TWI) of 4.4 ng/kg body weight was established for the sum of these four PFAS compounds (∑EFSA): PFOS, PFOA, PFNA and PFHxS. This level should also be too low for other effects of these substances on humans to occur.
As a result of this, new EU quality standards were established for these four PFAS substances in food in 2022 (EU, 2022), including specific PFAS standards for eggs, fish (filet), crustaceans and shellfish (see Table 2). For fish meat three quality standards were established: two more for specific freshwater or marine species, and a general standard for species not mentioned in these two categories. The general standard for fish meat of the sum of the four substances is 2 µg/kg WW, for the mainly marine species this is 8 µg/kg WW, and for the mainly freshwater species this is 45 µg/L (Table 2).

Table 2 *Overview of European quality standards for concentrations of PFAS in water and biota according to the European Water Framework Directive and food items . DW = dry weight; WW = wet weight; MAC = maximum allowable concentration; AA = annual average; EQS = Environmental Quality Standard. '∑EFSA' = the sum of PFOS, PFOA, PFNA and PFHxS.*

Water Framework Directive (EU, 2014)	**unit**	**PFOS**	**Dutch quality standard** (RIVM 2022)		**PFOA**	**GenX (HFPO-DA)**
Fish EQS-biota (whole-body 26% DW)	µg/kg WW	9.1	Fish	µg/kg WW	1.5	2.6
Freshwater (MAC-EQS)	µg/L	0.00065	Freshwater	µg/L	0.048	0.118
Freshwater (AA-EQS)	µg/L	36				
Marine water (MAC-EQS)	µg/L	0.00013	Marine water	µg/L	0.048	0.118
Marine water (AA-EQS)	µg/L	7.2				

Food safety (EU,2022)	**unit**	**PFOS**	**PFOA**	**PFNA**	**PFHxS**	**∑EFSA**
Eggs	µg/kg WW	1	0.3	0.7	0.3	1.7
Fish meat (filet) - general	µg/kg WW	2	0.2	0.5	0.2	2
Fish meat (filet) - mainly marine	µg/kg WW	7	1	2.5	0.2	8
Fish meat (filet) - mainly fresh water	µg/kg WW	35	8	8	1.5	45
Crustaceans and bivalve mollusks	µg/kg WW	3	0.7	1	1.5	5

The European quality standards (in µg per L or kg) are much stricter than the draft quality criteria for PFOS and PFOA in the freshwater environment that were recently proposed by the US EPA (in mg per L or kg) (*Table 3*; US-EPA 2022).

Table 3 Draft quality criteria for PFOA and PFOS as proposed by US-EPA. Data from US EPA, 2022.

US EPA	unit	PFOS	PFOA
Acute water column	µg/L	3000	49000
Chronic water column	µg/L	8.4	94
Invertebrate whole-body	µg/kg ww	937	1110
Fish whole-body	µg/kg ww	6750	6100
Fish muscle (filet)	µg/kg ww	2910	125

3 PFAS data in the Westerschelde estuary

To fulfil the obligations of the European Water framework Directive, PFOS concentrations in fish (whole-body) are monitored once every three years in the freshwater and coastal waters of the Netherlands. At five coastal/estuarine monitoring locations the flatfish flounder (*Platichthys flesus*) is sampled for this program. In addition to PFOS, at least PFOA, PFHxS and PFNA are monitored to allow calculation of the ∑EFSA. For all four PFAS compounds, the highest concentrations are found in fish from the Westerschelde (*Table **4***; Dogruer et al, 2023). The concentrations of PFOS and PFNA in flounder from the Westerschelde exceed the EQS from the European Water Framework directive for marine fish, which is also the case for most of the other locations, except Hollandse Kust. Also the ∑EFSA is exceeded in flounder at all sampled locations, except for Hollandse Kust, where levels (7.2 ug/kg ww) are just below the standard of 8.0 µg/kg ww.
Jonker (2021) also concluded that the Westerschelde is the PFAS hotspot in the Netherlands based on data from 2019 with the highest concentration of 170 µg/kg PFAS (of which 140 µg/kg PFOS) found in livers of flounders from locations in the Westerschelde. Jonker concludes that these levels are comparable with the high concentrations that can be found in fish from the Great lakes in Northern America.

Table 4 Concentrations of selected PFAS (µg/kg ww) measured in fish (whole-body, flounder) sampled at different locations in the Netherlands 2021 (data from Dogruer et al, 2022).

Location	PFOS	PFOA	PFHxS	PFNA	ΣEFSA
Westerschelde	52	1.3	2.2	1.8	57.3
Noordzeekanaal	42	0.1	0.9	0.6	43.6
Nieuwe Waterweg	14	0.1	0.5	0.6	15.2
Eems-Dollard	9.9	0.8	0.6	1.5	12.8
Hollandse kust (Noordwijk)	6.6	0.3	0.3	<LoQ	7.2

In the fall of 2021 a wide range of PFAS compounds was analysed in water, sediment and edible biota from the Westerschelde (Van den Heuvel-Greve et al, 2022). Eleven of the 21 PFAS compounds that were analysed were not found in any of the samples above the limit of quantification (LoQ). Of the remaining 10 compounds PFOS made up 50-100% of all PFAS in sediment and organisms, lamb's lettuce, shellfish, shrimp and fish (*Table **5***). In shrimp, the contribution of other PFAS compounds than PFOS, was higher than in the other biota. Byns et al. (2022) also found high concentrations of PFOA in shrimp relative to fish in samples from the Belgium North Sea. Also Zafeiraki et al.(2019) found relatively high PFAS concentrations in shrimp.

During the 2021 sampling (Van den Heuvel-Greve et al, 2022) only traces of PFOS, and at a single location PFOA, were detected above the LoQ in sediment samples. Differences between sediment types were not found which could be due to the small sampling sizes. For PFOS it has been described that the affinity with sediment increases with salinity and organic matter content (Chen et al., 2012). Water samples contained low (max 8.5 ng/L) concentrations of all four EFSA PFAS (with chain length C6 to C9), and at some locations slightly higher (max 87 ng/L) concentrations of the short chained (C4), and therefore in general better water soluble, PFBS and PFBA.

In the PFAS concentrations, especially in bivalves but also in water, a decreasing trend could be detected towards the west where the estuary meets the North Sea (Van den Heuvel-Greve et al, 2022).
From an analyses of 250 biota samples, collected at the North Sea between 2012 and 2018, it was concluded that PFAS levels in fish were lower in the northern North Sea than in the southern North

Sea. PFOS mostly dominated the profile, whereas other long-chain PFASs were also detected frequently, but short-chain PFASs were rarely found. The highest PFOS concentration of 67 ng/g ww was detected in an eel sample from the Ghent-Terneuzen canal (Zafeiraki et al., 2019).

Table 5 Range of PFAS concentrations in edible fish (filets of flounder, whiting, smelt and seabass), shell fish (mussel, oyster), shrimp and plants (lamb's lettuce) sampled in 2021 from the Westerschelde (van den Heuvel-Greve et al., 2022). Presented are PFAS compounds that were detected above the limit of quantification (LoQ). PFHxA, PFHpA, PFDS, PFPeA, PFTrDA, PFTeDA, GenX, FBSA, NaDONA, 11Cl-PF3OUdS, and 9Cl-PF3ONS were <LoQ in all samples. The four compounds in bold together form the ∑EFSA.

	µg/kg ww Fish	µg/kg ww Shell fish	µg/kg ww Shrimp	µg/kg ww Plant	µg/kg ww Sediment	ng/L Water
PFOS	1.5 - 35.3	0.4 -0.8	7.0-15.7	0.07-0.10	<0.08 - 0.4	<3.0 - 8.5
PFOA	<0.08 - 0.44	<0.2	0.23-0.47	<0.25	≤0.06	<1.8 - 7.4
PFHxS	<0.2-0.85	<0.20	0.30-0.34	<0.10	<0.08	<1.7 - 5.3
PFNA	<0.07 - 0.81	<0.2 - 1	0.36-0.92	<0.10	<0.06	<3.5
∑EFSA	1.5 - 37.4	0.4 - 1.8	7.9 - 17.4	0.07 - 0.10	<0.47 - 0.4	<10 - 21.2
PFBS	<0.2	<0.1	<0.06	<0.05-0.09	<0.08	<7 - 17
PFBA	-	<1.4	-	-	<0.7	<16 - 87
PFHpS	<0.07 - 0.49	<0.2	0.07-0.16	<0.050	<0.08	<1.8
PFDA	<0.3 -3.3	<0.7	1.2-1.7	<0.10	<0.2	<3.5
PFUnA	<0.2 - 0.89	<0.1 -0.2	0.64-0.9	<0.10	<0.09	<3.5
PFDoDA	<0.08 - 1.6	<0.1 -0.2	0.26-0.84	<0.25	<0.09	<1.9

In 2018 fish, shrimp and shellfish samples were collected at ten locations in the Belgian part of the North Sea, with the outflow of the Westerschelde estuary (line Vlissingen Breskens) as the most eastern location (Byns et al., 2022). In fish from this location PFAS concentrations in filet were reported to be between 2 and to 6 µg/kg WW, with PFOS being the most dominant compound. PFAS concentrations in liver and muscle of the fish were generally related, but up to ten times higher in liver than in muscle on wet weight basis. The location near Zeebrugge (south of the Westerschelde outflow) was identified as the location with the highest PFAS concentrations (Byns et al., 2022). Samples from this location did not only contain higher PFOS concentrations, but also remarkably high concentrations of PFTrA, a very long chained (C13) PFAS molecule, that was hardly detectable in the samples from the Westerschelde outflow. It was also not found above the LoQ in the Westerschelde sampling campaign in 2021 (van den Heuvel-Greve et al., 2022), which suggests that the Westerschelde estuary is not a source for this specific PFAS compound.

In an earlier study in 2007, eggs and adult common terns were collected in a breeding colony near Terneuzen in the Westerschelde estuary and, among others, analysed for PFOS. The average PFOS concentration in livers of the adult female birds was 367 µg/kg ww, while in the eggs 457 µg/kg ww was found (Van den Heuvel-Greve et al, 2010). In the same study blood samples of harbour seals from the same estuary were shown to contain 4947 µg/L PFOS, 20 µg/L PFOA and 233 µg/L PFBS. Around that time these PFOS concentrations were considered to be very high compared to seals from other locations (Dedert et al., 2015). It should be noted that PFAS concentrations in water of the Westerschelde, amongst other those of PFOS and PFOA, have decreased since then (*Figure 1* from Jonker, 2021).

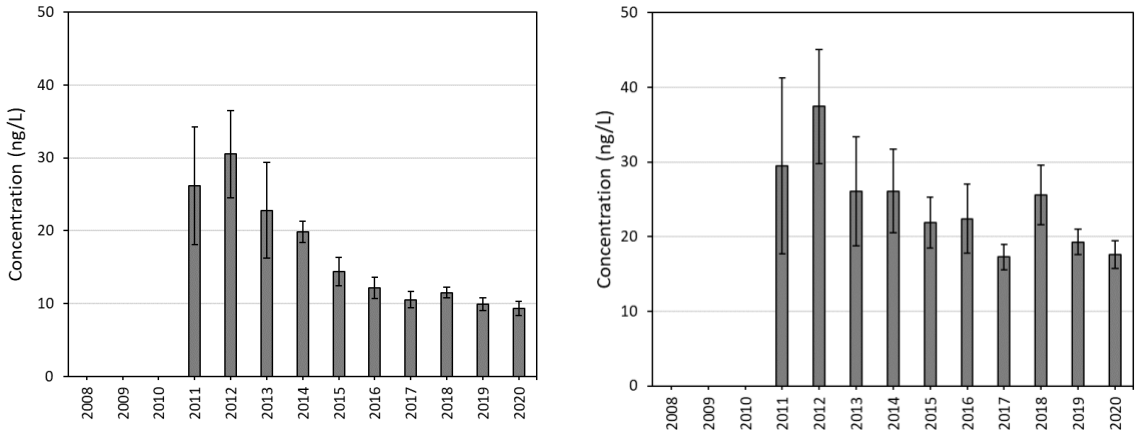

Figure 1 Concentrations of PFOS (left) and PFOA (right) in water samples from the Westerschelde estuary (location 'Schaar van Ouden Doel') in the period 2010 to 2020. Figure from Jonker 2021.

4 Effect types

A large amount of research has been published that is dedicated to the potential effects of PFAS on organisms, especially over the last decade. The types of effects that have been connected to PFAS exposure are very broad and still expanding (Panieri et al., 2022).

Reported endpoints in marine invertebrates are often limited to survival, growth and development of early life stages (Ma et al., 2022);(Hayman et al., 2021); (Sinclair et al., 2020); (Razak et al., 2023). In a rare case physiological responses are reported as well. For instance in green mussels (*Perna viridis*) an immunotoxic response was found to be correlated to PFAS tissue concentrations (Liu and Gin, 2018).

More sophisticated types of effects and underlying mechanisms (Beale et al., 2022) are predominantly studied in larger and more complex organisms, mainly consisting of fish (e.g. (Zhang et al., 2016) (Chen et al., 2019), and mice (Aghaei et al., 2022). The latter is also considered a model for humans. In addition to laboratory exposure studies, insight in effects of PFAS on humans has been gained from epidemiological studies (e.g (Cakmak et al., 2022).

Exposure studies are occasionally supported by or fully focussed on the biochemical response pathways as reviewed by Beale et al., (2022). They concluded that most laboratory exposure studies identify effects of PFAS only at high concentrations that are not realistic for the field situation. They therefore plea for the use of molecular markers in environmental monitoring to bridge the gap between laboratory and field.

Most research has been focussed on PFOS and PFOA as the most commonly detected PFAS, and even for these compounds still many uncertainties exist (Fenton et al., 2021) (*Figure **2***). Information on the toxicological effects of other PFAS compounds is scarce.

For the purpose of our study, common type of effects that have been related to PFAS exposure are summarised in Table 6. Given the broadness of the effect mechanism of the numerous PFAS compounds it was not possible to produce a complete overview within the current project. Therefore effect types were selected that we feel are the most relevant for the aim of the Westerschelde estuary field campaign. This includes both toxicological endpoints that have some potential to be included in the monitoring program, as well as effects that have the potency to have ecological implications. We selected effects on: early development; lipid metabolism and liver functioning; the endocrine system; the immune system and multiple stress situations. Some background information on each of these effect types, based on a variety of studies in laboratory organisms (fish, rats, mice, birds) and humans, is provided in the following paragraphs.

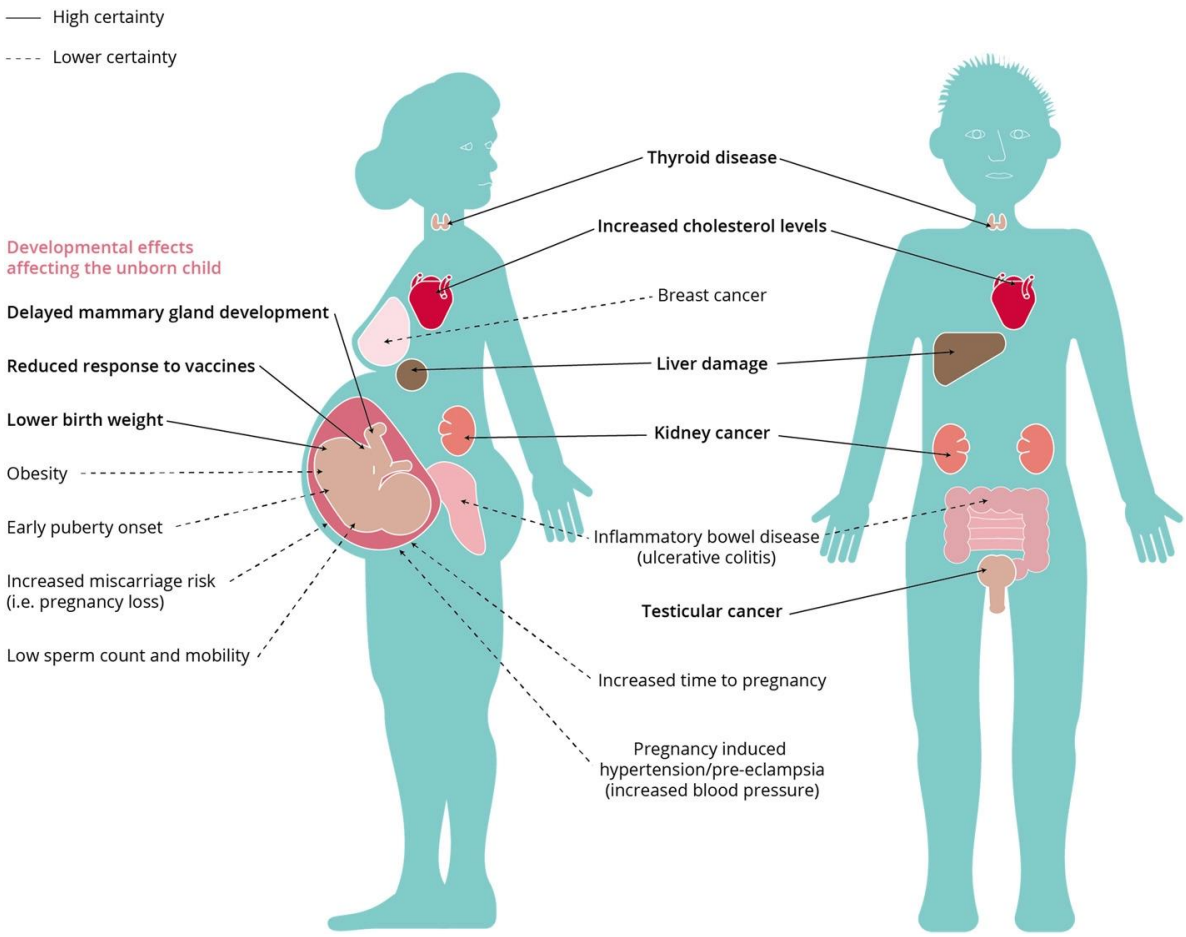

Figure 2 Types of effects associated with PFOS and PFOA exposure in humans (Figure from (Fenton et al., 2021). Note that more types of effects than presented in this figure have been associated with PFOS/PFOA exposure with a lower certainty.

4.1 Early development

Aquatic organisms:
Parental exposure of zebra fish to PFNA resulted in delayed hatching (Zhang et al., 2016), while exposure to PFOS and PFOA caused a wide variety of malformations to the off-spring and a retarded growth rate (Ma et al., 2023). Exposure of fish embryos to PFOA resulted in neurotoxicity (Guo et al., 2018). PFBS exposure of parent zebrafish resulted in higher mortality, delayed hatching, slower heart rate, reduced body weight and neurobehavioral disorders of their offspring (Tang et al., 2020).

Birds:
In 7 days old hatchlings from chicken eggs injected with PFOS pathological changes in liver were detected like inflammations and cell necrosis. Hatchability was negatively correlated with PFOS doses , but no indications were found that body or organ weights of the hatchlings were affected. The higher exposure levels caused embryo mortality (Molina et al., 2006).

Mice & humans:
From a review on mouse exposure studies with PFOS and PFOA, it was concluded that these substances can cross the placental barrier and reach the foetus (Aghaei et al., 2022). At high enough exposure concentrations, effects on the offspring were reported like reduced birth weight, disruption of the liver function, and the lipid metabolism like increased cholesterol levels.
In a study among 486 new born children in Taiwan a negative correlation was established between weight at birth and the PFOS concentration in the cord blood. It was hypothesised that DNA methylation changes in mesoderm-specific transcript (MEST) could be the underlying mechanism (Ku et al., 2022). A similar relation between PFOS exposure during pregnancy and lower weight at birth

was found by another study that followed 738 mother-child pairs in Denmark (Sevelsted et al., 2022). Pregnancy plasma PFOA concentrations were also negatively associated with weight at birth, and in addition correlated positively with the length of the child. At birth both PFOS and PFOA concentrations were corelated with lower BMI.

The above mentioned effects of PFAS exposure to offspring of mammals is likely partly related to a disruption of the placental development as has been shown in exposure studies with PFOS and PFOA in mice (Aghaei et al., 2022). From epidemiological studies exposure to PFOA, PFOS, PFHxS, PFHpA, PFBS, and PFNA has been correlated with hypertensive disorders of pregnancy in humans, and the researchers suggest that this could be caused by a disrupted placental function (Erinc et al., 2021).

4.2 Lipid metabolism and liver functioning

Aquatic organisms:
Frogs that were exposed via water to a PFAS mixture, including PFOS and PFOA, showed an increase in the hepatosomatic index, as a result of enhanced accumulation of fatty acids, especially triglyceride (Lin et al., 2022). Similar effects on lipid metabolism have been described for zebra fish and medaka (Ma et al., 2023), (Cheng et al., 2016), (Cui et al., 2017), and carp (Hagenaars et al., 2008). From the latter study it was concluded that the affected genes were mainly involved in energy metabolism, reproduction and stress response, and the available glycogen reserves of the exposed fish were significantly lower after 14 days of exposure.

Mice & humans:
In mice, dietary PFOS exposure resulted in liver inflammation and fat liver disease (Qin et al., 2022). In human datasets associations have been found between clinical biochemical measures of liver functioning and PFOA concentrations in blood, while concentrations of PFOS, PFOA, PFDA, PFNA, PFHxS were positively correlated with at least one biomarker of lipid metabolism, like triglycerides, low-density lipoprotein (LDL) cholesterol, total cholesterol, serum calcium (Cakmak et al., 2022). Disruption of the lipid homeostasis through PFAS exposure is suggested as a cause of obesity (Beale et al., 2022). However in a study among 210 persons from households, that were exposed through to a PFAS contaminated aquifer, no relation between BMI and PFAS serum concentrations was established (Blake et al., 2018). In another study a negative relation was found between BMI and PFOS, PFOA PFNA and PFTrA (Zhao et al., 2022).

From a dataset of 203 patients suffering from liver cancer and a similar number of control persons, (Cao et al., 2022) a correlation was established between the serum concentration of PFAS (mainly PFOS, PFOA and 6:2 Cl-PFESA) and the presence of biomarkers that indicate a risk for developing liver cancer.

4.3 Endocrine disruption

Aquatic organisms:
Exposure to PFOA and PFOS significantly reduced fecundity of the Japanese medaka (*Oryzias latipes*) (Lee et al., 2017). A mixture of PFOS, PFNA, PFOA and PFBS caused a declining gonadosomatic index (GSI) in the same species (Ma et al., 2023), while other researchers found a stimulated growth of the female gonads after exposure to PFTriDA, accompanied by a reduced number of eggs (Yu, 2011).
In zebra fish, chronic exposure (180 d 0.01-1 mg/L) to PFNA caused a reduction in the female egg production (Zhang et al., 2016). In the males from the same study, exposure resulted in a reduced GSI and an increase in the vitellogenin (VTG) concentration in the liver. VTG is a 'female' protein mainly related to egg production, and hence regarded a biomarker for estrogenic effects in males.

Gene expression indicated the possible interference of PFNA with hormone synthesis in the fish (Zhang et al., 2016). Similar effects were found after exposure of zebrafish to PFOS (Cheng et al., 2012). In Japanese medaka life-cycle exposure to PFBS at 'environmentally realistic concentrations' (1.0-9.5 µg/L) resulted in anti-estrogenic activity in females, and estrogenic activity in males. Female fish developed particularly small ovaries with decreased egg production, and after two generations the sex ratio shifted towards male dominance (Chen et al., 2019).

Humans:
In humans long-term exposure to PFAS has been associated with an increased risk of hormone related prostate and testicular cancer (Panieri et al., 2022).

Apart from sex hormones, PFAS also seem to have the potential to disrupt the thyroid hormone system. The hormones control the general metabolism of vertebrates, play a critical role in early life (neuro)development (Lee and Choi, 2017), metamorphosis (e.g. (Klaren et al., 2008), and the antioxidant defence mechanism (Kim et al., 2011). PFASs can cause thyroid disruption via different mechanisms which has been shown for both long chained (legacy) and short chained (new generation) PFAS (Coperchini et al., 2021; Lee and Choi, 2017) (De Toni et al., 2022; Dharpure et al., 2023). Data on PFAS induced thyroid disruption in humans is controversial (Coperchini et al., 2021), as demographic characteristics such as sex, age, and disease status appear to influence the associations between PFASs exposure and thyroid hormones (Lee and Choi, 2017). However, in a study of 210 individuals from households that had been chronically exposed to PFAS via a contaminated aquifer, serum PFOS concentrations were found to be associated with an increase in thyroid stimulating hormone (TSH) in both sexes, and total thyroxine (T4) in women (Blake et al., 2018).

Birds:
In Arctic seabirds (thick-billed murres, *Uria lomvia*) from northern Hudson Bay, PFAS blood plasma concentrations correlated with changes in the thyroid hormone levels (FT3 increase in all and a TT3 decrease in males only), and several long-chain PFASs were negatively correlated with body mass. In addition, serum concentrations of PFNA, PFDoA, PFTrDA, PFTeDA, and ∑PFAS were negatively correlated with hatching date (Choy et al., 2022).

4.4 Immunotoxicity

Aquatic organisms:
Liu and Gin, (2018) exposed green mussels for 7 days to PFOS, PFOA, PFNA,PFDA via water and observed a relation between the functioning of the immune system and PFAS body burden. Interestingly, this effect was reversable after a 7 day depuration period in clean water, in which the mussels lost the major part of the accumulated PFAS.
Fish larvae of the marine medaka (*Oryzias melastigma*) that were exposed as embryos to PFOS (1-4 mg/l) had a weak immune response when experimentally challenged (Fang et al., 2013). Repression of the immune system following PFOS exposure was also found in zebra fish (Guo et al., 2019), and in Tilapia when exposed to PFOS en PFOA (Han et al., 2012).

Humans:
In a cohort study including 155 patients with rheumatoid arthritis (RA) and a similar number of healthy controls it was found that PFAS concentrations in serum were higher in RA patients. These data suggest that exposure to PFASs may promote the disease activity of rheumatoid arthritis and the visceral lesions (Zhao et al., 2022).

4.5 Multiple stress situations

In general it can be stated that any toxic effect has the potential to make an organism less resilient against additional unfavourable conditions. PFOS exposure in carps resulted in increased energy expenditure and thereby negatively affected processes vital to survival (Hagenaars et al., 2008). This reduces the potency of the exposed indivudual to cope with challenging conditions.
There are indications in literature that PFAS can potentially impact multiple stress situations in a more specific way via immunotoxicity (see 4.4) or synergistic mixture toxicity. Some examples are briefly presented below.

4.5.1 Mixture toxicity

Aquatic organisms:
The potential of PFOS to increase the toxicity of other compounds has been shown in *in vitro* studies (Keiter et al., 2012). This was confirmed *in vivo* by zebra fish larvae. When pre-exposed to PFOS larvae were more sensitive to subsequent exposure to cadmium. This was reflected by a significantly lower survival rates and growth. The authors suggest that pre-exposure to PFOS affected the antioxidant defence mechanisms and as such potentially increased the toxicity of cadmium (Kim et al., 2011).
(Keiter et al., 2012) tested the hypothesis that the presence of PFOS increases the bioavailability of Bisphenol-A in water, which would result in a higher endocrine potential of the mixture. In a long term exposure covering three generations of zebra fish they found an impact of PFOS (100 & 300 µg/L) on growth, liver function, and vitellogenin levels, but no indication of synergistic effects of the mixture of PFOS and Bisphenol-A were observed. No chemical analyses in tissues were performed.
Synergistic effects were established with PFOS and ZnO- nanoparticles (50 mg/L) in a zebrafish study. Early life development was more severely affected by the mixture than when exposed to the single compounds, and the nanoparticles caused more serious histological damage in the adult fish (Du et al., 2016). It is possible that the surface active characteristic of PFOS facilitated the bioavailability of the nano-particles in this study.

Another mechanism by which PFAS could increase the impact of other toxicants is impairing the multi xenobiotic resistance (MXR) mechanism that marine organisms use to pump contaminants out of their cells. The high potency of PFOS to block this mechanism was shown in echinoid (*Psammechinus miliaris*) larvae (Anselmo et al., 2012).

Table 6 Summarised overview of type of effects of PFAS (compounds) reported in recent literature. Codes indicate the taxonomic group where the effect was observed: F = fish, H = human, A = amphibian, B = bird, M = mollusc, R = rat/mouse. PFAS =undefined mixture of PFAS compounds.

			C=4	C=6	C=8	C=8	C=8	C=8	C=9	C=10
Target	**Group**	**PFAS**	**PFBS**	**PFHxS**	**PFOS**	**PFOA**	**PFNA**	**PFESA**	**PFDA**	**PFUnDA**
Early development	F, B, H	x			x	x	x			
Placental development	M, H		x	x	x	x	x			
Lipid metabolism	A, H			x	x	x	x	x	x	x
Liver functioning	F, B, R, H	x			x					
Sex hormones	F		x		x		x			
Thyroid hormones	B, H	x								
Immune system	M, F				x	x	x		x	
Oxidative stress	F				x	x				
Rheumatoid arthritis	H	x								
Epigenetic effects	F, R, H	x			x	x				

5 External effect concentrations

A quick search in the US-EPA database (https://cfpub.epa.gov/ecotox/search.cfm, visited 25/01/2023) using the filters 'Per- and Polyfluoroalkyl Substances (PFAS)' and 'Salt Water' resulted in a total of 2208 records with toxicity data for marine invertebrates and fish. A further selection was made focusing on data that are indicative for the Lowest Observed Effect Concentrations, by selecting only LOEC (Lowest Observed Effect Concentration) and EC20 values, and thus excluding NOEC (No Observed Effect Concentration), EC10 and EC50 values. Finally only those records were selected that referred to measured concentrations with PFAS as active ingredient. This resulted in 384 entries that considered PFOS (273 records), PFOA (75 records), PFNA and PFDA (both 18 records). These data were used to create a simple Species Sensitivity Distribution (SSD), and to obtain for each of the four compounds an indicative estimation of a test concentration in water that would cause effects to 5% of the species (HC5). HC5-values were 0.002 mg/L for PFOA and PFDA, 0.01 mg/L for PFNA and 0.1 mg/L for PFOS (*Figure 3*).
Realising that the datasets covered endpoints ranging from biochemical or physiological responses to mortality, and a wide variety in exposure conditions and duration, these HC5-values should only be considered as a rough estimate. Nonetheless they give a first indication of which PFAS concentrations in water can cause effects in marine invertebrates and fish during laboratory exposure studies. These point at effect concentrations in the range of 2 to 100 µg/L.

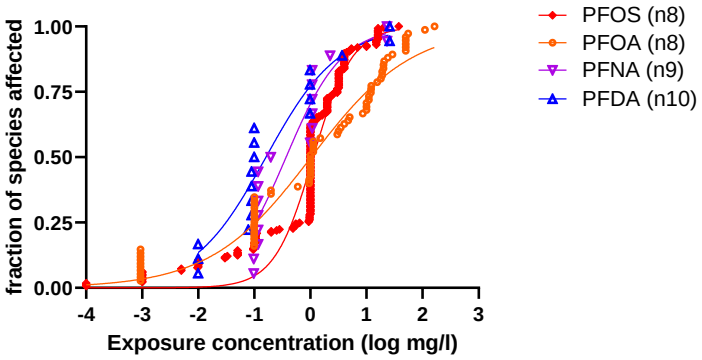

Figure 3 Species Sensitivity Distribution of PFOS, PFOA, PFNA and PFDA, based on LOEC/EC20 data from the US EPA database (https://cfpub.epa.gov/ecotox/search.cfm)

To our knowledge, the highest PFAS concentration ever reported in surface water was 13 µg/L (mainly PFOA) in a Chinese estuary exposed to direct emissions from fluorochemical industrial parks (Zhao et al., 2020). A systematic review and meta-analysis of toxicity data indicated that such PFAS concentrations can cause reduced growth rates and body sizes in aquatic secondary consumers (Banyoi et al., 2022). From PFBS it has been shown that life-cycle exposure to 9.5 µg/L can cause adverse endocrine effects in zebrafish (Chen et al., 2019).
The observed concentration of 13 µg/L should be regarded as an extremely high concentration, especially for the longer chained PFAS like PFOA and PFOS. Most field concentrations remain in the ng/L range (Sinclair et al., 2020). The locations in the Westerschelde estuary with the highest sum PFAS concentrations measured a maximum value of 155 ng/L (see also paragraph 3) that was dominated by PFBA and PFBS, both PFAS compounds with a relative short carbon chain (C4) (Van den Heuvel-Greve et al., 2022). In 2020 PFBA en PFBS were detected in concentrations of 162 and 91 ng/L respectively, while concentrations PFOA an PFOS were 9 and 18 ng/L respectively (Jonker, 2021). Reported effects of PFAS at environmentally relevant concentrations are very limited and Sinclair et al. (2020) suggest that 'many reported toxic effects of PFAS are, theoretically, unlikely to occur outside the laboratory'. Rusconi et al.(2015) studied macrobenthic communities in a river up- and downstream an industrial discharge point of a mixture of PFAS. No differences in community structures could be

detected. They were able to identify a significant divergence in a genetic study, but this could not be associated to a specific effect induced by PFAS.

6 Internal effect concentrations

Effects on an individual level occur when target organs become exposed to levels of a toxicant above a substance specific threshold. The development of an effect therefore is related to the internal concentration of the toxicant in the tissue of the organisms (Critical Body Residue or Critical Body Burden), rather than to the external exposure concentration. There are no indication that PFAS toxicity in aquatic organisms is significantly influenced by the exposure route. In other words the effects are similar if the organism takes up the PFAS via the water, sediment or food (Lee et al., 2020).
For bioaccumulative substances such as -especially longer chained- PFAS, tissue concentrations will increase with exposure duration. Hence long enough exposure times can eventually induce effects, even at low exposure concentrations.

PFAS in biota can accumulate when partitioned to membrane phospholipids or bound to proteins, and in general PFAS accumulation potential increases with the carbon chain length. The physicochemical properties such as KOW (distribution between octanol-water) and KPW (protein-water), are however limited for the comprehension of bioaccumulation in different tissues (Liu et al., 2011) (Chen et al., 2021).
Experiments with green mussels exposed to four different long chained PFAS in concentrations of 1 and 10 µg/L revealed a broad range in bioaccumulation factors (BAF; the steady state concentration ratio between organism's tissue µg/kg, and water µg/L) for the tested PFAS with chain lengths C8 to C10 (*Table **7***; (Liu et al., 2011)). The BAF for PFOS was found to be 20 times higher than for PFOA, despite the similar carbon chain length. Although less extreme, a substantially lower BAF for PFOA compared to PFOS was also found in zebrafish (Table 7; (Han et al., 2021).
Both studies further revealed that for all PFAS tested the BAF was density dependent and lower at higher exposure concentrations. It should be noted that these experiments were performed with concentrations of 10 and 1 µg/L, that largely exceed field relevant exposure conditions.
Jonker (2021) calculated BAF values for several PFAS for flounder collected from the field between 2017 and 2019 and by using yearly average water concentrations. This resulted in BAFs that were at least 10 times higher than generated by (Han et al., 2021) for zebrafish. At least part of this difference can be explained by the fact that the fish analyses in the BAF calculations of Jonker (2021) were performed on the liver that can contain 10 times higher PFAS levels than the tissue (Byns et al., 2022). For the filet of eels Kwadijk et al (2010) calculated lower BAFs for some PFAS based on field data.
The dataset revealed a relation between BAF and chain length and a difference between PFAS acids and sulphonates, with the latter showing a higher accumulation potential at the same chain length. This is clearly illustrated by a 5 to 25 times higher BAF for PFOS when compared to PFOA, both having a carbon chain length of C=8 (Table 7).

Tissue concentrations are thus the resultant of exposure concentration and bioaccumulation capacity of the specific compound and organism. The relative contribution of substances (PFAS compounds) in biota is therefore not representative for the relative contribution of these substances in the environment. Although concentrations in the water can be higher, the level of bioaccumulation determines whether or not high levels in biota can be observed. PFAS substances with low bioaccumulation potential, like PFAO will not reach the high levels in biota that can be found for substances with higher bioaccumulation potential like PFOS.

In marine predators like pinnipeds, cetaceans and polar bears, PFOS is generally the dominant PFAS. An average proportion of PFOS to total PFAS was calculated to be approximately 60% in these organisms (Chen et al., 2021). They also noted that PFUnDA and PFNA on average accounted both for around 10% of the total PFAS burden in predators.

Table 7 Bioaccumulation factors (BAF, kg/L) of PFAS compounds in green mussel (data from (Liu et al., 2011), zebrafish (Han et al., 2021) after laboratory exposure at 1 or 10 µg/L, and for flounder's liver (Jonker, 2021) and eels filet (Kwadijk et al., 2010) from field locations in the Netherlands.

		Green mussel		Zebrafish		Flounder	Eel
	Chain length	**BAF at 1 µg/L**	**BAF at 10 µg/L**	**BAF at 0.5 µM**	**BAF at 5 µM**	**Field exposure**	**Field exposure**
PFTDA	C14	-	-	-	-	107152	-
PFDoA	C12	-	-	-	-	22909	-
PFUndA	C11	-	-	-	-	21380	-
PFDA	C10	838	464	-	-	12023	1820
PFNA	C9	144	109	153	73	4677	331
PFOA	C8	15	12	32	17	437	12
PFHxA	C6	-	-	6.6	3.5	-	-
PFBA	C4	-	-	2.6	1	-	-
PFOS	C8	378	235	271	91	6761	2138
PFHpS	C7	-	-	-	-	4898	-
PFHxS	C6	-	-	-	-	525	355
PFBS	C4	-	-	2.9	2.8	-	18

During the reproduction process part of the accumulated PFAS are absorbed to sperm, or accumulated in eggs and/or the offspring thereby decreasing contaminant levels in the adult individual (Holmström and Berger, 2008). In mammals the production of breastmilk also forms a route for poorly water soluble substances to leave the mother's body (Dietz et al., 2018). It is rather obvious that these pathways related to reproduction can eventually impact the viability of the offspring.
Holmström and Berger, 2008 found that the ratio of levels in egg/female liver of guillemots, increased with increasing PFAS chain length. The concentration of PFOS in the liver of guillemots nestlings was approximately 2.5 times higher than that in adults.

Half live times of PFAS in mammals seem not directly related with the BAF values in *Table 7*, as (Pizzurro et al., 2019) listed that that half-lives decrease in the order of PFHxS (C=6)> PFOS (C=8) > PFOA (C=8) > PFBS (C=4)> PFBA (C=4), and in the order of humans > monkeys > rodents. It has been proposed that complex protein binding models are needed to further assess the accumulation potential of PFAS (Chen et al., 2021) (Han et al., 2021).

6.1 Invertebrates

For invertebrates hardly any data were found in literature that relate observed effects of PFAS to internal body concentrations. An exception is the work by (Liu and Gin, 2018) who exposed green mussels (*Perna viridis)* to PFOS, PFOA, PFNA, PFDA and found a linear relationship between the immunotoxicity response and PFAS body burden. These linear relationships were based on mussels that were exposed for 7 days to concentrations between 10 and 100 µg/L, resulting in body burdens of 2-5 µg PFOS/kg, 0.1-0.2 µg PFOA /kg, 1-2.4 µg PFNA /kg, and 3-9 µg PFDA/kg. Effects were found to be reversable after the mussels were placed 7 days in clean water.

6.2 Fish

A shift in sex ratio towards female dominance was observed after 5 months' exposure of zebrafish (from embryo to reproducing adult) to PFOS (250 µg/L), which resulted in whole body tissue concentrations between 7.7 (females) and 11.1 (males) mg/kg wet weight (Wang et al., 2011). These

tissue concentrations were also related to a reduced body length and weight in both sexes, and a reduced sperm density. A body burden of 5 mg PFOS/kg ww in the adult females resulted in 40% larval mortality. Other publications that connect PFAS tissue concentrations to effects were not found within the scope of this review.

6.3 Birds

Following PFOS injection of eggs, hatchlings of white leghorns developed increased spleen mass, and shorter wing length at serum concentrations of 154 µg PFOS/kg, increased liver mass at 284 µg PFOS/kg serum, and increased body length at 1607 µg PFOS/kg serum (Peden-Adams et al., 2009). In a comparable study it was found that hatchability of leghorns eggs was negatively affected at PFOS concentrations of 1 mg/g egg, while 4.9 mg/g egg was calculated as the median lethal dose (LC50) (Molina et al., 2006).

Based on dietary PFOS exposure studies with northern bobwhite quail and mallard, NOAEL (No Observed Adverse Effect Levels) of PFOS were determined for birds with a distinct difference between male and female birds (Newsted et al., 2005). In liver the NOAEL was 4900 and 88000 µg /kg WW for male and female bird respectively, while for blood serum these were 141000 and 8700 µg /L. The NOAEL for egg yolk was determined at 62000 µg PFOS/kg WW. Taken into account uncertainty factors they calculated toxic reference values for bird liver of 2400 and 140 µg/kg ww (male and female respectively), in blood serum 3900 and 240 µg/L, and for egg yolk 1700 µg /kg ww (Newsted et al., 2005).

6.4 Mammals

There is clear evidence that PFAS can disrupt the functioning of the immune system. The internal threshold for PFOS in studies with mice show a large variation ranging from 91 to 111000 ng/ml serum (DeWitt et al., 2012). This wide range might be related to the use of two different mouse strains; the lowest two values (91 and 670 ng/ml) were determined with B6C3F1 mice, the higher values (7130 -111000 ng/ml) with mice C57BL/6. The latter strain was also used in a single study with PFOA that revealed a threshold level of 74000 ng/ml serum (*Table 8).* DeWitt et al. (2012) do not suggest reasons for the differences in threshold levels between the two mice strains.
For polar bears the critical concentration of PFOS in the liver that were assessed to cause immune repression was calculated to be 9356 ng/g ww using physiologically-based pharmacokinetic modelling (Dietz et al., 2018). In the same study it was assessed that PFOS concentrations in liver of polar bears above 4678 ng/g ww could affect reproduction, and above 955 ng/g ww could increase the risk of carcinogenicity.
For Indo-Pacific dolphins Lam et al., (2016) estimated NOAEL liver concentrations for PFOS to be 775 ng/g WW based on data derived from rats and monkeys.

Table 8 Lowest observed adverse effect levels (LOAEL) for immune alterations and corresponding serum perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) concentrations in mice. Data from (DeWitt et al., 2012)

	Serum concentration (ng/mL or ng/g)	References
PFOS	91.5	Peden-Adams et al. 2008
PFOS	670	Guruge et al. 2009
PFOS	7130	Dong et al. 2009
PFOS	10300	Dong et al. 2011
PFOS	24500	Dong et al. 2010
PFOS	111000	Zheng et al. 2009
PFOA	74000	DeWitt, Copeland, Strynar, et al. 2008

(Cakmak et al., 2022) looked for correlations and trends in a dataset of concentrations of 6 PFAS in blood and health biomarkers of almost 7000 Canadian people in age of 3 to 79 years. A summary of the outcome of this study is given in *Table 9*, showing which health functions were related to a concentration of the PFAS that was twice the median concentration of the dataset. Although it must be taken into account that the correlating PFAS is most likely not the sole compound responsible for the deviation of the health function, and that a clinical non exposed control group is not included, the data at least give an indication at what order of magnitude an effect can be expected (in this case 2 times the median blood concentration). Compared to the NOAEL levels from mice studies that were reviewed by DeWitt et al., 2012 (*Table 8*), these human effect levels are extremely low. This could be related to the fact that the human data set revealed already significant differences when the biomarker response was only minimally affected, in some cases only 0.6%, but never over 15% (Cakmak et al., 2022). Having said this, the dataset shows that all tested PFAS can be associated with changes in lipid metabolism which is, among others, expressed by changes in the cholesterol concentration and composition. This is the only health function that could be related to PFUnDA. The other five PFAS are associated with kidney functioning and calcium metabolism. PFOA and PFHxS could be associated with most health functions.

Table 9 The biochemical markers of human health status that were significantly related to twice the median PFAS concentration in blood. Each '√' indicates an individually affected marker for a health function. Numbers between brackets indicate the total number of biomarkers tested for that health function. Data from an epidemiological study by (Cakmak et al., 2022).

	PFOA	PFOS	PFDA	PFNA	PFUnDA	PFHxS
Median blood conc. (ng/mL)	1.9	5.3	0.2	0.6	0.1	1.5
Health function						
Liver function (5)	√√√√	√√√	√	√	-	√
Kidney function (1)	√	√	-	-	-	√
Lipid metabolism (5)	√√	√√	√√√	√	√	√√
Thyroid hormone (2)	√√	-	-	-	-	√
Calcium metabolism (2)	√	√	√	√	-	√√
Glucose metabolism (4)	√	-	-	-	-	-

7 Conclusions and recommendations for the field campaign

In general
The results of this review are summarised in two tables:
Table 10 provides an overview of the relevant European Quality Standards, and the obtained effect concentrations of PFAS compounds in water and tissue of invertebrates, fish, birds and mammals. This table can help with the interpretation of results of PFAS analyses in environmental samples.
Table 11 lists what PFAS have been detected in biota from the Westerschelde in recent research, the compounds that are included in the standard PFAS chemical analysis at WMR, and for which PFAS Quality Standards and or (indicative) effect levels are described (see Table 10).
This overview can be used to check which PFAS compounds are relevant for inclusion in a field campaign to assess potential effects of PFASs on the marine and estuarine health based on current knowledge. It should however be noted that data about fate and biological effects are only available for a very small fraction of the thousands of PFAS compounds that exists, and although research is focussed on these compounds, that are known or suspected of being the worst, it cannot be excluded that some other hazardous PFAS compounds so far remained unnoticed.

What are the relevant current quality standards for PFAS in biota?
The European Water Framework Directive mentions 'PFOS and its derivatives' as priority substance, for which environmental quality standards (EQS) for water and/or biota have been determined that form the threshold for a good chemical status of the water body. The EQS-biota is set at 9.1 µg/kg based on fresh weight in fish (whole organism), which is standardised to a dry weight percentage of 26% (Table 10). This EQS-biota is related to the risk of secondary poisoning.

On 9 July 2020, the European Union adopted the scientific opinion from the European Food and Safety Authority that concluded that PFOS, PFOA, PFNA and PFHxS can cause developmental effects in humans and may have adverse effects on serum cholesterol, the liver and the immune system and birth weight. Several EU food security standard were developed for PFAS. The general standard for fish meat of the sum of these four substances is 2 µg/kg WW, for the mainly marine species 8 µg/kg WW, and for the mainly freshwater species 45 µg/L, and for crustaceans and bivalve molluscs 5 µg/kg WW (*Table 10*).

Which type of effects have been associated with PFAS exposure?
A broad range of effect types have been connected to PFAS exposure, and is still expanding. PFAS effects that may have consequences for the quality of nature include effects on early development, on lipid metabolism and liver functioning, as well as endocrine disruption and immunotoxicity. Reported endpoints in marine invertebrates are often limited to survival, growth, development of early life stages and sometimes physiological responses (*Table 10*).

At what exposure (water) concentrations have effects been reported?
A quick analysis of data from the US-EPA database indicated that PFAS concentrations in water can cause effects in marine invertebrates and fish during laboratory exposure studies in the range of 2 to 100 µg/L (*Table 10*).

At what internal (biota) concentration have effects been reported?
On an individual level effects occur when target organs become exposed to levels of a toxicant above a substance specific threshold. The development of an effect therefore is related to the internal concentration of the toxicant in the tissue of the organisms. There are no indication that PFAS toxicity in aquatic organisms is significantly influenced by the exposure route (water, sediment or food).
For invertebrates hardly any data were found in literature that relate observed effects of PFAS to internal body concentrations, except for green mussels (*Perna viridis)* that were exposed to PFOS,

PFOA, PFNA,PFDA and showed a linear relationship between the immunotoxicity response and PFAS body burden. In fish a shift in sex ratio towards female dominance, reduced body length and weight and reduced sperm density was observed after long term exposure of zebrafish to PFOS. Other/more publications that connect PFAS tissue concentrations to effects were not found within the scope of this review. For birds and mammals (including humans) more data are available. All relevant internal body concentrations are summarized in *Table 10*.

Advice for the Westerschelde field campaign

- The WMR standard list of PFAS scheduled for analysis covers the most relevant PFAS compounds, based on the information as compiled for this review (Table 11). Based on previous campaigns in the Westerschelde, PFBS, PFTeDA, PFDS, PFPeA, PFHxA and PFHpA may not be detected in biota above limits of quantification. However, since these compounds are part of the standard analysis set, these data will be collected without additional costs.
- The additional measurement of biochemical biomarkers may be of interest for the Westerschelde field campaign as the can be used to get a first insight into a potential inducement as a consequence of exposure to PFAS. However, also other environmental parameters and contaminants can play a role in this and a clear relationships between PFAS exposure and biomarker response can therefore be hard to detect in field situations. Nonetheless, we advise to perform a selection of biomarkers in blood samples from seals and harbour porpoises. This concerns biomarkers that are routinely applied in medical research to check functioning of the liver, kidneys, thyroid and the lipid metabolism that have been related to PFAS exposure in humans by CakMac et al., 2022 (Table 12).

Table 10 Overview of quality standards, external and internal effect concentrations of PFAS in organism types relevant for the Westerschelde field campaign. '-' indicates 'no data available or not applicable'; '(field study)' indicates that interference with other contaminants cannot be excluded. For more details see the cited references.

QUALITY STANDARDS	Species	Unit	PFOS	PFOA	PFNA	PFHxS	∑EFSA	PFDA	PFUnDA	GenX	reference
Marine water											
Marine water WFD EQS	-	µg/L	0.00013	-	-	-	-	-	-	-	EU, 2014
Marine water RIVM	-	µg/L	-	0.048	-	-	-	-	-	0.118	RIVM, 2022
Invertebrates											
Invertebrates EU Food safety	edible crustaceans and moluscs	µg/kg ww edible	3	0.7	1	1.5	5	-	-	-	EU, 2022
Marine fish											
Marine fish EU Food safety	various edible species	µg/kg ww filet	7	1	2.5	0.2	8	-	-	-	EU, 2023
Fish WFD EQS	Flounder	µg/kg ww whole	9.1	-	-	-	-	-	-	-	EU, 2014
Fish RIVM	not specified	µg/kg ww whole	-	1.5	-	-	-	-	-	2.6	RIVM, 2022
Birds											
Bird eggs EU Food safety	various edible species	µg/kg ww filet	1	0.3	0.7	0.3	1.7	-	-	-	EU, 2024
EFFECT CONCENTRATIONS											
External water											
acute LOEC SSD US-EPA	aquatic marine species	HC5 µg/L	100	2	10	-	-	2	-	-	data https://cfpub.epa.gov
Internal - invertebrates											
immunotoxicity	Mussel	CBR µg/kg ww	2-5	0.1-0.2	1-2.4	-	-	3-9	-	-	(Liu and Gin, 2018)
affected behaviour	Crayfish	CBR µg/kg ww	-	-	-	-	2440	-	-	-	(Coy et al., 2022)
Internal – fish											
endocrine and growth	zebra fish	CBR µg/kg ww	11000	-	-	-	-	-	-	-	(Wang et al., 2011)
endocrine and growth	zebra fish	CBR µg/kg ww	7700	-	-	-	-	-	-	-	(Wang et al., 2011)
affected behaviour	bluegill	CBR µg/kg ww	-	-	-	-	13900	-	-	-	(Coy et al., 2022)
Internal – birds											
shorter wings & increased spleen mass	Leghorns	µg/kg serum	154	-	-	-	-	-	-	-	Peden-Adams et al., 2009
increased liver mass	Leghorns	µg/kg serum	284	-	-	-	-	-	-	-	Peden-Adams et al., 2009

increased body length	Leghorns	µg/kg serum	1607	-	-	-	-	-	-	-	Peden-Adams et al., 2009
Reduced hatchability	Leghorns	µg/kg egg	1000000	-	-	-	-	-	-	-	Molina et al., 2006
Embryo mortality	Leghorns	µg/kg egg	4900000	-	-	-	-	-	-	-	Molina et al., 2006
Reduced blood protein and triglyceride	Great tit (field study)	µg/kg egg	19-5635	-	-	-	-	-	-	-	Parolini et al., 2022
Reduced cholesterol and triglyceride	Great tit/Blue tit (field study)	µg/kg liver	86-3322	-	-	-	-	-	-	-	Parolini et al., 2022
reduced shell thickness	Great tit (field study)	µg/kg egg	5111-187032	3.4-359	-	-	-	-	-	-	Parolini et al., 2022
Internal – mammals											
immunotoxicity	Mice	µg/kg serum	91-111000	74000	-	-	-	-	-	-	DeWitt et al., 2012
liver function	Human (field study)	µg/kg blood	10.6	3.8	1.2	3	-	-	-	-	Cakmak et al., 2022
kidney	Human field study)	µg/kg blood	10.6	3.8	-	3	-	-	-	-	Cakmak et al., 2022
lipid metabolism	Human (field study)	µg/kg blood	10.6	3.8	1.2	3	-	-	0.2	-	Cakmak et al., 2022
thyroid hormone	Human (field study)	µg/kg blood	-	3.8	-	3	-	-	-	-	Cakmak et al., 2022
calcium metabolism	Human (field study)	µg/kg blood	10.6	3.8	1.2	3	-	-	-	-	Cakmak et al., 2022
glucose metabolism	Human (field study)	µg/kg blood	-	3.8	-	3	-	-	-	-	Cakmak et al., 2022
birth weight	Human (field study)	µg/L cord blood	0.00407	1.05	-	-	-	-	-	-	Xu et al., 2019

Table 11 Overview of PFAS compounds that were analysed in recent Westerschelde surveys (van den Heuvel et al 2022, Cara et al 2022, Jonker 2021), with indicated if they were found above the limit of detection (LoQ) in biota. Also indicated is which of these compounds are included in the standard analytical set of WMR, if quality standards have been established or indications of effect concentrations were found (see Table 10), and if further information about this compound is included in this review report.

Compound name	**Acronym**	>LoQ in biota vdHeuvel 2022	>LoQ in biota Cara et al 2022	>LoQ in biota Jonker 2021	WMR set	QS/effect conc (Table 10)	Info in report
Perfluorooctane sulphonic acid (c= 8)	PFOS	Y	Y	Y	v	Y	v
Perfluorooctanoic acid (c= 8)	PFOA	Y	Y	Y	v	Y	v
Perfluorononanoic acid (c= 9)	PFNA	Y	Y	Y	v	Y	v
Perfluorodecanoic acid (c= 10)	PFDA	Y	Y	Y	v	Y	v
Perfluoroundecanoic acid (c= 11)	PFUnDA	Y	Y	Y	v	Y	v
Perfluorohexane sulphonic acid (c= 6)	PFHxS	Y	<LoQ	Y	v	Y	v
Perfluoroheptane sulphonic acid (c= 7)	PFHpS	Y		Y	v		v
Perfluorododecanoic acid (c= 12)	PFDoDA	Y	Y	Y	v		v
Perfluorotridecanoic acid (c= 13)	PFTrDA	<LoQ	Y	Y	v		v
Perfluorobutane sulphonic acid (c= 4)	PFBS	<LoQ	<LoQ	<LoQ	v		v
Perfluorotetradecanoic acid (c= 14)	PFTeDA	<LoQ	<LoQ		v		v
Perfluorodecane sulphonic acid (c= 10)	PFDS	<LoQ	<LoQ		v		
Perfluoropentanoic acid (c= 5)	PFPeA	<LoQ	<LoQ	<LoQ	v		
Perfluorohexanoic acid (c= 6)	PFHxA	<LoQ	<LoQ	<LoQ	v		v
Perfluoroheptanoic acid (c= 7)	PFHpA	<LoQ	<LoQ	<LoQ	v		v
Perfluorobutanoic acid (c= 4)	PFBA	Y	<LoQ	<LoQ			v
Hexafluoropropylene oxide dimer acid (c=3)	HFPO-DA, or GenX	<LoQ		<LoQ		Y	v
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	<LoQ					
Perfluorobutane sulfonamide (c=4)	FBSA	<LoQ					
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (c=10)	11Cl-PF3OUdS	<LoQ					
Perfluoro(2-((6-chlorohexyl)oxy)ethanesulfonic acid)	9Cl-PF3ONS	<LoQ					
6:2 Chlorinated polyfluorinated ether sulphonic acid (c= 6)	6:2 Cl-PFESA (trade: F-53B)						v

Perfluoropentanoic sulphonic acid (c= 5)	PFPeS			<LoQ			
6:2 fluortelomeric sulphonic acid (c=2)	6:2 FTS			<LoQ			
N-ethyl- Perfluorooctane sulfonamidoacetic acid (c=8)	EtFOSAA			<LoQ			

Table 12 Proposed biomarkers that can be measured in blood samples of seals and harbour porpoises, and that have been correlated with PFAS exposure in humans √ = significant correlation in database of 6768 human individuals (CakMak et al., 2022)

Biomarker in blood	**Represents :**	**PFOA**	**PFOS**	**PFDA**	**PFNA**	**PFUnDA**	**PFHxS**
Creatine	Kidney functioning	√	√				
Calcium	Calcium metabolism	√	√	√	√		√
GGT	Liver functioning	√	√	√	√		
Bilirubin	Liver functioning	√	√				
Cholesterol	Lipid metabolism	√	√	√			
Triglyceride	Lipid metabolism			√		√	
T4	Thyroid functioning	√					
TSH	Thyroid functioning	√					√

Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2015 certified quality management system. The organisation has been certified since 27 February 2001. The certification was issued by DNV.

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Justification

Report C037/23
Project Number: 4316100304

The scientific quality of this report has been peer reviewed by a colleague scientist and a member of the Management Team of Wageningen Marine Research

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Annex 1 Overview of PFASs and acronyms

Overview of PFASs and acronyms, based on (Nakayama et al., 2019).

Compound name	Acronym
Perfluoroalkyl sulphonic acids (PFSAs)	**PFSAs**
Perfluorobutane sulphonic acid (n = 4)	PFBS
Perfluoropentane sulphonic acid (n = 5)	PFPeS
Perfluorohexane sulphonic acid (n = 6)	PFHxS
Perfluoroheptane sulphonic acid (n = 7)	PFHpS
Perfluorooctane sulphonic acid (n = 8)	PFOS
Perfluorononane sulphonic acid (n = 9)	PFNS
Perfluorodecane sulphonic acid (n = 10)	PFDS
Perfluorododecane sulphonic acid (n = 12)	PFDoDS
Perfluoroalkyl carboxylic acids (PFCAs)	**PFCAs**
Trifluoroacetic acid (n = 2)	TFA
Perfluoropropanoic acid (n = 3)	PFPrA
Perfluorobutanoic acid (n = 4)	PFBA
Perfluoropentanoic acid (n = 5)	PFPeA
Perfluorohexanoic acid (n = 6)	PFHxA
Perfluoroheptanoic acid (n = 7)	PFHpA
Perfluorooctanoic acid (n = 8)	PFOA
Perfluorononanoic acid (n = 9)	PFNA
Perfluorodecanoic acid (n = 10)	PFDA
Perfluoroundecanoic acid (n = 11)	PFUnDA
Perfluorododecanoic acid (n = 12)	PFDoDA
Perfluorotridecanoic acid (n = 13)	PFTrDA
Perfluorotetradecanoic acid (n = 14)	PFTeDA
Perfluorohexadecanoic acid (n = 16)	PFHxDA
Perfluorooctadecanoic acid (n = 18)	PFODA
Perfluoroalkyl phosphonic acids (PFPAs)	**PFPAs**
Perfluorohexane phosphonic acid (n = 6)	PFHxPA
Perfluorooctane phosphonic acid (n = 8)	PFOPA
Perfluorodecane phosphonic acid (n = 10)	PFDPA
Perfluoroalkyl phosphinic acids (PFPiAs)	**PFPiAs**
6:6 Perfluoroalkyl phosphinic acid (m = 6, n = 6)	6:6 PFPiA
6:8 Perfluoroalkyl phosphinic acid (m = 6, n = 8)	6:8 PFPiA
8:8 Perfluoroalkyl phosphinic acid (m = 8, n = 8)	8:8 PFPiA

Annex 1continued

Perfluoroalkane sulphonamides (FASAs)	**FASAs**
Perfluorooctane sulphonamide (n = 8, R_1 = H, R_2 = H)	FOSA
N-Methyl fluorobutane sulphonamide (n = 4, R_1 = H, R_2 = H)	MeFBSA
N-Methyl fluorooctane sulphonamide (n = 8, R_1 = CH_3, R_2 = H)	MeFOSA
N-Ethyl fluorooctane sulphonamide (n = 8, R_1 = C_2H_5, R_2 = H)	EtFOSA
N-Alkyl perfluoroalkane sulphonamido acetic acids (FASAAs)	**FASAAs**
Perfluorooctane sulphonamidoacetic acid (R_1 = H)	FOSAA
N-Methyl fluorooctane sulphonamido acetic acid (R_1 = CH_3)	MeFOSAA
N-Ethyl fluorooctane sulphonamido acetic acid (R_1 = C_2H_5)	EtFOSAA
N-Alkyl perfluoroalkane sulphonamido ethanols (FASEs)	**FASEs**
2-(N-Methyl fluorooctane sulphonamido)-ethanol (R_1 = CH_3)	MeFOSE
2-(N-Ethyl fluorooctane sulphonamido)-ethanol (R_1 = C_2H_5)	EtFOSE
Perfluoroalkyl iodides (PFAIs)	**PFAIs**
Perfluorohexyl iodide (n = 6)	PFHxI
Perfluorooctyl iodide (n = 8)	PFOI
Perfluorodecyl iodide (n = 10)	PFDI
Perfluoroether sulphonic acids (PFESAs)	**PFESAs**
6:2 Chlorinated polyfluorinated ether sulphonic acid (n = 6)	6:2 Cl-PFESA (trade name: F-53B)
8:2 Chlorinated polyfluorinated ether sulphonic acid (n = 8)	8:2 Cl-PFESA
10:2 Chlorinated polyfluorinated ether sulphonic acid (n = 10)	10:2 Cl-PFESA
Perfluoroether carboxylic acids (PFECAs)	**PFECAs**
Hexafluoropropylene oxide dimer acid	HFPO-DA (trade name: GenX)
Hexafluoropropylene oxide trimer acid	HFPO-TA
4,8-Dioxa-3H-perfluorononanoic acid	ADONA
Perfluorooctane sulphonamido ethanol-based phosphate esters (SAmPAPs)	**SAmPAPs**
Phosphate diester of N-ethylperfluorooctane sulphonamido ethanol (R_1 = R, R_2 = R, R_3 = H)	SAmPAP diester
Phosphate triester of N-ethylperfluorooctane sulphonamido ethanol (R_1 = R, R_2 = R, R_3 = R)	SAmPAP triester
Cyclic perfluoroalkyl sulphonic acids (cyclic PFSAs)	**cyclic PFSAs**
Perfluoromethylcyclohexane sulphonic acids (R_1 = CH_3)	PFMeCHS
Perfluoroethylcyclohexane sulphonic acids (R_1 = C_2H_5)	PFECHS

Annex 1 continued

Fluorotelomer sulphonic acids (FTSAs)	**FTSAs**
n:2 Fluorotelomer sulphonic acids (n = 4, 6, 8, 10)	n:2 FTSA
Fluorotelomer carboxylic acids (FTCAs)	**FTCAs**
n:2 Fluorotelomer carboxylic acids (n = 6, 8, 10)	n:2 FTCA
n:3 Fluorotelomer carboxylic acids (n = 5, 7)	n:3 FTCA
Fluorotelomer unsaturated carboxylic acids (FTUCAs)	**FTUCAs**
n:2 Fluorotelomer unsaturated carboxylic acids (n = 6, 8, 10)	n:2 FTUCA
Fluorotelomer olefins (FTOs)	**FTOs**
n:2 Fluorotelomer olefins (n = 6, 8, 10)	n:2 FTO
Fluorotelomer alcohols (FTOHs)	**FTOHs**
n:2 Fluorotelomer alcohols (n = 4, 6, 8, 10, 12)	n:2 FTOH
Fluorotelomer iodides (FTIs)	**FTIs**
n:2 Fluorotelomer iodides (n = 4, 6, 8)	n:2 FTI
Fluorotelomer acrylates (FTACs)	**FTACs**
n:2 Fluorotelomer acrylates (n = 4, 6, 8, 10, 12)	n:2 FTAC
Fluorotelomer methacrylates (FTMACs)	**FTMACs**
n:2 Fluorotelomer methacrylates (n = 6, 8)	n:2 FTMAC
Polyfluoroalkyl phosphate monoesters (monoPAPs)	**monoPAPs**
n:2 Polyfluoroalkyl phosphate monoesters (n = 4, 6, 8, 10)	n:2 monoPAP
Polyfluoroalkyl phosphate diesters (diPAPs)	**diPAPs**
n:2 Polyfluoroalkyl phosphate diesters (m = n = 4, 6, 8, 10)	n:2 diPAP
4:2/n:2 Polyfluoroalkyl phosphate diesters (m = 4, n = 4, 6)	4:2/n:2 diPAP
6:2/n:2 Polyfluoroalkyl phosphate diesters (m = 6, n = 6, 8, 10, 12, 14)	6:2/n:2 diPAP
8:2/n:2 Polyfluoroalkyl phosphate diesters (m = 8, n = 8, 10, 12)	8:2/n:2 diPAP
10:2/10:2 Polyfluoroalkyl phosphate diesters (m = 10, n = 10)	10:2/10:2 diPAP

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With knowledge, independent scientific research and advice, **Wageningen Marine Research** substantially contributes to more sustainable and more careful management, use and protection of natural riches in marine, coastal and freshwater areas.

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