Substance Name: Perfluorobutane sulfonic acid and its salts

EC Number: -

CAS Number: -

MEMBER STATE COMMITTEE

SUPPORT DOCUMENT FOR IDENTIFICATION OF PERFLUOROBUTANE SULFONIC ACID AND ITS SALTS AS SUBSTANCES OF VERY HIGH CONCERN BECAUSE OF THEIR HAZARDOUS PROPERTIES WHICH CAUSE PROBABLE SERIOUS EFFECTS TO HUMAN HEALTH AND THE ENVIRONMENT WHICH GIVE RISE TO AN EQUIVALENT LEVEL OF CONCERN TO THOSE OF CMR[1] AND PBT/vPvB[2] SUBSTANCES (ARTICLE 57F)

Adopted on 11 December 2019

[1] CMR means carcinogenic, mutagenic or toxic for reproduction

[2] PBT means persistent, bioaccumulative and toxic; vPvB means very persistent and very bioaccumulative

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TABLES

Abbreviations

11-KT	11-Ketotestosterone
ACC	Acetyl-Coa Carboxylase
Ach	Acetylcholine
AEC	Anion Exchange Capacity
AFFF	Aqueous Film-Forming Foam
AIX	Anion Exchange
AOR	Adjusted Odds Ratio
AR	Androgen Receptor
ASBT	Apical Sodium-Dependent Bile Salt Transporter
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
BMF	Biomagnification Factor
BMI	Bodymass Index
BW	Body Weight
C/EBPα	CCAAT/Enhancer-Binding Protein A
CAR	Constitutive Androstane Receptor
CC16	The 16-Kda Club Cell Secretory Protein
cDNA	Complmenetary DNA
CEN	Chicken Embryonic Neuronal
ChAT	Choline Acetyl Transferase
ChG	Choriogenin
CHO	Chinese Hamster Ovary
CI	Confidence Interval
Cl-PFAES	Chlorinated Polyfluorinated Ether Sulfonate
COD	Chemical Oxygen Demand
CTD	Characteristic Travel Distance
CYP19	Cytochrome P-19
DA	Dopamine
DEP	Differentially Expressed Protein
DHT	5 Alpha-Androstan-17-Beta-Ol-3-One
DMSO	Dimethylsulphoxide
DOC	Dissolved Organic Carbon
dpf	Days Post Fertilization
dw	Dry Weight
E2	Estrogen/17-Beta-Estradiol
EbC50	Effect Concentration Algal Biomass
EC50	Effect Concentration
ED-RIA	Direct Equilibrium Dialysis Followed By Radioimmunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
ER	Estrogen Receptor
ErC50	Effect Concentration Algal Growth
ERK	Extracellular Signal–Regulated Kinase
ETS	Environmental Tobacco Smoking
F0	Parental Generation

FAS	Fatty Acid Synthase
FBBR	Biological Fixed-Bed Bioreactor
fEPSP	Field Excitatory Postsynaptic Potential
FOB	Functional Observational Battery
FSH	Follicle Stimulating Hormone
GAC	Granular Acticated Carbon
GD	Gestation Day
GLP	Good Laboratory Practice
GnHR	Gonadotropin Releasing Hormone
GPCR	G-Protein Coupled Receptor
GSI	Gonadosomatic Index
HDP	Hypertensive Disorders Of Pregnancy
HEK293	Human Embryonic Kidney 293
HGEN	Herring Gull Embryonic Neuronal
hMSC	Human Mesenchymal Stem Cells
hpf	Hours Post Fertilization
HPG	Hypothalamus-Pituitary-Gonad
HPT	Hypothalamus-Pituitary-Thyroid
HSI	Hepatosomatic Index
IC50	Inhibitory Concentration
IL	Interleukin
IQR	Interquartile Range
KPFBS	Potassium Perfluorobutane Sulfonate
LBD	Ligand Binding Domain
LC50	Lethal Concentration
LH	Luteinizing Hormone
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit Of Detection
LOQ	Limit Of Quantification
LRTP	Long-Range Transport Potential
LSAF	Leaf/Soil Accumulation Factor
LTB4r	Leukotriene Receptor B4
LTP	Long-Term Potentiation
MAPK	Mitogen-Activated Protein Kinase
MDL	Method Detection Limit
Me-FBSA	N-Metylperfluorobutane Sulfonamide
MEK	MAPK/ERK Kinase
MEOGRTS	Medaka Extended One-Generation Reproduction
mRNA	Messenger-RNA
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
NTCP	Na+/Taurocholate Cotransporting Polypeptide
OATPs	Organic Anion Transporting Polypeptides
Oct-1	Octamer Motif-Binding Factor 1
P4	Progesterone
PAPs	Polyfluoroalkyl Phosphate Esters

PBSF	Perfluorobutane Sulfonyl Fluoride
PFAAs	Perfluoroalkyl Acids
PFAS	Per- And Polyfluoroalkyl Substance
PFBA	Perfluorobutanoic Acid
PFBS	Perfluorobutane Sulfonic Acid
PFCAs	Perfluoroalkyl Carboxylic Acid
PFDA	Perfluorodecanoic Acid
PFDoDA	Perfluorododecanoic Acid
PFHxA	Perfluorohexanoic Acid
PFHxS	Perfluorohexane Sulfonic Acid
PFNA	Perfluorononanoic Acid
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctane Sulfonic Acid
PFSAs	Perfluoroalkane Sulfonic Acids
PFTeDA	Perfluorotetradecanoic Acid
PFTrDA	Perfluorotridecanoic Acid
PFUnDA	Perfluoroundecanoic Acid
PND	Postnatal Day
POD	Peroxidase Dismutase
POP	Persistent Organic Pollutants
PPAR	Peroxisome Proliferator-Activated Receptor
PTFE	Polytetrafluoroethylene
QSAR	Quantitative Structure-Activity Relationship
RIA	Radioimmunoassay
ROS	Reactive Oxygen Species
ROS	Reverse Osmosis
RT	Reverse Transcriptase
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SCF	Shoot Concentration Factor
SOD	Superoxide Dismutase
STP	Sewage Treatment Plant
T3	Triiodothyronine
T4	Thyroxine
TBG	Thyroxine-Binding Globuline
TF	Transfer Factor
TH	Tyrosine Hydroxylase
ThOD	Theoretical Oxygen Demand
TNF-α	Tumor Necrosis Factor-A
TSH	Thyroid Stimulating Hormone
TTR	Transport Protein Transthyretin
Vdss	Volume Of Distribution
VTG	Vitellogenin
WBA	Whole-Body Autoradiography
ww	Wet Weight
WWTP	Wastewater Treatment Plant

IDENTIFICATION OF A SUBSTANCE OF VERY HIGH CONCERN ON THE BASIS OF THE CRITERIA SET OUT IN REACH ARTICLE 57

Substance name: Perfluorobutane sulfonic acid (PFBS) and its salts

EC Number: -

CAS Number: -

- The substances are identified as equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of Regulation (EC) No 1907/2006 (REACH) according to Article 57(f) of REACH Regulation.

Summary of how the substances meet the criteria set out in Article 57 of the REACH Regulation

Perfluorobutane sulfonic acid (PFBS) and its salts are identified as substances of very high concern in accordance with Article 57(f) of Regulation (EC) 1907/2006 (REACH) as there is scientific evidence of probable serious effects to the environment and human health which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of REACH.

Substance identification

PFBS-salts are fully indistinguishable from PFBS in the environment as the salts exist in their dissociated anionic sulfonate form, just like PFBS itself, and they are all a part of an acid-base equilibrium in water. Hence, all conclusions on different end-points apply to any and all salt forms, as well as PFBS itself.

Intrinsic properties of PFBS

PFBS is very persistent in the environment. Based on the available data abiotic or biotic degradation of PFBS at relevant environmental conditions is expected to be very slow or negliglible. This is supported by read-across to perfluoroalkane sulfonic acids with both shorter and longer chain lengths, which also have a very low degradability. PFBS shows a preference for distribution to the aqueous phase due to its high solubility in water (52.6 g/L at 22.5-24 °C for the potassium salt), its low sorption potential (log K_{OC} 1.2 to 2.7) and it is considered highly mobile in the environment.

The very high persistence, together with low adsorption potential and high mobility, imply a very high potential for increasing environmental concentrations and potential irreversible exposures of wildlife and of humans via the environment. Long-term, low dose exposure may potentially lead to currently unexpected or even still unknown effects. In particular, endocrine disturbances may be of relevance when considering such exposure. PFBS is bioavailable via the aqueous environment. Together, these environmental fate properties lead to a high potential for irreversible effects. Furthermore, there are high costs and technical challenges related to the removal of PFBS using end-of-pipe treatment.

The high global transport potential (characteristic travel distance, CTD 17616 km, P_{OV} = 220 days), is demonstrated by detection of PFBS in samples of surface water, snow, ice, air and marine water from remote areas such as the Arctic and the Antarctic. This is supported by scientific assessments of the mobility of PFBS, together with QSAR modelling data. PFBS has also been found in biota like dolphins and whales, as well as green turtles

and polar bears, which are both threatened species. This shows that PFBS is bioavailable, and that exposure may occur throughout the food chain and via drinking water and that this is already taking place worldwide.

Toxicological data relevant for human health assessment include effects on thyroid hormone disturbances observed in both rats and mice. These effects are serious and of particular concern since the developing foetus is dependent on maternal production of thyroid hormones. Evidence of effect on development and delay in pubertal onset was observed in mice and disturbed estrus cyclicity was observed in mice and rats. In addition, effects on liver, kidney and haematological system were observed in rats.

A serum elimination half-life of around one month (up to 46 days) has been measured in humans, which is considerably longer than the half-lives measured for rodents (less than 1 day). The limited data show that PFBS has at least a moderate bioaccumulation potential in humans. In pigs an average half-life of 43 days has been estimated.

The ecotoxicological data showing effects on reproduction in F0 marine medaka fish (lowered GSI, delayed oogenesis and reduced fecundity) at 9.5 µg/L PFBS fulfils the T criteria for the environment of Annex XIII of REACH (i.e. NOEC or EC10 for marine or freshwater organisms less than 10 µg/L). Exposure of wheat seedlings to PFBS resulted in reduced chlorophyll a content and shoot biomass together with a decrease in biomass and oxidative stress, which indicates a potential for phytotoxicity. Furthermore, PFBS has been found to cause effects on mRNA expression of hormone receptors in tadpoles and in genes associated with the thyroid pathway in avian neuronal cells, while thyroid hormonal disturbances have been observed in exposed marine medaka fish. Ecotoxicological studies, supported by *in vivo* studies in rodents and in vitro studies, provide evidence for adverse effects.

Overall, PFBS has a high potential to cause effects in wildlife and in humans exposed via the environment worldwide, due to its very high persistence, high mobility, potential for long-range transport, and observed adverse effects that are relevant for human health and the environment, and exposure via drinking water and food. The continuous and increasing exposure in human populations cannot be avoided if releases are not minimised. Similarly, wildlife populations cannot be protected from the total quantity of the substance released.

In addition, the potential for combined exposure to similar PFAAs substances is considered a supportive concern.

Scientific evidence of probable serious effects to human health and the environment is as follows:

- a moderate bioaccumulation potential in humans
- thyroid hormonal disturbances in rodents
- reproductive development deficiencies in mice
- disturbed estrus cyclicity in rodents
- effects on liver, kidney and haematological system in rats
- effects on reproduction in marine medaka (Environmental T)
- thyroid hormonal disturbances in marine medaka
- effects on mRNA expression of hormone receptors in tadpoles

The effects on thyroid hormones are serious since the foetus is dependent on maternal production of thyroid hormones important for e.g. growth, metabolism, reproductive organ and brain development. PFBS is also transferred to the foetus. The developmental effects are serious because they affect the embryos.

Based on the reported effects on reproduction in marine medaka PFBS fulfils the T criteria for the environment of Annex XIII of REACH (i.e. NOEC or EC10 for marine or freshwater organisms less than 10 µg/L). Thyroid hormonal disturbances in marine medaka are also observed. These environmental effects are supported by *in vivo* studies in rodents and *in vitro* studies, and provide evidence for adverse effects. In addition potential effects on hormone receptor expression in tadpoles are reported.

All these human health and environmental effects are serious because, in conjunction with environmental fate properties of PFBS (e.g. very high persistence, high mobility and long-range transport potential), they are potentially irreversible.

Equivalent level of concern

The level of concern is considered very high in particular due to the combination of the following concern elements:

- Potential for irreversible and increasing presence in the environment
- Potential for irreversible and increasing contamination of surface water, marine water and groundwater
- Continuous presence in water results in continuous bioavailability
- Worldwide occurrence
- PFBS enters the biosphere via several routes
- Intergenerational effects, observed mother-to-offspring transfer
- Potential for delay of effects
- Potential for causing serious effects although those would not be observed in standard tests
- Derivation of future exposure levels and safe concentration limits will be highly uncertain
- High societal concern for the presence of PFBS in drinking water sources

PFBS has been detected in humans worldwide and in different species of wildlife, including in endangered species and in remote areas. The substance has been found to transfer from mother to offspring in humans, whales and in birds and may disturb development at sensitive life stages and in vulnerable populations. It may be difficult in practice to manage exposures due to the high mobility of PFBS and the fact that exposures may take place at a different location than where releases occurred and at a different moment in time.

The very high persistence and high mobility of PFBS together lead to a concern for co-exposure with other contaminants with similar effects on human health and the environment. It may be expected that PFAAs cause similar effects, and hence that their individual contributions add up to the total effect. Co-exposure may lead to additive effects and may last for a very long time, because natural degradation processes for these

substances are slow or negligible. This is brought into the weight-of-evidence as supportive information.

Limitations of the available remediation techniques raise a concern that the removal of PFBS from drinking water may only be possible with high societal costs. Remediation of environmental pollution may even be practically impossible due to the high mobility of the substance. Furthermore, PFBS will quickly diffuse from contaminated sites.

In conclusion

The combined intrinsic properties justifying the inclusion as a substance for which there is scientific evidence of probable serious effects to human health and the environment which give rise to an equivalent level of concern are the following: very high persistence, high mobility in water and soil, high potential for long-range transport, and difficulty of remediation and water purification as well as moderate bioaccumulation in humans. The observed probable serious effects for human health and the environment are thyroid hormonal disturbances and reproductive toxicity seen in rodents, and effects on liver, kidney and haematological system in rats, hormonal disturbances and effects on reproduction in marine medaka fish and effects on expression of hormone receptors in tadpoles. Together, these elements lead to a very high potential for irreversible effects.

Registration dossiers submitted for the substance? Yes

Justification

1. Identity of the substance and physical and chemical properties

PFBS belongs to the group of per- and polyfluoroalkyl substances (PFAS). It consists of a C4 perfluoroalkyl chain attached to a sulfonic acid group. PFBS belongs to the short-chain PFASs. PFBS is similar to the more well-known perfluorooctane sulfonic acid (PFOS), but PFOS carries a C8 perfluoroalkyl chain. In its pure form, PFBS is a corrosive liquid that causes severe skin burns and eye damage.

As a sulfonic acid, PFBS is a strong acid that readily forms sulfonate salts with bases, e.g. sodium, potassium and ammonium salts. In aqueous solution the salt forms will exist in equilibrium with PFBS itself. In the environment PFBS will quickly be deprotonated by available bases, and it will be present dissolved on its anionic form. Salts of PFBS are also dissolved in the water phase and the PFBS-part will be present in its anionic sulfonate ion form. It is usually not differentiated between PFBS and its anionic form or salt forms in analyses and quantifications of PFBS. In the literature, the concentrations reported in environmental and human monitoring studies will always be a sum of the acid PFBS, its anionic sulfonate form and its salt forms, which are all part of the same equilibrium.

For clarity, it is usually referred to PFBS in the discussions and conclusions on health and environmental effects in this document. However, based on the reasoning above, the conclusions are considered equaly valid for any PFBS salt as well.

As the scope of the present SVHC dossier we have selected PFBS and any/all of its salts. These entities are indistinguishable in the environment and will all contribute to the PFBS levels.

It should be noted that the potassium salt, KPFBS, is the salt form most widely manufactured, used and studied among the PFBS salts. In many experimental studies the test material has been KPFBS, which is available and convenient to employ in studies. In the physicochemical properties section, Section 1.5, we have included the properties of KPFBS in addition to PFBS itself, in order to illustrate the differences between the free acid and a salt form.

1.1. Name and other identifiers of the substance

Data on the substance identity of PFBS and its potassium salt KPFBS are presented in Table 1 and Table 2, respectively.

Table 1: Substance identity, PFBS.

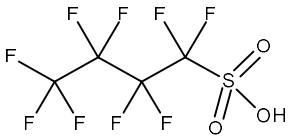
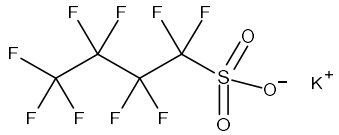
EC number:	206-793-1
EC name:	1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulfonic acid
CAS number (in the EC inventory):	375-73-5
CAS name:	--
IUPAC name:	Nonafluorobutane-1-sulfonic acid
Index number in Annex VI of the CLP Regulation:	
Molecular formula:	$C_4HF_9O_3S$
Molecular weight:	300.10 g/mol
Synonyms:	Perfluorobutane sulfonic acid, PFBS
Chemical structure:	
SMILES notation:	OS(=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F

Table 2: Substance identity, KPFBS – the most widely used and studied salt form of PFBS.

EC number:	249-616-3
EC name:	Potassium 1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulfonate
CAS number (in the EC inventory):	29420-49-3
CAS name:	--
IUPAC name:	Potassium nonafluorobutane-1-sulfonate
Index number in Annex VI of the CLP Regulation:	
Molecular formula:	$C_4F_9O_3SK$
Molecular weight:	338.19 g/mol
Synonyms:	Potassium perfluorobutane sulfonate, Perfluorobutane sulfonic acid potassium salt, KPFBS, K-PFBS, PFBSK+,
Chemical structure:	
SMILES notation:	[K+].[O-]S(=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F

1.2. Composition of the substances

This dossier applies to PFBS and any/all of its salts. The salt forms included in ECHA's database, either registered or notified, are listed in Table 3, Section 1.3. However, the dossier is not limited to the currently registered and notified salt forms. It applies to any salt form of PFBS, either currently existing or developed in the future.

Name: Perfluorobutane sulfonic acid, PFBS

Substance type: mono-constituent

1.3. Identity and composition of structurally related substances

1.3.1. Salt forms - grouping

Salt forms of strong acids, such as the sulfonic acid PFBS, are closely related to its parent compound, in this case the free acid PFBS. The acid, the corresponding deprotonated, anionic sulfonate ion and the salt forms are all part of the same acid/base equilibrium. All salt forms of PFBS released, contribute to the overall levels of PFBS in the environment, and therefore to the overall risk for health effects, when exposure is via the environment.

In the present dossier PFBS and its salt forms are grouped together in a common proposal for listing on the Candidate List. The salt forms of PFBS included in ECHA's database, either registered or notified, are listed in Table 3. These PFBS salts are members of the group of substances addressed in this dossier, but any other salt form of PFBS is included as well. According to the above argument, any salt form of PFBS will contribute to PFBS-concentrations through the acid-base equilibrium.

Table 3: Salt forms of PFBS in ECHA's database.

Perfluorobutane sulfonic acid (PFBS)				
CAS No	375-73-5		EC No	206-793-1
Molecular formula	$C_4HF_9O_3S$		Molecular weight (g/mol)	300.10
Potassium perfluorobutane sulfonate (KPFBS)				
CAS No	29420-49-3		EC No	249-616-3
Molecular formula	$C_4F_9O_3SK$		Molecular weight (g/mol)	338.19
Tetraethylammonium perfluorobutane sulfonate				
CAS No	25628-08-4		EC No	NA
Molecular formula	$C_{12}H_{20}F_9NO_3S$		Molecular weight (g/mol)	429.34
Tetrabutylphosphonium perfluorobutane sulfonate				
CAS No	220689-12-3		EC No	444-440-5
Molecular formula	$C_{20}H_{36}F_9O_3PS$		Molecular weight (g/mol)	558.52
Triphenylsulfanium perfluorobutane sulfonate				
CAS No	144317-44-2		EC No	478-340-8
Molecular formula	$C_{22}H_{15}F_9O_3S_2$		Molecular weight (g/mol)	562.47
Dimethyl(phenyl)sulfanium perfluorobutane sulfonate				
CAS No	220133-51-7		EC No	452-310-4
Molecular formula	$C_{12}H_{11}F_9O_3S_2$		Molecular weight (g/mol)	438.33
Bis(4-t-butylphenyl) iodonium perfluorobutane sulfonate				
CAS No	NA		EC No	432-660-4
Molecular formula	$C_{24}H_{26}F_9IO_3S$		Molecular weight (g/mol)	692.42
Ammonium perfluorobutane sulfonate				
CAS No	68259-10-9		EC No	269-513-7
Molecular formula	$C_4H_4F_9NO_3S$		Molecular weight (g/mol)	317.13
Lithium perfluorobutane sulfonate				

CAS No	131651-65-5		EC No	NA
Molecular formula	$C_4F_9LiO_3S$		Molecular weight (g/mol)	306.03
Magnesium perfluorobutane sulfonate				
CAS No	507453-86-3		EC No	NA
Molecular formula	$C_8F_{18}MgO_6S_2$		Molecular weight (g/mol)	622.49
Morpholinium perfluorobutane sulfonate				
CAS No	503155-89-3		EC No	NA
Molecular formula	$C_8H_{10}F_9NO_4S$		Molecular weight (g/mol)	387.22
1-(4-Butoxy-1-naphthalenyl)tetrahydrothiophenium 1,1,2,2,3,3,4,4,4-nonafluoro-1-butanesulfonate				
CAS No	NA		EC No	468-770-4
Molecular formula	$C_{22}H_{23}F_9O_4S_2$		Molecular weight (g/mol)	586.53

In the environment all the salts will exist in their dissociated anionic sulfonate form, just like PFBS itself, and they will be indistinguishable from one another. Hence, all conclusions on different end-points apply to any and all salt forms, as well as PFBS itself.

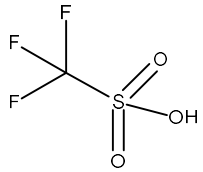
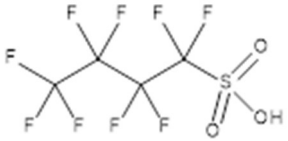
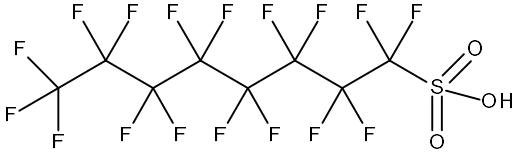
1.3.2. **Perfluoroalkane sulfonic acids used in read-across**

In the assessment of the persistence of PFBS read-across to structurally related perfluoroalkane sulfonic acids (PFSAs) have been applied. PFBS, a C4 PFSA, has been compared to one shorter (C1) and one longer (C8) homologue PFSA, trifluoromethane sulfonic acid and perfluorooctane sulfonic acid (PFOS), for which experimental data are available. In ECHAs database trifluoromethane sulfonic acid, the potassium salt of PFBS, and the tetraethylammonium salt of PFOS are registered, and information on their degradation is found on the ECHA dissemination website.[3] These data have been compared in a read-across approach in order to investigate the influence of perfluoroalkyl chain length on persistence of PFSAs.

In Table 4 information on the identity and composition of these substances are presented. The read-across assessment is found in Section 3.1.4.

[3] https://echa.europa.eu/registration-dossier/-/registered-dossier/5311
https://echa.europa.eu/registration-dossier/-/registered-dossier/22432
https://echa.europa.eu/registration-dossier/-/registered-dossier/10980

Table 4: Identity and composition of substances used in read-across

Chain length	**C1**	**C4**		**C8**	
Molecular formula	CF_3SO_3H	$C_4F_9SO_3H$	$C_4F_9SO_3K$	$C_8F_{17}SO_3H$	$C_8F_{17}SO_3N(C_2H_5)_4$
Name	Trifluoromethane sulfonic acid	Perfluorobutane sulfonic acid	Potassium perfluorobutane sulfonate	Perfluorooctane sulfonic acid	Tetraethylammonium perfluorooctane sulfonate
Acronym	Triflic acid	PFBS	KPFBS	PFOS	PFOS-NEt_3
CAS No	1493-13-6	375-73-5	29420-49-3	1763-23-1	56773-42-3
EC No	216-087-5	206-793-1	249-616-3	217-179-8	260-375-3
Molecular weight (g/mol)	150.08	300.10	338.19	500.13	629.37
Structure		 PFBS		 PFOS	

1.3.3. **PFBS related substances**

PFBS is the final degradation product of a range of PFBS-precursors, or PFBS-related substances, that may degrade to PFBS during use, in the waste stage, or in the environment. Hence, contributions to the environmental concentrations of PFBS will come from the production and use of PFBS itself, as well as production and use of PFBS-related substances for various applications. The concern for PFBS relates to the overall concentrations of PFBS in the environment, and therefore to the combined use and emissions of PFBS, its salts and PFBS-related substances. The manufacture and use of PFBS is limited compared to the related substances.

A comprehensive evaluation of the degradation of PFBS-related substances to PFBS was included in a literature study performed at the University of Oslo (Nielsen, 2017) and elaborated upon in the RMO analysis for PFBS, its salts and related substances (Norwegian Environment Agency, 2018).[4] The degradation study identified several classes of substances that should be considered as precursors to PFBS. Furthermore, COWI A/S performed an investigation, commissioned by the Norwegian Environment Agency in 2017, of sources to PFBS in the environment (Lassen et al., 2017).

Perfluorobutane sulfonyl fluoride (PBSF) is a key intermediate in the production of PFBS-related substances. Most PFBS-related substances may be prepared from PBSF. PBSF is a reactive substance used in manufacture. When PBSF is treated with alcohols or amines in a production process, sulfonic esters and sulfonamides are formed, respectively. PBSF reacts with water in a hydrolytic process with the formation of PFBS. The relationship between PBSF, PFBS-related substances and PFBS is illustrated in Figure 1.

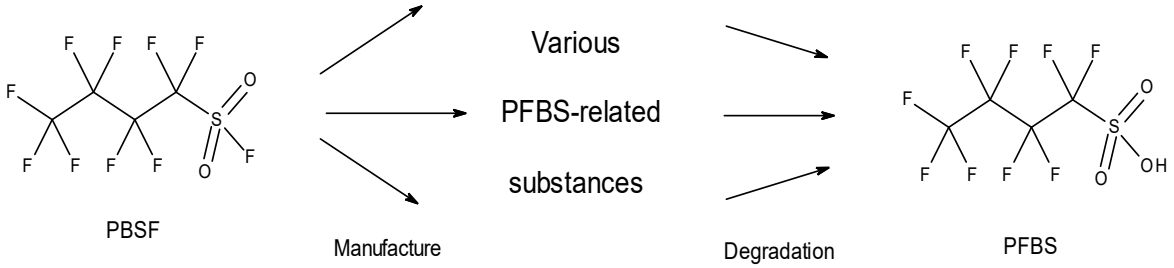


Figure 1: Relationship between PBSF, PFBS-related substances and PFBS

The PFBS-related substances identified in the study by Nielsen, 2017, include PFBS salts, sulfonic acid halides, sulfonic alkyl/olefinic/aryl esters, sulfonamides, sulfones and side-chain fluorinated polymers containing the PFBS moiety. Perfluorobutane sulfinic acid also represents a precursor to PFBS through oxidation to the required sulfonic acid group (Nielsen, 2017). The chemical structures of PFBS-related substances are shown in Figure 2.

[4] https://echa.europa.eu/rmoa/-/dislist/details/0b0236e1809fd422

R = any chemical group, in particular R = -OH, -F, -Cl, -OR', -NR'R" where R' and R" represent any chemical group.

Related substances include e.g. sulfonic halides, sulfonic acid salts, sulfonic esters, sulfonamides, sulfones and sulfinic scid.

Figure 2: Chemical structure of PFBS-related substances

In summary, there is a concern related to concentrations of PFBS in the environment. Contributions to the overall amount of PFBS in the environment come from production, use and emissions of PFBS and its salts, as well as from degradation of PFBS-related substances during use, in the waste stage, or in the environment. However, the concern is first and foremost associated with the ultimate degradation product PFBS and its salts.

1.4. Other PFASs

Kotthoff and Bücking (2018) have looked at the trends in PFAS chemistry over the last years. They found that the portfolio of detected anthropogenic PFAS in products encountered in daily life has expanded. Yet no clear picture of the full range of individual substances that comprise PFASs is available and this challenges authorities who struggle to cope with uncertainties in managing risk of harm posed by PFASs. This is said to be a result of an incomplete understanding of the range of compounds that are used in different products. Yeung and Mabury (2016) found that after the year 2000, the fraction of unidentified organofluorine in plasma samples is increasing. This suggests that humans are being exposed to an increasing number of new and unidentified fluorinated products.

The number of PFASs on the world market was estimated at more than 3000 in a survey by Swedish authorities in 2015 (Swedish Chemicals Agency, 2015). The OECD/UNEP Global PFC Group (2018) performed a new count in 2018 and identified 4730 different PFAS-related CAS-numbers.

Several PFASs have been identified as substances of very high concern (SVHC), including perfluorohexane sulfonic acid (PFHxS), and the C8 to C14 perfluorinated carboxylic acids: perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA) and perfluorotetradecanoic acid (PFTeDA). In addition, PFOS is identified as a Persistent Organic Pollutant under the Stockholm Convention (UNEP, 2006).

1.5. Physicochemical properties

PFBS is a strong acid, and it is predominately present as a dissociated anion in the environment (Arp and Slinde, 2018). The substance has a hydrophilic anionic sulfonate head group, and a hydrophobic perfluoroalkyl end group.

As is always the case for acids/salts, the pure phase properties, like physical state, melting point, boiling point, density, vapour pressure and water solubility, are intrinsically dependant on whether they are determined for the neutral species or the salt. All properties involving water, such as water solubility, K_{OW} and K_{aw} are dependent on the pH-value. PFBS is an acid, and in water it dissociates from the neutral form $C_4F_9SO_3H$ to the anionic $C_4F_9SO_3^-$ form. Over ambient pH ranges, there is a convention to use the symbol "D" instead of "K" for acids and bases for partitioning processes to indicate the pH dependence of the property. The pH dependence is governed by the acid-dissociation constant, pK_a. For monoprotic acids like PFBS, the relationship between K_{OW} and the pH-dependant D_{OW} follows from Equation 1:

Equation 1 $$D_{OW} = K_{OW} / (1+10^{(pH - pKa)})$$

In general, perfluorinated surfactants are much more surface active than hydrocarbon surfactants. The substitution of fluorine atoms for hydrogen atoms decreases their surface activity for aqueous solutions, which promotes micellisation at lower concentrations and lowers the surface tension relative to that of other hydrocarbon analogues (Kissa, 2001; Moody and Field, 2000). It has been found that the surface film of PFBS has a lower stability due to the greater water solubility (Campbell et al., 2009). The aqueous surface tension as a function of the PFBS concentration was measured and compared with PFBA, PFHxA and PFHxS and is illustrated in Campbell et al., (2009). Furthermore, it was found that PFBS, PFHxS and PFOS become insoluble prior to reaching their critical micelle concentration, Campbell et al. (2009); Solubility of KPFBS: 52.6 g/L at 22.5-24 °C. Hence, PFBS is not expected to form micelles in water.

Physicochemical data for PFBS as compiled by Arp and Slinde (2018) are shown in Table 5. For information, the table also includes data for the potassium salt, KPFBS, as the most widely used and studied PFBS salt.

Table 5: Physicochemical data for PFBS, as well as its potassium salt KPFBS

Property	Salt/Neutral Form	Description of key information	Reference/source of information
Physical state at 20°C and 101.3 kPa	KPFBS	White powder	Experimental, ECHA dissemination website
	PFBS	Liquid	Elvers, 2011
Melting/freezing point	KPFBS	> 280 °C	Experimental, ECHA dissemination website
	PFBS	-21 °C	Experimental, ECHA dissemination website
Boiling point	KPFBS	Decomposes before boiling	Experimental, ECHA dissemination website
	PFBS	198 °C	Experimental, ECHA dissemination website; Grayson, 1978
Vapour pressure	KPFBS	< 1.22E-05 Pa at 20°C ± 1°C	Experimental, ECHA dissemination website
	PFBS	7 Pa at 20°C	Experimental, ECHA dissemination website
		2.8 Pa	Wang et al., 2011; Estimated using COSMOtherm
Density	KPFBS	2.248 g/cm^3 at 20 °C	Experimental, ECHA dissemination website
	PFBS	1.824 g/cm^3 at 20°C	Experimental, ECHA dissemination website
Water solubility	KPFBS	52.6 g/L at 22.5-24 °C	ECHA dissemination website
	PFBS	Fully miscible at 20°C	Experimental, ECHA dissemination website; This conclusion is also expected over the entire ambient pH range based on an estimated water solubility of the neutral species (30 g/L) and the estimated pKa of -3.94
Partitioning coefficient	KPFBS	< 2	ECHA dissemination website, following OECD Guideline 106

Property	Salt/Neutral Form	Description of key information	Reference/source of information
soil/water (log Koc)	PFBS	1.2 1.79 2.2 2.7 1.2	Pereira et al., 2018; Guelfo and Higgins, 2013; Kwadijk et al., 2010; Vierke et al., 2014; Milinovic et al., 2015;
Dissociation constant pK_a	PFBS	-3.94	Wang et al., 2011; Estimated using COSMOtherm
Partition coefficient n-octanol/water (log Kow / pH dependent Dow value)	KPFBS	-1.8 at 23 °C	Experimental, ECHA dissemination website
	PFBS	3.9 (neutral form) -4.0 to 0.0 (pH 4) -7.0 to -3.0 (pH 7) -8.0 to -4.0 (pH 8)	Wang et al., 2011; Estimated using COSMOtherm One experimental value is -0.34 in a 0.01 mol/L concentration (pH 1.7) at 23°C, from Experimental, ECHA dissemination website
Partition coefficient air/water (log Kaw/ pH dependant Daw value)	PFBS	-2.59 (pH << 0)	Wang et al., 2011; Estimated for neutral (nonionised species) using COSMOtherm
		-2.6 (neutral form) -10.5 to -6.5 (pH 4) -13.5 to -9.5 (pH 7) -14.5 to -10.5 (pH 8)	Estimated using log Kaw of -2.6 for the neutral species and pKa -3.94 and 0.14, respectively, and the equation Daw = (1/(1+10^(pH – pKa)))Kaw (for monoprotic acids)
Partitioning coefficient n-octanol/air (log Koa)	PFBS	6.49	Wang et al., 2011; Estimated using COSMOtherm

2. Harmonised classification and labelling

There is no harmonised classification available for PFBS or its salts. However, there are four different self-classifications for PFBS and six for KPFBS.

The free acid and the salt forms may have quite different physicochemical properties when they are still in their respective forms. However, in the environment or when mixed in a chemical system (e.g. water with buffer salts), the substances will be indistinguishable.

3. Environmental fate properties

The Norwegian Geotechnical Institute (NGI) carried out a study of PFBS in the environment with emphasis on monitoring data and the physical-chemical properties of the substance (Arp and Slinde, 2018). The study was funded by the Norwegian Environment Agency and is an important part of the basis for the evaluation of the environmental fate of PFBS.

The studies referred to in this chapter are according to standard tests, OECD test guidelines or GLP compliant if stated so. Otherwise they are studies published in scientific peer-reviewed journals or public reports.

3.1. Degradation

The perfluorinated substances are among the most stable organic compounds. According to Kissa (2001), this is due to the high electronegativity and low polarisability of fluorine, which results in a high bond energy of the C-F bond. It is not expected that the length of the perfluoroalkyl chain has a major impact on the inherent stability of PFASs. Hence, it is unlikely that a C4 perfluorobutyl compound should be considerably less stable as compared to e.g. the corresponding C8 perfluorooctyl compound (PFBS vs. PFOS in this case).

The C-F bond is among the strongest covalent bonds known, and it is resistant to acids, bases, oxidation and reduction, even at high temperatures. The strength of the C-F bond increases with increasing fluorine substitution at the carbon atom. Perfluoroalkane sulfonic acids (PFSAs) are remarkably stable, with an outstanding thermal and chemical stability. Anhydrous PFSAs are stable at 400 °C in the absence of air, but they may form hydrogen fluoride at this temperature when moisture is present. The sulfur atoms in PFSAs are at their maximum oxidation state, and cannot be oxidised further (Arp and Slinde, 2018).

The Global PFC Group refers to PFSAs as highly persistent in the environment, while their potential precursors are transformed into PFSAs abiotically or biotically (OECD/UNEP Global PFC Group, 2013). Due to the high resistance to heat and chemical agents, the perfluoroalkyl substances have been frequently used in products with high versatility, strength, resilience and durability. However, the high persistence allows for a wide distribution in the environment, and many PFSAs have been detected globally in the environment.

Brendel et al. (2018) recently summarised the current knowledge on the environmental stability of short chain PFASs and concluded that perfluoroalkyl acids (including PFBS) are extremely persistent, and that they do not undergo abiotic or biotic degradation at all under environmental conditions (short-chain PFSAs defined as PFSAs with less than 6 C atoms). It was further pointed to that the extreme stability in the environment is by some regarded as an incalculable hazard itself, as short-chain perfluoroalkyl acids will stay in the environment for decades to centuries.

Ateia et al. (2019) point out that the short-chain PFASs (for PFSAs, 4 to 6 C-atoms) are equally persistent as their long-chain counterparts, and that the high solubility of short-chain PFAS in water, low/moderate sorption to soils and sediments and resistance to biological and chemical degradation has resulted in their widespread presence in various aquatic environments.

In a review paper Cousins et al. (2016) looked at the precautionary principle and chemicals management in relation to perfluoroalkyl acid contamination of groundwater. The authors argue that all PFASs entering groundwater, irrespective of their perfluoroalkyl chain length and bioaccumulation potential, will result in poorly reversible exposures and risks, as well as further clean-up costs for society. In order to protect groundwater resources for future generations, the authors call for a precautionary approach and prevent the use and release of highly persistent and mobile chemicals such as PFASs. In a different study it is proposed that high persistence alone should be established as a sufficient basis for regulation of a chemical (Cousins et al., 2019). The authors argue that if a chemical is highly persistent, its continuous release will lead to continuously increasing contamination irrespective of the chemical's other physical and chemical properties. The increasing concentrations will result in increasing probabilities for known and unknown effects. Once adverse effects are identified, it will take decades, centuries or even longer to reverse contamination and therefore the effects. PFASs are one of three classes of highly persistent compounds addressed in the study.

Parsons et al. (2008) reviewed the biodegradation of perfluorinated compounds. The authors pointed out that the most theoretically plausible degradation pathway for PFASs, such as PFBS, is through reductive defluorination, which could occur under anaerobic conditions. The same authors reported for PFOS that no biodegradation was observed under aerobic conditions, while there were some observations of degradation of PFOS under anaerobic conditions though no metabolites were measured in these studies. In principle, it cannot be ruled out that some degradation of PFBS under anaerobic conditions can occur (e.g. in hypoxic groundwater, marine water or sediments), or will occur in the future if bacteria adapt to utilise the energy present in the PFAS substrates. Indications for such bacterial behaviour were found when a PFOA-degrading bacterial strain was isolated from soil near a PFAS production plant (Yi et al., 2016). The PFOA-degradation has been demonstrated at lab conditions with a low degradation efficiency only. The findings are promising with regards to a future bioremediation technique for PFOA-polluted areas. However, the rates of these processes under environmental conditions are unknown, and potentially very slow or negligible, and have yet to be observed in the environment (Arp and Slinde, 2018).

Defluorination of fluorinated sulfonates by a *Pseudomonas* strain was investigated by Key et al. (1998). Trifluoromethane sulfonate, PFOS and some related not fully fluorinated substances were subjected to biodegradation by *Pseudomonas* under aerobic, sulfur-limiting conditions. Growth and defluorination were observed for the compounds containing hydrogen on the carbon chain, while it is reported that trifluoromethane sulfonate and PFOS were not degraded. Hence, both longer and shorter chain PFSAs, as compared to PFBS, were found to be inert to biodegradation in the study.

Sáez et al. (2008b) studied the degradation of PFASs, including both PFBS and PFOS, in closed bottle tests with municipal sewage sludge. Bacterial communities from sewage sludge were exposed to a mixture of PFASs under aerobic and anaerobic conditions. Individual PFAS concentrations were determined after solid phase extraction. The experiments were based on the OECD guideline 301D (closed bottle test) with slight modifications. It was found that the PFASs tested in these experiments are non-biodegradable under the conditions used. In particular, no degradation was observed for PFBS or PFOS.

The microbial toxicity and biodegradability of PFOS and shorter chain PFASs were investigated by Ochoa-Herrera et al. (2016). PFOS and PFBS, together with other PFASs, were subjected to biodegradation by micoorganisms present in sludge obtained from various industrial and municipal wastewater treatment processes under anaerobic conditions for 110 weeks. No fluoride release was observed, indicating that the substances resisted biodegradation. It was concluded that the substances are highly resistant to microbial degradation.

Chetverikov et al. (2017) found that a strain of *Pseudomonas* isolated from soil contaminated with waste from petrochemical production in Russia, was able to degrade PFOS to perfluoroheptanoic acid (PFHpA) in laboratory experiments. The bacterium was able to use PFOS as the only source of carbon and energy, while free fluoride ions were released to the medium. The transformation was observed with a pure bacterial culture under optimised conditions. The authors suggested that the results may be developed further for use as a biotechnological method for the transformation of fluoroorganic molecules.

Chetverikov and Loginov (2019) reported an experiment wherein a bacterial strain of *Ensifer adhaerens* was isolated after enrichment culture techniques when bacteria were grown with PFOS as the sole source of carbon and energy. The isolated strain was demonstrated to have the ability to degrade both PFOA and PFOS with the formation of perfluoroheptanoic acid and fluoride. The transformation observed in the lab was only under optimised conditions with a bacterial isolate. The same transformation has not been observed in the environment.

PFOS was also degraded in a laboratory experiment when exposed to laccase enzyme and 1-hydroxybenzotriazole as a mediator (Luo et al., 2018). Fluoride ions were released in the process, but the other degradation products were not identified. The authors note that enzyme-catalyzed oxidative humification reactions (ECOHRs) may be effective in natural water and soil systems to transform and incorporate PFOS into the natural organic matter, thus detoxifying and immobilizing PFOS. However, it should be noted that this degradation mechanism has not yet been demonstrated to occur for PFOS in environmental conditions. The method may be developed into a viable approach to remediate PFOS contamination.

Hence, several studies report that the PFSAs are resistant to biodegradation, while a few recent studies report degradation of PFSAs by isolated bacterial strains under laboratory conditions or by a specific enzyme when incubated with a mediator substance in laboratory conditions. The latter results show that bacteria may adapt to utilise the energy present in the PFAS substrates. However, the rates of these processes under environmental conditions are unknown, and potentially very slow or negligible and have yet to be observed in the environment. As is pointed out in the studies mentioned above, the methods may be developed into a viable approach to remediate PFSA contamination.

3.1.1. **Abiotic degradation**

3.1.1.1. Hydrolysis

A study of KPFBS hydrolysis as a function of pH is reported on ECHA's dissemination website for KPFBS (according to EU method C.7, Degradation: Abiotic Degradation: Hydrolysis as a Function of pH). The substance was studied in pH 4.0, 7.0, and 9.0 buffer systems and at 50 °C. The registrant assessed the study as GLP compliant with a Klimisch reliability 2. No changes in the concentration of the substrate were observed after 5 days at any of the different pH values. Since less than 10% hydrolysis occurred, the test substance is considered hydrolytically stable with a hydrolytic half-life, $t_{1/2}$ at 25 °C of more than 1 year.

Comparison of the hydrolytic stability of the C1, C4 (PFBS) and the C8 PFSAs based on experimental data in a read-across approach is presented in Section 3.1.4.

PFOS has been concluded to be hydrolytically stable with a hydrolytic half-life of more than 41 years (UNEP, 2006). PFHxS is also considered to be hydrolytically stable under environmental conditions based on read-across to PFOS and PFOA (ECHA, 2017).

3.1.1.2. Phototransformation in air

The QSAR model AOPWIN v1.92 of the EPISuite tool was used to assess degradation in the atmosphere. The model estimates the rate constant for the atmospheric, gas-phase reaction between hydroxyl radicals and organic chemicals. The rate constants estimated by the program are then used to calculate atmospheric half-lives for organic compounds. The model is preset with the following: 1.5×10^6 molecules (radicals)/cm^3 per 12 hours of daylight, which was also used for this assessment. The predicted degradation rate constant for PFBS was 0.1400×10^{-12} cm^3/(molecule*sec), which is equal to an atmospheric half-life of 76.4 days. It should be emphasised that perfluoroalkyl substances are not fully covered by the EPISuite models, and the estimated overall OH rate constant and half-life are based only on "*reaction with N, S and OH-*".

Thus, the results should be interpreted with caution. However, the data can be used in a weight of evidence approach indicating that the degradation half-life of PFBS in the atmosphere is above the threshold of two days, which indicates that the substance has a potential for long-range transport.

Photolysis of perfluoroalkane sulfonates in the atmosphere is generally considered negligible under environmental conditions (Vecitis et al., 2009). An outlying study is one by Taniyasu et al. (2013) which reported observing photolysis in air at high altitudes (>2500 m) of PFOS, which reacted via dealkylation to form PFBS. It was concluded that PFBS was more recalcitrant and stable. It should be noted that this photodegradation of PFOS has been disputed by Wang et al. (2015a), who argue that PFOS is too stable to undergo atmospheric photolysis. Nevertheless, PFBS, as such, is considered to remain stable to photolysis under environmental conditions, and this has been supported by all studies thus far.

PFOS has an expected half-life in the atmosphere greater than two days and fulfils the POP-criteria (UNEP, 2006). For PFHxS read across to PFOS and PFOA has been used, concluding on an atmospheric half-life which also exceeds two days (ECHA, 2017).

3.1.1.3. Phototransformation in water

A study on the photolysis of PFOS in water has been conducted, where no evidence of direct or indirect photolysis was observed under any of the conditions tested. The indirect photolytic half-life of PFOS at 25°C was calculated to be more than 3.7 years (UNEP, 2006).

Similar data for PFBS have not been found, but based on the structural similarities, PFBS would be expected to have a similar behaviour as PFOS.

3.1.1.4. Oxidation

PFBS was included in a study of the degradation of new substitutes for perfluorinated surfactants (Quinete et al., 2010). The study tested for the abiotic degradation of PFBS using advanced oxidation processes through ultraviolet radiation, hydrogen peroxide, or both. No significant oxidative degradation of PFBS was observed in the tests.

In summary, no hydrolysis was observed in a hydrolysis test. An atmospheric half-life of 76.4 days has been predicted, which by far exceeds the criterion in the Stockholm Convention of > 2 days. Although the value was not from a model validated for such chemistry, it is indicative of a potential for long-range transport. The available information demonstrates that PFBS is stable towards hydrolysis, oxidation and photodegradation and is very persistent under environmentally relevant conditions.

3.1.2. **Biodegradation in water**

3.1.2.1. Estimated data

Biodegradation of PFBS was modelled using the EpiSuite BIOWIN v4.10 model in EpiSuite (US EPA, 2000-2017). BIOWIN estimates the probability of rapid aerobic and anaerobic biodegradation of an organic compound in the presence of mixed populations of microorganisms and contains seven separate models. The screening criteria for persistence in the environment are BIOWIN2 <0.5 or BIOWIN6 <0.5 and BIOWIN3 <2.25. For substances where BIOWIN3 indicates a value between 2.25 and 2.75 more degradation relevant information is generally required. The outcome of the estimation is provided in Table 6.

Table 6: Results from BIOWIN estimations of PFBS biodegradation.

Model	Result	Limit	Conclusion
BIOWIN 1, linear probability model	-0.3592		Does not biodegrade fast
BIOWIN 2, non-linear probability model	**0.0052**	<0.5	Does not biodegrade fast
BIOWIN 3, expert survey ultimate biodegradation model	**1.5793**	<2.25	Recalcitrant
BIOWIN 4, expert survey primary biodegradation model	2.8572		Weeks-months
BIOWIN 5, MITI linear model	0.1786	≥0.5	Does not biodegrade fast
BIOWIN 6, MITI non-linear model	**0.0000**	<0.5	Does not biodegrade fast
BIOWIN 7, anaerobic biodegradation model	-0.1666		Does not biodegrade fast
Ready biodegradability prediction			NO

The QSAR estimates for PFBS are all below the screening criteria for being rated as "readily biodegradable" and indicate that PFBS is potentially persistent or very persistent according to ECHA Guidance on PBT/vPvB assessment (ECHA, 2017a). PFBS and PFASs are not always represented well in QSAR estimations, and the results should be interpreted with caution. For example, the main structural difference in the perfluoroalkane sulfonic acid series is the number of "carbons with 4 single bonds and no hydrogens", and in BIOWIN 1-4 these contribute to a more stable structure, while in BIOWIN 5-6 they provide less

stability. Hence, comparison with shorter or longer homologues in the perfluoroalkane sulfonic acid group, will give different results for BIOWIN 1-4 and 5-6. However, although the small differences in the estimates among closely related PFAS substances are uncertain, all models clearly suggest that PFBS does not biodegrade fast. This is taken as a strong indication that PFBS will not fulfil the screening criteria for being rated as "readily biodegradable" and PFBS can be regarded as potentially persistent or very persistent according to ECHA Guidance on PBT/vPvB assessment (ECHA, 2017a). In any case, BIOWIN predictions are used only as support for other evidence for persistence of PFBS.

3.1.2.2. Screening studies in aquatic compartment

A screening test on ready biodegradability of KPFBS is provided as key study on ECHA's dissemination webpage. The test was performed according to the test guideline OECD 301E (Modified OECD Screening Test), was GLP compliant, and assessed by registrants with Klimisch reliability 2. The purity of the test substance was 98.7% and a test concentration of 20 mg/L dissolved organic carbon (DOC) was incubated with secondary effluent of a sewage treatment plant (STP) receiving predominantly domestic sewage. Experimental bottles were run in duplicates for test suspension, inoculum blank and procedure control. Based on the properties of PFBS, volatilisation, adsorbtion and toxicity of the test substance seems unlikely. No abiotic control, toxicity control and adsorption control were included in the test set up. The test was carried out at 22 ± 2 °C, in darkness and under aerobic conditions for 28 days.

The conclusion of the screening test for PFBS is that the substance is not readily biodegradable as the degradation is less than 60% after 28 days measured as decrease in DOC. There is an apparent 14% biodegradation reported in this test for PFBS after 28 days, see Table 7. However, whether this can be regarded as evidence of biodegradation must be considered in light of the precision of the chemical analysis and the experimental test set up which is validated for testing whether a chemical reaches 60% degradation or not. Both the variance in DOC concentration in the blank control (apparently both increasing and decreasing during the course of the test period) and the negative degradation values in the first part of the test gives indication that the analytical precision of the method used is not sufficient to evaluate low variance in concentrations. Assuming the increase in DOC from day 14 (1 mg/L) to day 21 (2 mg/L) and day 28 (3 mg/L) is an analytical error and instead assuming the blank DOC remains at the level from day 14 (1 mg/L), the calculated biodegradation on day 28 would have been only 4%. The OECD 301 E guideline does not include specific validity criteria for the inoculum blank. The reference compound was degraded by 95% after 7 days and fulfilled the validity criteria (70 % after 14 days). Likewise, the validity criterion of less than 20% difference of extremes of replicate values of the test chemical at the end of the test seems to be fulfilled. Taken all that into account the study can be regarded as reliable with restriction (Klimisch reliability 2).

Table 7: Percent biodegradation of KPFBS in OECD 301 E (Modified OECD Screening Test)

	Replicate	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 27**	**Day 28**
Blank values reported mg/L DOC mean		3	2	1	2	2	3
KPFBS % biodegradation	A		-3 %	0 %	5 %	10 %	15 %
	B		-8 %	-29 %	8 %	8 %	13 %
	Average		**-5 %**	**-14 %**	**7 %**	**9 %**	**14 %**

Further biodegradation screening tests are available on ECHAs dissemination webpage, where Quinete et al. (2010) tested KPFBS for biodegradation in OECD 301 F (manometric respirometry) and OECD 301 D (closed bottle test).

For the 301 F test activated sludge from a domestic non adapted sewage treatment plant in Germany was used as bacterial inoculum. The study was run with two parallels including test suspension (containing 100 mg/L PFBS, inoculum and mineral medium), inoculum blank and procedure control with sodium acetate as reference compound. No toxicity control and abiotic control were included in the study. Flasks were sealed, assembled with Oxi Top and the bottles were incubated for 40 days at 20 ± 0.2 °C in an incubation cabinet. Oxygen consumption was measured daily and samples were analysed in duplicates. PFBS was biodegraded by 1% after 40 days based on oxygen consumption.

The study is valid if the percentage degradation of the reference compound has reached the pass level of 60% ThOD (theoretical oxygen demand) by day 14, oxygen uptake of the inoculum blank is not greater than 60 mg/l in 28 days and extremes of replicates for test chemical are within 20%. Based on Quinete et al. (2010) the ThOD of the reference substance was over 60% at day 14 and calculated to 70% at day 28, and the authors deemed the test as valid. Registrants assessed the study as not reliable (Klimisch reliability 3) and described several shortcomings "The test was conducted on several fluorochemical surfactants as well as PFBS. The figure shows roughly equivalent %ThOD for one surfactant (Zonyl) and PFBS. Nevertheless, the report states a biodegradation of 13% for the surfactant and <1% for PFBS. Both values are contradicted by the depicted biodegradation curve, in which both materials have %ThOD values in the range 5-10%. In addition, the stated % ThOD for the reference substance, 70.4%, is not supported by the biodegradation curve." The description of the results in the text (<1% biodegradation of PFBS) of Quinete et al. (2010) does indeed not correspond with the result in figure 5 (around 5-10 % biodegradation of PFBS). The study should therefore be regarded as not reliable (Klimisch reliability 3) due to missing documentation which is not sufficient for assessment.

A closed-bottle test, OECD 301 D, was performed in the dark at 20 ± 1 °C for 28 days, where 73 mg/L PFBS was incubated using water from Rhine River as inoculum. Experimental bottles were run in duplicate for test suspension, inoculum blank and procedure control, however a toxicity control was missing. Sodium acetate was used as reference compound, but no more information has been reported if the validity criteria for the OECD 301 D test were met. However, for another test substance, 10-(trifluoromethoxy)decane-1-sulfonate, a biodegradation of 80% within 28 days was demonstrated, indicating that the microbial activity of the inoculum was sufficient. PFBS was biodegraded < 3% (based on oxygen consumption) within 28 days. This study was not GLP compliant but followed a test guideline of relevance. Data of replicates and reference substance were not available. However, evidence of biodegradation of other test substances was provided. The study should be regarded as not reliable (Klimisch reliability 3) since the documentation is not sufficient for assessment.

A biological fixed-bed bioreactor (FBBR) test with KPFBS has been reported on ECHAs dissemination page as a simulation test and has been published by Quinete et al. (2010). This study cannot be regarded as sewage treatment simulation test but as a non-guideline screening test as explained in Section 3.1.2.3.

In the FBBR study a glass column filled with glass beads forms the main part of an FBBR, enabling microorganisms derived from surface water of the Rhine River to accumulate on the surface. The water was recirculated at a flow rate of 16 mL/min through the glass column to allow a biofilm to develop. The duration of biofilm development was not stated. The experiment was then performed with 100 mg/L PFBS and the FBBR was used as inoculum in 5 L fresh water from a pristine creek near Biebesheim, Germany. A blank experiment with the same test conditions but without test substance was run in parallel.

Aeration was provided and the study was performed in the dark at room temperature, at neutral pH and for 28 days. Samples were taken weekly and analyzed by HPLC/MS/MS. No reference compound was tested but a second fluorinated surfactant (10-(trifluoromethoxy)decane-1-sulfonate) was examined in a parallel study. PFBS was not measurably biodegraded (RSD of HPLC peak areas ≤7%) over the test interval, whereas the fluorinated surfactant was degraded >60% during the same period. It can be concluded that PFBS is not primary biodegradable. The study is considered as reliable with restrictions (Klimisch reliability 2).

Sáez et al. (2008b) studied the degradation of PFASs, including both PFBS and PFOS, in closed bottle tests with municipal sewage sludge. Bacterial communities from sewage sludge were exposed to a mixture of PFASs under aerobic and anaerobic conditions. Individual PFAS concentrations were determined after solid phase extraction and analysed using HPLC/MS. The experiments were based on the OECD guideline 301D (closed bottle test) with slight modifications. Information on fulfilment of validity criteria were missing, especially no information of biodegradation of a reference compound was available, and the study is considered as not reliable (Klimisch reliability 3). It was found that the PFASs tested in these experiments are non-biodegradable under the conditions used. In particular, no primary degradation was observed for PFBS or PFOS.

In buffered media, biodegradation testing on the free acid is equivalent to testing on the salt. Therefore, results for KPFBS are directly applicable to PFBS.

In total five biodegradation screening test are availabe for KPFBS/PFBS with varying reliability, demonstrating no primary biodegradation and that the substance is not readily biodegradable.

Comparison of ready biodegradability of the C1, C4 (PFBS) and the C8 PFSAs based on experimental data in a read-across approach is presented in Section 3.1.4. No information is available for the short-chain PFASs perfluorobutanoic acid (PFBA) and perfluoropentanoic acid (PFPeA).

The C8 homologue PFOS has been established as not readily biodegradable (UNEP, 2006), and a read-across to PFOS has also been used to conclude on no "ready biodegradablility" for PFHxS (ECHA, 2017).

3.1.2.3. Simulation tests (water and sediments)

A FBBR test with KPFBS has been reported as a sewage treatment simulation test on ECHAs dissemination page and has been published by Quinete et al. (2010).

Simulation tests aim at mimicking actual environmental conditions such as redox potential, pH, temperature, microbial community, concentration of test substance and occurrence and concentration of other substrates according to the OECD Guideline for Testing of Chemicals, Part 1: Principles and Strategies Related to the Testing of Degradation of Organic Chemicals (OECD, 2005). The OECD 303 (simulation test- aerobic sewage treatment) is the relevant test guideline for a sewage treatment simulation test.

Although indicated as a sewage treatment simulation test in the ECHA webpage, compared to the OECD 303 (simulation test- aerobic sewage treatment), the test does not use a relevant inoculum for a sewage treatment plant, nor an organic test medium and the number of sampling points are too few (preferably at least 15 valid values in the plateau phase). Compared to the OECD 309 (Aerobic Mineralisation in Surface Water), the test concentration is too high (100 mg/L vs 1-100 µg/L) and metabolites were not analysed. In both simulations tests, the test medium contribute more organic carbon than the test substance to allow for co-metabolism of the test substance. In the FBBR study, the test

substance is added as the major carbon source, thus it should be regarded as a screening study and not as a simulation test.

The results demonstrating no biodegradation of PFBS confirm that PFBS can be regarded as not readily biodegradable. Since no experimental simulation tests were performed, no biodegradation half-lifes could be derived.

3.1.3. **Biodegradation in soil**

There are no experimental soil degradation tests available for PFBS or KPFBS. The C8 homologue PFOS has been found to be persistent in soil cultures (UNEP, 2006) and read-across to PFOS has been used to conclude that also PFHxS is persistent and very persistent in soil (ECHA, 2017). Although certain bacterial strains isolated from soil, and enzymes related to humification reactions, are able to degrade PFOS under optimised laboratory conditions, see Section 3.1, this cannot be expected under environmentally relevant conditions.

3.1.4. **Read-across to similar PFSAs**

In general, the read-across approach can be applied in the assessment of a property when the substances compared have physicochemical and/or toxicological and/or ecotoxicological properties that are likely to be similar or follow a regular pattern as a result of structural similarity. According to ECHA's practical guide 6 "How to report read-across and categories"[5] similarities may be due to a common functional group, common precursor or breakdown products, constant pattern in changing potency or common constituents or chemical class.

The group of perfluoroalkane sulfonic acids (PFSAs) consists of closely related substances with very similar structures and properties. The PFSAs include a perfluoroalkyl group of varying carbon chain length, attached to a sulfonic acid group. Hence, the different substances in the group differ only in the number of CF_2-units in the perfluoroalkyl chain, whereas the other structural features are the same. The perfluoroalkyl chain is persistent due to the high stability of the C-F-bond. The sulfonic acid group is at its highest oxidation state and cannot be oxidised further in the environment (without breaking the C-S bond). It is not expected that the sulfonic acid group will make the attached perfluoroalkyl chain more susceptible to chemical transformations. If the sulfonic acid group was to have an influence on the perfluoroalkyl chain, it would be on the neighbouring C- and F-atoms, and they are common for all the PFSAs. Hence, the persistence of the members in this class of substances could be expected to be comparable, and an evaluation of the persistence of PFBS in comparison with trifluoromethane sulfonic acid and PFOS in a read-across approach is justified.

In the study by Key et al. (1998) it was found that trifluoromethane sulfonate and PFOS did not degrade when subjected to *Pseudomonas*. The study by Sáez et al. (2008b) showed no aerobic or anaerobic biodegradation of PFBS or PFOS in a closed bottle test with sewage sludge with test compound analysis. The study by Ochoa-Herrera et al. (2016) showed that PFOS and PFBS did not degrade when exposed to micoorganisms present in sludge obtained from various industrial and municipal wastewater treatment processes under anaerobic conditions for 110 weeks. All three studies are presented in Section 3.1.

[5] https://echa.europa.eu/documents/6362380/7127661/pg_report_readacross_en.pdf/69860e5b-c669-4a0d-b868-72f5dba5b560

Hence, in experimental biodegradation studies in which different perfluorinated sulfonates have been studied in the same test system, the persistence of the members in this class of substances has been comparable. It is noted that these studies have features which decrease their environmental relevance (Key et al. (1998) study is a pure culture study and Ochoa-Herrera et al. (2016) studied only anaerobic conditions), or reliability (Key et al. (1998) and Saez et al. (2008b) have limited documentation). However, these results as a whole, together with the structural considerations presented above and the results of the other biodegradation tests (Table 8), indicate that an evaluation of the persistence of PFBS, in comparison with trifluoromethane sulfonic acid and PFOS in a read-across approach is justified.

In general, when comparing experimental and estimated data, it can be assumed that with increasing chain length, water solubility decreases and the sorption potential increases (ECHA, 2015). Consequently, a short-chain PFSA with a higher solubility in water could potentially be more readily available for aqueous organisms and biodegradation. Furthermore, a short perfluoroalkyl chain would represent a smaller steric bulk of the substance and make it more easily available to enzymes and bacteria and more susceptible to biodegradation. However, no biodegradation has been observed for trifluoromethane sulfonic acid in screening tests, see Table 8. The persistence of PFHxS and PFOS has been confirmed. PFOS was in 2006 identified as a Persistent Organic Pollutant and listed in the Stockholm convention (UNEP, 2006). PFOS is considered extremely stable in the environment and does not hydrolyse, photolyse or biodegrade under any environmental conditions tested (OECD, 2002). PFHxS was established as P and vP by the Member State Committee in 2017 when PFHxS was identified as a substance of very high concern based on read-across to PFOS and PFOA, (ECHA, 2017).

At the ECHA dissemination page there is data for the hydrolytic stability and biodegradability of the C1, C4 and C8 perfluoroalkane sulfonic acids from the registration dossiers for the following substances: trifluoromethane sulfonic acid (C1), PFBS potassium salt (C4) and PFOS triethylammonium salt (C8). The three substances belong to the same PFAS subclass and are different only in the length of the perfluoroalkyl chain. There is no difference in testing the persistence of the free acid or a salt form, as the salts will be in equilibrium with the corresponding acid in the aqueous phase.

The identity and composition of the substances used in the read-across assessment is found in Section 1.3.2, while physical-chemical data for the substances are presented in Annex I. A comparison of the stability data found on the ECHA dissemination webpage for the three substances is shown in Table 8.

Table 8: Hydrolytic stability and biodegradation screening test data for C1, C4 and C8 perfluoroalkane sulfonic acids (data reported at the ECHA dissemination page, if no reference is indicated).

	C1: CF_3SO_3H	**C4: PFBS/KPFBS**	**C8: PFOS NEt_4-salt[6]**
Hydrolytic stability	OECD Guideline 111, Hydrolysis as a function of pH (pH 4.0, 7.0 and 9.0): Hydrolytically stable at all pH values with estimated half-life greater than 1 year	Hydrolysis as a function of pH (4.0, 7.0 and 9.0), EU Method C.7 (EG-guideline 92/69): Less than 10% hydrolysis observed. Hydrolysis half life considered to be > 1 year under environmental	Study not conducted as the substance shows no chemical moieties that may be subject to hydrolytical reactions.

[6] Tetraethylammonium perfluorooctane sulfonate

	C1: CF_3SO_3H	**C4: PFBS/KPFBS**	**C8: PFOS NEt_4-salt[6]**
	at 25 °C	conditions (25°C).	
Biodegradability	OECD Guideline 301 D, (Closed Bottle Test) for ready biodegradability: There was 0% degradation of test item based on O_2 consumption. after 28 days. Substance not readily biodegradable.	OECD Guideline 301 E, (modified OECD screening test) for ready biodegradability: 14% biodegradation observed based on DOC removal. Substance concluded not readily biodegradable. OECD Guideline 301 F, (Manometric Respirometry Test) for ready biodegradability : Less than 1% degradation based on O_2 consumption. PFBS not readily biodegradable. OECD Guideline 301 D, (Closed Bottle Test) for ready biodegradability: Less than 3% degradation observed, based on O_2 consumption. PFBS is not readily biodegradable OECD Guideline 301 D, (modified Closed Bottle Test), ready biodegradability, no primary degradation observed based on chemical analysis. (Saez et al. 2008) Non-guideline screening test: Biological fixed bed bioreactor. No biodegradation observed based on substance specific analysis. PFBS not primary biodegradable	OECD Guideline 301E (modified OECD screening test) for ready biodegradability: Biodegradation after 27 days is given as 0%. based on DOC removal. Substance not readily biodegradable OECD Guideline 301 D, (modified Closed Bottle Test, PFOS potassium salt tested), ready biodegradability, no primary degradation observed based on chemical analysis., (Saez et al. 2008)

With regards to the hydrolytic stability, PFBS and the C1 compound were found to be stable at all tested pH values with an estimated half-life greater than 1 year at 25 °C, while PFOS was considered to have no chemical moieties that may be subject to hydrolytical reactions. All three compounds were concluded to be not readily biodegradable in the tests performed. As the other biodegradability tests for PFBS, as well as the tests for the shorter (C1) and longer (C8) PFSAs demonstrate very low or negliglible biodegradation, the conclusion that PFBS is not readily biodegradable is considered reliable. It is emphasised in particular that in a ready biodegradability test no biodegradation (based on oxygen consumption) was observed for trifluoromethane sulfonic acid which may be expected to be the most susceptible to biodegradation of the PFSAs due to the low steric bulk of the molecule.

In summary, based on experimental results from screening tests on KPFBS, which are supported by BIOWIN predictions, and read-across between the C1, C4 (PFBS) and the C8 PFSAs in tests of hydrolytic stability and ready biodegradability, it can be concluded that PFBS is not readily biodegradable and is meeting the screening criteria for "potentially P/vP" according to ECHA Guidance on PBT/vPvB assessment (ECHA, 2017a).

3.1.5. **Summary and discussion of degradation**

The perfluorinated substances are among the most stable organic compounds due to the high bond energy of the C-F bond, which makes the substances resistant to acids, bases, oxidation and reduction, even at high temperatures. The sulfonic acid group is at its highest oxidation state and cannot be oxidised further under environmentally relevant conditions. The high stability of PFASs, combined with their mobility, allows for a wide distribution in the environment, and many PFSAs have been detected globally in the environment.

PFBS is stable towards hydrolysis, oxidation and photodegradation in the atmosphere, and no abiotic degradation has been reported at environmental conditions. In a hydrolysis test no hydrolysis was observed. Reductive defluorination has been suggested as a plausible degradation pathway for PFASs, but evidence that PFBS undergoes such processes in the environment has not been found.

In total five biodegradation screening test are available for KPFBS/PFBS with varying reliability, demonstrating no primary biodegradation and that the substance is not readily biodegradable. When also taking into account read-across to similar measurements for the corresponding C1 and C8 sulfonic acids, as well as supportive evidence from BIOWIN predictions, it can be concluded that PFBS is not "readily biodegradable" but meets the screening criteria for "potentially persistent/very persistent" according to ECHA Guidance on PBT/vPvB assessment (ECHA, 2017a).

A few recent studies have reported degradation of PFOS under optimised laboratory conditions by bacteria or specific enzymes, but the rates of these processes under environmental conditions are unknown, and potentially very slow or negligible. Based on the close structural relationship between PFBS and PFOS, similar transformations may be expected for PFBS, but this has not yet been reported. Furthermore, biodegradation of PFSAs have not been observed in the environment.

In summary, based on the available data, abiotic or biotic degradation of PFBS at environmentally relevant conditions is expected to be very slow or negliglible.

3.2. Environmental distribution

3.2.1. **Sorption to the organic fraction of soil**

Information from the ECHA dissemination website for KPFBS refers to a study of adsorption - desorption using a Batch Equilibrium Method (OECD Guideline 106). The study was performed on the free acid form of PFBS in the pH-range 5.96 to 7.77, as specified by the test guideline. Adsorption and desorption testing on the acid is equivalent to testing on the potassium salt. The measured adsorption coefficient was 0 for the tested soils and sediment, and 0.5 for the tested sludge. The low sorption of PFBS to all five matrices tested (three soils, sediment, and sludge) was evaluated by the registrant as placing PFBS in the very high mobility class (K_{OC} = 0 - 50).

When conceptualising sorption/desorption to soil for neutral organic molecules, one has to consider mainly van der Waal type interactions, i.e. non-ionic sorption interactions (Arp and Slinde, 2018). For organic ions, like PFBS, this is more complex, and one has to additionally consider ionic interactions between the substance and soil. Soils exhibit wide variation in their anion exchange capacity (AEC), and hence their ability to retain negatively charged molecules like PFBS.

The complexity of the PFAS chemistry is high, and it is difficult to predict the sorption of the substances from a single sorbent bulk property, such as for example organic carbon (OC) content (Pereira et al., 2018). How various sorbent-specific properties, such as pH and surface-bound cations, interact to determine the binding of PFASs to soils is still not fully understood. At environmental pH, natural organic matter (NOM) carries a negative net charge due to the presence of dissociated carboxylic and phenolic acid groups. The sorption of anionic, organic contaminants is promoted by a decrease in pH and by an increase in the concentration of cations.

Pereira et al. (2018) investigated the effect of solution pH and concentrations of Al^{3+}, Ca^{2+} and Na^{+} on the sorption of PFASs in soils. They found that perfluoroalkane sulfonic acids sorbed more strongly than perfluoroalkyl carboxylic acids. The PFAS sorption was further found to increase with increasing perfluorocarbon chain length with 0.60 log K_{OC} units per CF_2 moiety for C_3-C_{10} PFCAs and 0.83 log K_{OC} units per CF_2 moiety for C_4, C_6, and C_8 PFSAs. Short-chained PFASs, including PFBS, were weakly sorbed (less than 10% on average), while long-chained PFASs sorbed strongly (on average, 99-100%). Log K_{OC} for PFBS was determined to be in the interval -0.7 to 2.2 at different pH-values and cation concentrations, with an average log K_{OC} at 1.2.

Though adsorption/desorption to soils is commonly normalised to the organic carbon fraction, i.e. K_{OC} value, a common discussion point regarding the adsorption/desorption of ions is the role of clays and minerals (Droge and Goss, 2013). Clays and minerals can have widely differing available surface areas for sorption and AEC values. It is therefore challenging to include a generic parameter to account for clay sorption.

Guelfo and Higgins (2013) studied the subsurface transport potential of perfluoroalkyl acids in batch sorption experiments with various soils in the presence of co-contaminants relevant to aqueous film-forming foam (AFFF)-impacted sites. The results indicated that PFAA groundwater transport will depend on the solid phase characteristics at the site as well as PFAA concentration and chain length. Detailed site-specific information will likely be needed to accurately predict PFAA transport at AFFF-impacted areas. A log K_{OC} value for PFBS at 1.79 ± 0.10 was reported, together with a range of log K_d values for different soil types, -0.55 to 0.21 (K_d = concentration-specific solid-water distribution coefficient, L/Kg).

Kwadijk et al. (2010) examined the sorption of PFAS contaminated areas in the Netherlands and derived a log K_{OC} of 2.2 for PFBS, and a log K_d at 1.42 ± 0.50. Vierke et al. (2014) investigated the transport of perfluoroalkyl acids in a water-saturated sediment column under near-natural conditions. The authors concluded that short-chain PFCAs and PFSAs, like PFBS, may be problematic if contaminated surface waters are to be used for drinking water production via riverbank filtration as the short-chain substances will pass through the sediments. A log K_{OC} of 2.7 for PFBS was reported.

In a study of the sorption behaviour of three perfluoroalkyl substances (PFBS, PFOS and PFOA) in six different soils with contrasting characteristics, it was found that PFBS and PFOA showed a considerably lower sorption than PFOS in soils (Milinovic et al., 2015). This was attributed to the varying hydrophobicity of the substances, which is derived from the length of their perfluorinated carbon chain and the functional hydrophilic group, i.e. sulfonic vs. carboxylic acid. Furthermore, the sorption of PFOS was found to be highly

irreversible, while PFOA and PFBS showed much higher desorption yields. For PFBS a log K_{OC} of 1.2 was determined.

The Reach Guidance, Chapter R.7b: Endpoint Specific Guidance defines a log K_{OC} of 3.0 as threshold when a substance can be considered to have a high potential for adsorption. At lower log K_{OC}, the adsorption is low, while the preference for the water phase is correspondingly higher. Hence, with log K_{OC} in the range 1.2 to 2.7, PFBS will be distributed relatively easily within and between environmental compartments and should be considered as highly mobile in the environment.

Arp and Slinde (2018) considered a log K_{OC} of 2.2 (equal to a K_{OC} of 158 L/Kg) and pointed out that this indicates that PFBS is quite mobile in the aquatic environment. The definition of K_{OC} is given in Equation 2:

Equation 2 $$K_{OC} = C_{soil}/(C_{water} * f_{OC})$$

where C_{soil} (ng/Kg) is the soil concentration, C_{water} (ng/L) is the water concentration and f_{OC} the fraction of organic carbon. At 2% f_{OC} one can calculate as presented in Equation 3:

Equation 3 $$C_{soil}\ (ng/kg) = K_{OC} * C_{water} * 0.02 = 3.16 * C_{water}\ (ng/L)$$

When water flows through soil, water with dissolved PFBS is continuously replaced with fresh water, and PFBS with a C_{soil}/C_{water} ratio of ca. 3 is effectively washed out of the soil. PFBS can be more readily transported compared to substances with a higher log K_{OC} value. For example, if the calculation is performed for a more classical PBT-substance like HBCDD with a log K_{OC} = 4.66 (ECHA, 2008), corresponding to K_{OC} = 46000, C_{soil} = 920 * C_{water}.

In summary, the high complexity of the PFAS chemistry makes it difficult to predict the sorption of the substances from a single sorbent bulk property. Properties such as pH, identity of the soil/sediment and surface-bound cations interact to determine the binding of PFASs. The sulfonic acids tend to sorb more strongly than carboxylic acids, and the PFAS sorption tends to increase with increasing perfluorocarbon chain length. However, PFBS has been found to be a weakly sorbing substance with a high mobility in the environment. PFBS has a preference for distribution to the aqueous phase and is relatively readily transported when water flows through soil. Log K_{OC} for PFBS has been reported in the range 1.2 to 2.7.

3.2.2. **Sorption to the mineral fraction of soil**

In addition to the highly complex PFAS chemistry that makes it difficult to predict their sorption, the highly complex nature of the soil complicates matters further. Soils consist of organic matter, minerals and pore spaces filled with air and water (Brady and Weil, 2010). Sand, silt and clay all provide minerals and surface area for the sorption of PFAS. Sand, silt and clay differ in their particle size, and smaller clay particles have colloidal properties carrying positive and/or negative charges. Both silicates, iron and aluminium oxides drive the soils chemical and physical activity (Brady and Weil, 2010).

Equation 4 describes the sorption (represented by K_D) of ionic species to minerals (e.g. Milinovic et al., 2015):

Equation 4 $$K_D = f_{OC}K_{OC} + f_{minerals/clays}\,K_{minerals/clays}$$

Where f_{OC} is the fraction of organic carbon in the soil, $f_{minerals/clays}$ is the fraction of minerals and clays in the soil, and the corresponding K values represent partitioning coefficients for the sorption of ionic species to both the organic carbon (K_{OC}) and the minerals and clays ($K_{minerals/clays}$).

A more specific model for ion exchange sites on minerals and clays has been developed for cations (Droge and Goss, 2013a), and this may be extended to anions, Equation 5.

Equation 5 $K_D = f_{OC}K_{OC} + f_{IEC}\,K_{IEC\text{-}clays}$

Where f_{IEC} is the fraction of ion exchange capacity sites of a soil per volume, and $K_{IEC\text{-}clays}$ the compound specific sorption to the ion exchange sites.

An important consideration for sorption of ions like PFBS, is that the soil solution chemistry will also play a role. This is especially true if ion exchange mechanisms dominate sorption, as the pH and salt type and concentration can affect the availability of ion exchange sites on the soil or mineral surfaces (Higgins and Luthy, 2006), as well as potentially influencing the ionic state of PFBS via protonation. Both Equation 4 and Equation 5 indicate that sorption to a soil is the sum of sorption to the organic carbon and to mineral component. In cases where sorption to minerals is very strong, or at very low organic carbon content (for example freshwater and marine sediments), electrostatic interactions with mineral phases increases and may become the dominate sorption phase (Barzen-Hanson et al., 2017).

Despite the organic matter comprising between 2 and 5 % of the soils volume and the minerals making up between 45 and 58 % (Brady and Weil, 2010; ECHA Guidance Chapter R.16 on Environmental Exposure Assessment), previous studies investigating the sorption of PFAS to soil have often concluded that sorption is most strongly correlated with the organic matter fraction. For example, Qian et al. (2017) concluded that soil organic matter enhanced the sorption of PFOS to the greatest extent in soils with varying clay, and therefore oxide and silicate contents (soil organic matter contents of 4.91-11.83 g/kg). In another study investigating the sorption of PFOS to soil, three Ferrsols, two Cambosols and one Isohumosol were selected (Wei et al., 2017). These soils varied in terms of pH, organic carbon content, Fe_2O_3 content, Al_2O_3 content, soil texture, specific surface area, average pore diameter and total pore volume. The results from a linear regression analysis revealed a positive correlation of PFOS sorption and Al_2O_3, Fe_2O_3 and organic carbon contents (most significant relationship being with Al_2O_3 content). Li et al. (2018) conducted a meta-analysis in order to investigate whether sorption to soils and sediments could be explained by the physicochemical properties of the soils or sediments. These authors concluded that a combination of organic carbon, pH and clay content had a significant role on sorption. A previous study investigating the sorption of PFBS to the aluminium oxide mineral boehmite (AlOOH) and humic acid found that sorption to humic acid and even humic acid coated boehmite was much stronger than to boehmite. This study nicely shows that even very low organic matter contents can dominate PFBS sorption (Wang et al., 2015b).

Milinovic et al. (2015) characterised sorption of PFAS to soils with a broad range of organic carbon (dissolved organic carbon from 180 to 7250 mg/kg) contents. The authors concluded that the soil mineral fraction had a negligible role in the sorption of PFBS to the soils tested, and that the organic matter dominated. In direct contrast to this, Li et al. (2019) reported that the sorption of PFBS to six soils with distinctly different physicochemical properties was not driven by the total organic carbon content of the soil which was between 2580 and 32800 mg/kg, but by the content of proteins, anion exchange capacity and the content of iron oxides (Fe_2O_3 content 1.51 – 9.21 mg/g). In a study specifically focusing on fluorotelomer sulfonates, correlations of sorption with pH, CEC, AEC and organic carbon content were investigated (Barzen-Hanson et al., 2017). These authors reported a contribution to sorption from the mineral phase for the soils with the lowest organic carbon content (percent organic carbon 0.098-7.7).

A simplistic way to describe the sorption of PFBS to minerals is to consider sorption to the silicates which are generally negatively charged and sorption to the iron and aluminium oxides which can be positively charged depending on the soil water chemistry. Silicates

repel organic anions like PFBS, while positively charged metal oxides could potentially sorb PFBS. Sorption to metal oxides occurs via ion exchange interactions (as described above), with a contribution from hydrogen bonding ligand exchange and surface complexation (Li et al., 2019a). This has been demonstrated for PFOS which sorbed to alumina but not to silica (Hellsing et al., 2016). Further, the sorption behaviour and mechanisms of PFAA to the clays: montmorillonite, kaolinite and hematite were investigated by Zhao et al. (2014). The authors reported sorption to all three minerals, with the sorption strength order being hematite (an iron oxide) > kaolinite (an alumino-silicate) > montmorillonite (a phyllosilicate). Adsorption to alumina (Al_2O_3) has also been demonstrated for PFOS and PFOA with the authors of the study postulating that the protonated alumina surface rendered it accessible for compounds with negative charges to attach themselves as a result of electrostatic interactions. Sorption was sensitive to changes in pH and ionic strength, particularly the presence of Ca^{2+} ions (Wang and Shih, 2011). PFOS and PFOA have also been reported to sorb to organo-montmorillonites ($Na_3(Al, Mg)_2Si_4O_{10}(OH)_2.4H_2O$) (Zhou et al., 2010), goethite (Lath et al., 2018) and boehmite (AlOOH) (Wang et al., 2012).

In summary, the influence of minerals and soil chemistry on the sorption of PFBS is complex, and modelling of the processes and mechanisms involved is difficult. High organic carbon contents in soils most often dominate sorption, but even trace amounts of organic carbon can have the same effect. The contribution of minerals to sorption occurs in addition to that of organic carbon, and this only becomes substantial when mineral oxides dominate the clay fraction and the appropriate solution chemistry is present. In the complete absence of organic carbon in soils or sediments, sorption to minerals would be a function of the complex solution chemistry.

3.2.3. **Volatilisation**

PFBS is a strong acid which will be fully deprotonated at environmentally relevant conditions (Arp and Slinde, 2018). The anion in solution, as well as dissolved PFBS salts do not volatilise. However, the non-deprotonated, neutral PFBS does volatilise to ca. 2.8-7 Pa. This implies that traces of PFBS fumes would be present in a room containing neutral PFBS and poor ventilation.

The pH dependant air-water partition coefficient, log D_{aw}, was estimated at -6.5 to -14.5 (see

Table 9). This indicates that volatilisation from water is negligible. At pH 8, essentially all PFBS is ionic, and the substance would remain exclusively in water.

Because of negligible volatilisation of PFBS, its presence in the air would be primarily due to direct emissions of the substance or its salts into the air. Another possibility is through emissions of contaminated particles or water droplets (e.g. contaminated marine aerosols). When in the atmosphere, PFBS is expected to readily partition with surface water and water droplets (rain, cloud droplets, fog droplets, etc.) based on the very low log D_{aw} value. Therefore, it will undergo efficient removal from the atmosphere via wet-deposition.

The Henry's Law constant describes the tendency of a substance to volatilise from water. It can be calculated by Equation 6 (ECHA Guidance Chapter R.16 on Environmental Exposure Assessment):

Equation 6 $\quad HENRY = VP * MOLW / SOL$

Where HENRY is the substance specific Henry's Law constant, VP is its vapour pressure, MOLW is molecular weight and SOL is water solubility.

Using the physical chemical properties reported in Section 1.5 (measured VP = 7 Pa; MOLW 300.1 g/mol; measured SOL = 52.6 g/L as reported on the ECHA dissemination page) the Henry's Law constant of PFBS is 0.040 Pa * m^3/mol.

The calculated value for the Henry's Law constant for PFBS, 0.040 Pa * m^3/mol, is far below the limit value for volatile chemicals, >250 Pa * m^3/mol. This shows that PFBS has a low tendency to volatilise from water and that the aqueous environmental compartment is preferred for PFBS, compared to the atmosphere.

3.2.4. **Distribution modelling**

Distribution modelling for PFBS in comparison with other perfluoroalkane sulfonic acids (PFHxS, PFOS and PFDS) was performed by the Norwegian Geotechnical Institute (Arp and Slinde, 2018).

Distribution modelling of anionic substances like PFBS and other PFAS is not straightforward. Most distribution models were developed for neutral substances, wherein distribution modelling is generally described using three main partitioning coefficients: K_{OW}, K_{oa} and K_{aw}. However, octanol as a surrogate phase for soil/sediment/aerosol organic matter, or biological membranes, is inappropriate for anionic substances like PFBS, because octanol cannot make ionic interactions. One approach that has been used to solve this for PFOS is to apply the experimental K_{OC} value in distribution modelling, rather than the K_{OW} (Armitage et al., 2009), as the experimental K_{OC} inherently includes some ionic interactions to a naturally occurring phase. In this case, basic distribution modelling can be done using the pH-dependant K_{aw}, K_{OC} and the organic carbon-air partitioning coefficient $K_{OC, a.}$ For the selected perfluoroalkane sulfonates, values for these terms are presented in

Table 9.

Table 9: Physiochemical properties needed to estimate environmental distribution for PFBS and homologues.

Parameter		PFBS	PFHxS	PFOS	PFDS	References
log K_{aw}	(neutral)	-2.6	-2.4	-1.7	-1.2	Wang et al., 2011
log D_{aw}	(pH 8)	-14.5	-13.8	-13.1	-12.0	Derived from Wang et al., 2011
log K_{OC}		2.2	2.6	3.0	3.4	PFBS: Kwadijk et al., 2010 PFOS: Zareitalablad et al., 2013 PFHxS and PFDS extrapolated (read-across)
log $K_{OC, a}$		4.8	5.0	4.7	4.6	Calculated from the thermodynamic triangle: log $K_{OC, a}$ = log K_{OC} - log K_{aw}

The log K_{OC} values spread from 2.2 (PFBS) to 3.4 (PFDS), which would imply PFDS, PFOS and PFHxS sorb stronger than PFBS by factors of 16, 6 and 2.5 respectively.

The Globo-POP model (Wania, 2003) has been established to model the global distribution of neutral substances. In Figure 3 below, output from the Globo-POP model is used to illustrate the partitioning properties of substances that, globally, are predominantly in the air, water or soil phases, based on the following assumptions: the substance is non-degradable (which is appropriate for PFBS), 10 years of continuous emissions has

occurred, emissions occur equally to soil, water, air (1/3rd each), and the global distribution of emissions is zonally distributed similarly to the human population. In this figure, lines are overlaid that represented the physical chemical properties of PFBS, PFHxS, PFOS and PFDS (Table 9), though using the pH-dependant K_{aw}, K_{OC} and the organic carbon-air partitioning coefficient $K_{OC,a}$ which is more appropriate for negatively charged substances.

As is indicated by Figure 3, PFBS is tightly clustered along with PFHxS, PFOS and PFDS, which indicates that they are all primarily in the water phase, while PFBS shows the highest preference for water.

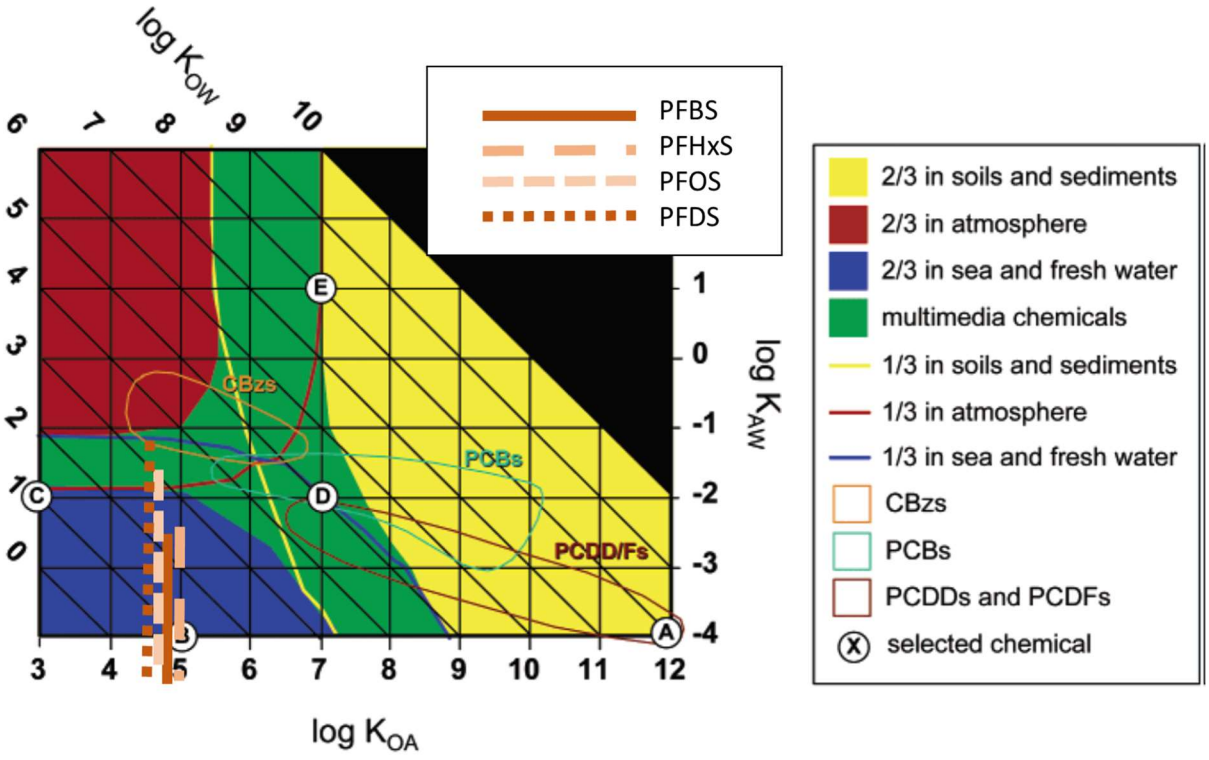


Figure 3: Global POP chemical space distribution for non-degradable substances being emitted into air-water-soil at equal levels for the 10 years, with a similar zonal distribution the human population. The location of PFBS, PFHxS, PFOS and PFDS is overlaid, using log K_{OC} for log K_{OW}, log $K_{OC,\,a}$ instead of log K_{oa} and considering the pH dependence of log K_{aw}. Note that at ambient pH, the log K_{aw} is even lower than presented in this chart, and the substances would be strongly within the sea and freshwater media area of the chart. Other substances presented in this figure for comparison are CBzs (chlorobenzenes), PCBs (polychlorinated biphenyls) and PCDDs and PCDFs (dioxins). This Figure is modified and reprinted with permission from Wania and the American Chemical Society.

3.2.5. **Field data**

There are numerous studies reporting PFBS data in the environment compiled from peer-reviewed scientific research papers, thesis and reports. Although monitoring data encompass some uncertainties and variability, the reliability of the published data is considered to be of sufficient quality. Arp and Slinde (2018) gathered a non-exhaustive list of monitoring data for PFBS in surface water, ground water, drinking water, wastewater and leachate from landfill.

3.2.5.1. PFBS in surface fresh water

PFBS has been found in surface fresh waters throughout Europe, Asia and North America. In Table 10 PFBS concentrations from surface water not connected to known point sources have been compiled.

Table 10: Environmental concentrations of PFBS in surface fresh water samples (ng/L).

Location	Date	Mean +/- SD	Min. – Max. (Median)	n (det. Freq), LOD	Reference
Baltic sea	2013-2014	0.098		4/4 (100 %) LOD = 0.04	Gebbink et al., 2016a
Elbe River	2014	2.3	0.80 - 3.60 (2.40)	(100 %) LOD = 0.13	Heydebreck et al., 2015
France	2009	<1	<1 - 3 (<1)	(1 %) LOD = 4	Boiteux et al., 2012
France	2012		<0.02 - 29.00	(65 %) LOD = 0.02	Munoz et al., 2015
Germany Bad Godesberg	2008-2009	<0.01	<0.01	0 % LOD = 0.01	Wilhelm et al., 2010
Germany Bimmen-Lobith	2008-2009		0.00 - 0.08 (0.02)	(89 %) LOD = 0.01	Wilhelm et al., 2010
Germany Düsseldorf-Flehe	2008-2009		0.00 - 0.10 (0.03)	(91 %) LOD = 0.01	Wilhelm et al., 2010
Germany Möhne river	2008-2009		0.017 - 0.032 (0.023)	35 (100 %) LOD = 0.01	Wilhelm et al., 2010
Germany WkSt Rhein-Nord Kleve	2008-2009		0.00 - 0.06 (0.03)	(92 %) LOD = 0.01	Wilhelm et al., 2010
Germany WkSt Süd/Bad Honnef	2008-2009	<0.01	<0.01 - 0.03 (<0.01)	(7 %) LOD = 0.01	Wilhelm et al., 2010
Germany, Rhine river	2006	14.3	<LOD - 46.00 (12.50)	38 LOD = 2	Skutlarek et al., 2006
Germany, Rhine watershed	2008		<0.08 - 181.00	LOD = Not reported	Möller et al., 2010
Germany, Rhine River	2013	15.6	1.40 - 40.00 (15.00)	(100 %) LOD = Not reported	Heydebreck et al., 2015
Germany, Rhine River	2016	21.9	0.46 - 146.00 (20.1)	20 (100 %) LOD = 0.03	Pan et al., 2018

Location	Date	Mean +/- SD	Min. – Max. (Median)	n (det. Freq), LOD	Reference
Germany, Ruhr river	2006	16.7	<LOD - 71.00 (13.00)	27 LOD = 2	Skutlarek et al., 2006
Germany, River Elbe	2007	6.3	3.40 - 17.70 (5.40)	LOD = 0.50 (LOQ= 1.67)	Ahrens et al., 2009a
Italy (river basin survey)	2010-2013	8.90 ± 8.90	<LOD - 31.40 (5.60)	104 (74 %) LOD = 1	Valsecchi et al., 2015
Norway, lake	2004	nd		5 LOD = 0.03	Kallenborn et al., 2004
Norway, lake	2004	42.8	5.64 - 112.00	6 LOD = 0.03	Kallenborn et al., 2004
Norway, River Alna River Drammens-elva River Glomma	2016		 0.05- 0.74 0.05-0.1 0.2-0.5	LOD = Not reported	Skarbøvik et al., 2017
Nordic countries, rivers and lakes	2017		<0.02-1.62	(36 %) LOD = Not reported	Kärrman et al., 2019
Finland from 47 different sites	2014-2019		<0.1-3.6	222 (68 % > LOQ)	Finnish Environment Institute, 2018
Spain	2009	1.22 ± 1.55	n.d. - 5.50	12 (17 %) LOD = 0.02-1.5	Pico et al., 2012
Spain	2009	1.33 ± 3.01	<0.10 - 10.10 (0.20)	30 (57 %) LOD = Not reported	Domingo et al., 2012
SW along River Elbe	2007	2.2	0.00 - 3.40 (2.30)	30 (100 %) LOD = 0.5	Ahrens et al., 2009b
Sweden, Mälaran Lake	2016	1.43	0.75 - 1.92	10 (100 %) LOD = 0.05	Pan et al., 2018
Sweden, river	2015	0.24 ± 0.10	0.11 - 0.35 (0.23)	4 (100 %) LOD = 0.2425	Tröger et al., 2018
Sweden, lake	2015	0.54 ± 0.46	0.19 - 1.20 (0.39)	2 (100 %) LOD = 0.5425	Tröger et al., 2018
Swedish rivers	2013	9.5	0.03 - 19.00	40 (59 %) LOD = Not reported	Nguyen et al., 2017

Location	Date	Mean +/- SD	Min. – Max. (Median)	n (det. Freq), LOD	Reference
Switzerland, Glatt river	2007	4.3	2.30 - 7.70 (2.80)	3 LOD = Not reported	Huset et al., 2008
UK, Thames River	2016	5.06	3.26 - 6.75	6 (100 %) LOD = 0.05	Pan et al., 2018
Xiaoqing River	2014	nd		LOD = Not reported	Heydebreck et al., 2015)
China, Chao Lake	2016	15.4	1.50 - 81.50	13 (100 %) LOD = 0.05	Pan et al., 2018
China, Huai River	2016	0.83	0.52 - 1.59	9 (100 %) LOD = 0.05	Pan et al., 2018
China, Liao River	2016	0.94	0.43 - 2.16	6 (100 %) LOD = 0.05	Pan et al., 2018
China, Pearl River	2016	4.49	0.21 - 21.50	13 (100 %) LOD = 0.05	Pan et al., 2018
China, Shuangtaizi Estuary	2013	4.7	0.95 - 12.60 (4.45)	21 (100 %) LOD = 0.06-0.403	Shao et al., 2016
China, Tai Lake	2016	2.02	0.17 - 4.85	15 (100 %) LOD = 0.05	Pan et al., 2018
China, Taihu Lake	2012	3.76 ± 0.72		LOD = Not reported	Fang et al., 2014
China, Yangtze River	2016	1.84	0.22 - 4.68	35 (100 %) LOD = 0.05	Pan et al., 2018
China, Yellow River	2016	0.99	0.07 - 2.23	15 (100 %) LOD = 0.05	Pan et al., 2018
Hong Kong	2008-2010	6.83 ± 2.40	2.18 - 8.69 (7.30)	12 (100 %) LOD = Not reported	Loi et al., 2011
Korea, Han River	2016	2.27	1.34 - 3.17	6 (100 %) LOD = 0.05	Pan et al., 2018
South Korea, Shihwa Industrial zone	2004	2.5	4.27	LOD = 0.05	Rostkowski et al., 2006
Vietman	2013-2015		0.10 - 8.28 (4.19)	LOD = 0.03-0.52	Lam et al., 2017
Canada, urban	2012-2016	2.52 ± 1.04	1.40 - 4.00 (2.50)	5 (100 %) LOD = 0.05	D'Agostino and Mabury et al., 2017

Location	Date	Mean +/- SD	Min. – Max. (Median)	n (det. Freq), LOD	Reference
Canada, rural	2012-2016	0.78 ± 0.90	0.00 - 1.70 (0.70)	4 (50 %) LOD = 0.05	D'Agostino and Mabury et al., 2017
NJ, Delaware River	2016	2.19	0.52 - 4.20	12 (100 %) LOD = 0.05	Pan et al., 2018

The PFBS concentrations measured in surface water vary widely. In many cases the concentrations are at the low end, or even not detected. However, in other cases elevated levels are measured. The highest PFBS concentration reported is 180 ng/L, measured in the River Rhine watershed in Germany (Möller et al. 2010).

The ubiquity of PFBS in water samples shows that it is highly mobile and well distributed in the freshwater environment, even apart from point sources.

PFBS concentrations found in surface water close to PFAS production facilities or other PFAS hot spots are presented in Table 11.

Table 11: Concentrations of PFBS in surface water near PFAS production facilities or known hotspots (ng/L).

Location	Date	Mean +/- SD	Min-Max. (Median)	n (det. Freq) LOD	Remark	Reference
Italy (Bormida, Tanaro)	2010-2013	6.8 ± 5.5	<LOD - 17.0 (5.9)	33 (80 %) LOD = 0.2-5	River basin survey	Valsecchi et al., 2015
Italy (Brenta, Fratta-Gorzone)	2010-2013	878.0 ± 764.0	56.0 - 1,665.7 (894.2)	4 (100 %) LOD = 0.2-5	River basin survey	Valsecchi et al., 2015
Italy	2010-2013	11	0.0 - 66.0 (2.0)	(74 %) LOD = 0.2-5	Industrialised river	Castiglioni et al., 2015
Netherlands	2016	20.3 ± 3.7	12.0 - 27.0	18 (100 %) LOD = 0.04	River	Gebbink et al., 2017
Germany,	2006	319	<LOD - 1,450 (83)	12 LOD = 2	Moehne river	Skutlarek et al., 2006
Norway	2016		<7.5-1,150	66 % LOD = Not reported	Stream	Lassen et al., 2017

Location	**Date**	**Mean +/- SD**	**Min-Max.** **(Median)**	**n (det. Freq)** **LOD**	**Remark**	**Reference**
Finland from 47 different sites	2014-2019		<0.1–4,030	222 (73 % > LOQ)	Firefighting training sites or ditches and streams near airport area	Finnish Environment Institute, 2018
China, Daling River	2014	1090	nd - 3,780.0 (668.0)	LOD = Not reported	River	Wang et al., 2016b
China, Fuxin	2009	320	7.8 - 445.0 (353.0)	LOD = Not reported	River	Bao et al., 2011
China, Tangxun Lake	2013	4950	4,600.0 - 5,300.0	2 LOD = 0.5	WWTP effluent	Zhou et al., 2013
China, Tangxun	2013	3720	2,240.0 - 4,520.0	LOD = 0.5	Lake	Zhou et al., 2013
Taiwan	2013	0.3 ± 0.2		3 LOD = 0.1	Upstream WWTP	Lin et al., 2014
Taiwan	2013	329.0 ± 16.0		3 LOD = 0.1	WWTP	Lin et al., 2014
Taiwan	2013	392.0 ± 6.0		3 LOD = 0.1	Downstream WWTP	Lin et al., 2014
Taiwan	2013	282.0 ± 9.0		3 LOD = 0.1	Downstream WWTP	Lin et al., 2014
Taiwan	2013	262.0 ± 4.4		3 LOD = 0.1	Downstream WWTP	Lin et al., 2014
Canada	2012-2016	6.4 ± 4.5	0.8 - 13.0 (4.4)	14 (100 %) LOD = 0.05	AFFF source	D'Agostino and Mabury, 2017
Canada, lake	2010-2011	4.90 ± 1.00		5 LOD = 0.0091	local pollution from an airport	Lescord et al., 2015
Canada	2010 - 2011	0.07 ± 0.01		5	Lake	Lescord et al., 2015

Location	Date	Mean +/- SD	Min-Max. (Median)	n (det. Freq) LOD	Remark	Reference
				LOD = 0.0019		

The surface water concentrations of PFBS reported above were measured near PFAS production facilities in the Netherlands (Gebbink et al., 2017) and China (Wang et al., 2016b; Bao et al., 2011; Zhou et al., 2013). PFBS was also detected in the influent and effluent from a waste-water treatment plant near an electronic production facility in Taiwan (Lin et al., 2014), in fire-fighting foam contaminated areas in Canada (D'Agostino and Mabury, 2017) Norway (Lassen et al., 2017) and Finland (Finnish Environment Institute, 2018) as well as in a mixed industrial area that includes fluorochemical plants in Italy (Valsecchi et al., 2015; Castiglioni et al., 2015).

The PFBS concentrations reported from areas near point sources and industrial facilities are in many cases much higher compared to concentrations measured in surface waters without known PFBS sources. The highest concentrations of PFBS found, 5.3 µg/L, were found in Tangxu Lake in China, near a PFAS production facility. However, also in Europe high concentrations of PFBS have been found: 1.7 µg/L in water from the Fratta Garzone river in Italy which receives water from textile industry and a fluorochemical plant. In Finland up to 4.0 µg/L have been measured which are related to firefighting training sites or ditches and streams near airport areas.

3.2.5.2. PFBS in groundwater

Concentrations of PFBS measured in groundwater are presented in Table 12.

Table 12: Environmental concentrations of PFBS in groundwater (ng/L).

Location	Date	Mean +/- SD	Min-Max (Median)	n (det. Freq.) LOD	Remark	Reference
Europe - 26 countries	2008	0.3	25	164 (15 %) LOD = 0.3		Loos et al., 2010
France	2009		9	(4 %) LOD = 4		Boiteux et al., 2012
Netherlands Groundwater recharge area (several (PFAA) sources are present – former	2011		OW 1: 1.6-104 OW2: <LOQ-104	LOD = Not reported	Observation wells (OW, n=2) Pumping wells (PW, n=5)) – travel time through soil	Eschauzier et al., 2013

Location	Date	Mean +/- SD	Min-Max (Median)	n (det. Freq.) LOD	Remark	Reference
landfill, military base, small commercial/industrial area			PW:<LOQ LOQ= 0.01		from landfill to pumping wells > 25 years	
Vietnam	2013-2015	<0.3		LOD = 0.03-0.52		Lam et al., 2017
China, Daling River	2014	375	865	4 LOD = 0.03		Wang et al., 2016b
China, Fuxin	2009	235	1.2 – 872 (34.4)	LOD = Not reported		Bao et al., 2011
China, Fuxin	2017	11 010	64-21 200	LOD = Not reported		Bao et al., 2019

A European survey of groundwater from 2008 reported PFBS in 15% of the samples, with a maximum PFBS concentration at 25 ng/L and mean at 0.3 ng/L (Loos et al., 2010). In a French study PFBS concentrations up to 9 ng/L were reported, but the substance was found in 4% of samples only (Boiteux et al., 2012). In the Netherlands a maximum PFBS concentration of 104 ng/L was found in a groundwater recharge area, in an observation well located downstream from a landfill area.

Considerably higher PFBS levels were reported in groundwater collected near two PFAS production facilities in China, up to 21.2 µg/L (Wang et al., 2016b; Bao et al., 2011; 2019). A remarkable 24-fold increase in PFBS concentration from 0.87 to 21.2 µg/L in groundwater beneath one of the facilities was registered from 2009 to 2017 (Bao et al., 2019). Another study did not report PFBS in groundwater in Vietnam (Lam et al., 2017).

3.2.5.3. PFBS in drinking water

Drinking water samples, including finished water, bottled water, and water at different steps in the purification process, have been analysed for PFBS in areas near and far removed from PFAS source zones. Measured concentrations of PFBS in drinking water are listed in Table 13.

Table 13: Concentrations of PFBS in drinking water (ng/L)

Location	Date	Mean +/- SD	Min-Maks (Median)	n (det. Freq) LOD	Remark	Reference
Global	2015	0.1	1.6	38 (47 %) LOD = 0.02	Bottled water (Canada, Burkina Faso, Chile, Ivory Coast, France, Japan, Mexico, Norway, USA)	Kaboré et al., 2018
Global	2015	0.24	0.0 - 1.1 (0.2)	59 (88 %) LOD = 0.02	Tap water (Canada, Burkina Faso, Chile, Ivory Coast, France, Japan, Mexico, Norway, USA)	Kaboré et al., 2018
BE, DE, I, NL, NO, SE	2010		<0.09 – 18.8	(86 %) LOD = Not reported	Tap water samples in six European Countries (Sweden, Italy, Belgium, Netherlands, Norway, Germany) (n=7)	Ullah et al., 2011
France	2015	3.2	1.3 - 6.7 (2.9)	(32 %) LOD = 0.15-8.76	Bottled water	Schwanz et al., 2016
France	2015	6.8	2.0 - 15.0 (6.5)	(62 %) LOD = 0.15-8.76	Drinking water	Schwanz et al., 2016
Germany	2008-2009		<0.01 - 0.01(<0.01)	70 (3 %) LOD = 0.01	Drinking water	Wilhelm et al., 2010
Germany Ruhr river	2006	12.2	<LOD - 26.0 (13.5)	37 LOD = 2	Drinking water	Skutlarek et al., 2006
Italy	2010-2012	6.7 ± 11.3	<0.5 - 45.0 (1.8)	46 (63 %) LOD = Not reported	Drinking water	IRSA-CNR, 2013

Location	Date	Mean +/- SD	Min-Maks (Median)	n (det. Freq) LOD	Remark	Reference
Netherlands	2008	24.0 ± 10.0	10.0 - 47.0	6 LOD = Not reported	Infiltrated river water	Eschauzier et al., 2010
Netherlands	2008	2.4 ± 4.9	0.5 - 18.0	6 LOD = Not reported	Infiltrated rainwater	Eschauzier et al., 2010
Nether-lands	2016	4.94	0.5 - 19.0	6 (100 %) LOD = 0.04	Source zone: Drinking water near a PFAS production facility	Gebbink et al., 2017
Netherlands	2017	7.1	2.5-11.0 7.3	6 100 % LOD <1	Source zone: Drinking water , intake points within 25 km of PFAS production plant	Brandsma et al., 2019
Spain	2009	4.5 ± 14.5	<0.07 - 69.4 (0.4)	LOD = 0.07	Drinking water	Ericson et al., 2009
Spain	2015	nd		LOD = 0.15-8.76	Bottled water	Schwanz et al., 2016
Spain	2015	11	2.8 - 24.0 (11.0)	(31 %) LOD = 0.15-8.76	Drinking water	Schwanz et al., 2016
Sweden	2015	0.8 ± 0.3	0.3 - 1.3 (0.7)	11 (92 %) LOD = 0.01	Various parts of DW treatment production	Tröger et al., 2018
China, Shuangtaizi Estuary	2013	5.42	1.0 - 13.8 (4.4)	21 (100 %) LOD = 0.06-0.403	Bottled water	Shao et al., 2016
China	2008-2010		0.0 - 18.0 (2.8)	70	Drinking water	Mak et al., 2009

Location	Date	Mean +/- SD	Min-Maks (Median)	n (det. Freq) LOD	Remark	Reference
				(74 %) LOD = 0.04-1.6		
New Jersey, US	2009-2010	nd	6	30 (10 %) LOD = Not reported	Raw water	Post et al., 2013
US	2013-2015			(0.05%) 19 of 36,953	Drinking water samples	https://www.epa.gov/sites/production/files/2017-02/documents/ucmr3-data-summary-january-2017.pdf
Brazil	2015	3.4	3.1 - 3.6 (3.5)	(50 %) LOD = 0.15-8.76	Bottled water	Schwanz et al., 2016
Brazil	2015	4.4	0.5 - 16.0 (1.3)	(90 %) LOD = 0.15-8.76	Drinking water	Schwanz et al., 2016
China, Daling River	2014	25.4	97.8	4 LOD = 0.03	Source zone: Drinking water	Wang et al., 2016b
China, Fuxin	2009	0.47	0.2 - 0.7 (0.5)	LOD = Not reported	Source zone: Raw water	Bao et al., 2011
China, Fuxin	2009	0.54	<0.1 - 0.6 (0.5)	LOD = Not reported	Source zone: Finished water	Bao et al., 2011
China, Fuxin	2009	0.54	<0.1 - 0.6 (0.5)	LOD = Not reported	Source zone: Drinking water	Bao et al., 2011

Findings from the US contradict monitoring data from Europe and other continents. A data set from the third Unregulated Contaminant Monitoring Rule (UCMR3) has been provided, which measured PFBS and other PFAS at drinking water facilities between 2013 and 2015. In this dataset, PFBS was found above reporting levels (0.09 ug/L) in 19 out of 36,953

water samples collected (0.051%). PFBS was found at 10 of the 5,543 public water system facilities tested (0.18%).

However the majority of monitoring data in drinking water demonstrates that PFBS appears ubiquitously in drinking water, both from the tap and from bottles. Even in regions away from established PFAS source areas, widespread drinking water contamination can occur, with a France max of 15 ng/L (Schwanz et al., 2016), Germany max of 26 ng/L (Skutlarek et al., 2006), Italy max of 45 ng/L (IRSA-CNR, 2013), Spain max of 69 ng/L (Ericson et al., 2009), China max of 18 ng/L (Mak et al., 2009), and Brazil max of 16 ng/L (Schwanz et al., 2016). These concentrations even exceed those of some known source zones, such as 19.0 ng/L near a PFAS production facility in the Netherlands (Gebbink et al., 2017) , and 0.6 ng/L for drinking water near the PFAS production area of Fuxin, China (Bao et al., 2011). The highest concentration, however, was near a source zone, at 97.8 ng/L, along the Daling River, China (Wang et al., 2016b). This indicates that PFBS persists through drinking water production, and currently is ubiquitously present in global drinking water (Kaboré et al., 2018).

3.2.5.4. PFBS in effluents from wastewater treatment plants and landfills and occurrence in receiving waters

PFBS is commonly found in effluents from wastewater treatment plants (WWTP) and in landfill leachate. A list of PFBS concentrations measured is provided in **Error! Reference source not found.**.

Table 14: Concentrations of PFBS in WWTP effluent and in landfill leachate (ng/L).

Location	Date	Mean +/- SD	Min-Max (Median)	n (det. Freq) LOD	Remark	Reference
Faroe Islands	2004	0.2		LOD = 0.03	WWTP	Kallenborn et al., 2004
Finland	2004	64.6	<LOQ - 68.00	3 LOD = 0.03	Landfill	Kallenborn et al., 2004
Finland	2004	2.9	2.61 - 3.09	3 LOD = 0.03	WWTP	Kallenborn et al., 2004
Germany	2009		<0.39 - 1,356.00	20 LOD = 0.12	Landfill	Busch et al., 2010
Norway	2004	1.64	1.00 - 2.60	3 LOD = 0.03	WWTP	Kallenborn et al., 2004
Nordic countries	2017	5.9	0.92-13.1	100 % LOD = Not reported	WWTP	Kärrman et al., 2019
Sweden	2012-2015	1.9	3.7	LOD = 0.07	WWTP	Eriksson et al., 2017

Location	Date	Mean +/- SD	Min-Max (Median)	n (det. Freq) LOD	Remark	Reference
WWTP along River Elbe	2007	8.23	0.00 - 25.90 (5.10)	9 (100 %) LOD = 0.5	WWTP (9 wwtp, 18 samples)	Ahrens et al., 2009a
San Francisco	2014	2.70 ± 1.50		LOD = 1.9	WWTP	Houtz et al., 2016
USA (three temperate zones)	2013-2014	231.00 ± 577.33	3.44 - 3,410.00 (41.05)	87 (100 %) LOD = 2.6	Landfill (Ref organises data based on climate zone and age)	Lang et al., 2017
Singapore	2015 – 2016	N/A	161.6-1916.3 (752.6)	12/12 (100 %) LOD = 0.009	Landfill (raw leachate)	Yin et al., 2017
Ireland	June – November 2017	1100	<0.01 – 17,000 (79)	85 %	Landfill	Harrad et al., 2019

Concentrations of PFBS in landfill leachate are relatively high, with a max of 3410 ng/L measured in the US (Lang et al., 2017). High concentrations have also been found in Europe, with 1356 ng/L measured in untreated leachate at a landfill in Germany (Busch et al., 2010). The high level was explained by different uses of PFASs and different regulation and treatment processes on landfills in different countries. The effect of different treatment techniques was investigated in the study. Lowest PFAS concentrations were observed after membrane treatments (reverse osmosis and nanofiltration) and activated carbon, while the high PFAS levels were found after biological treatment and wet air oxidation (Busch et al., 2010).

Harrad et al. (2019) reported high concentrations of PFBS and other PFASs in samples of leachate from landfills in the Republic of Ireland. Leachate was collected from 40 municipal soilid waste (MSW) landfill sites across the country. Samples from sites without high density polyethylene (HDPE) liners (i.e. unlined sites) were pumped from "boreholes" (pipes inserted into the landfill body for collection and sampling of leachate), while samples from newer state of the art sites (i.e. mixed and lined sites) were collected from on-site leachate storage tanks. PFBS (arithmetic mean = 1100 ng/L) was the predominant compond among the measured PFASs. The concentrations of PFBS were sinificantly higher ($p<0.05$) in leachate samples from newer, lined landfills than in samples from unlined landfills.

PFBS concentrations in WWTP effluent are typically lower, with a maximum in this study of 26 ng/L (Ahrens et al., 2009a). Nguyen et al., (2019) investigated the temporal trend of PFAS in influents of two large WWTPs in Australia. Daily influent samples were collected over one week at different seasons from 2014 to 2017. Mean concentration of PFBS in influent of the two WWTPs rangend from 2.6-9.2 ng/L and 2.3-20 ng/L respectively, showing a decreasing trend in both WWTPs.

Evidently, landfills can be environmental hotspots for PFBS emissions which may result in a risk for the local environment. According to a Nordic screening study on PFAS by Kärrman et al. (2019), PFSAs constituted between 11% and 46% of total PFASs (27% on average) in WWTP effluent samples, and PFBS was the predominant compound detected in all samples with 37% of the PFSA class on average.

3.2.5.5. PFBS in marine water

PFBS has been reported in marine water throughout the world both in remote regions and in populated regions. Concentrations of PFBS measured are given in Table 15.

Table 15: Environmental concentrations of PFBS in marine water samples (ng/L).

Location	**Year**	**Mean +/- SD**	**Min-Maks (Median)**	**n (det. Freq)** **LOD**	**Remark**	**Reference**
Labrador Sea, the Mid Atlantic Ocean, the South Pacific Ocean, and the Japan Sea.	2002-2006		(0.01-0.04) subsurface layer (0.02-0.07) <2000 m depth	LOD= 0.0002	Labrador Sea	Yamashita et al., 2008
Mid-Atlantic	2009-2010	nd	nd-0.017	20 LOD = Not reported		Zhao et al., 2012
Baltic sea, Kattegat	2013	0.32	0.06 - 0.57	18 (65 %) LOD = 0.03		Nguyen et al., 2017
Baltic sea	2013-2014	0.10 ± 0.00		(100 %) LOD = 0.04		Gebbink et al., 2016a
German coast Baltic Sea	2007		0.01-6.51 0.26–0.88	LOD = 0.01		Ahrens et al., 2010b
Northern Europe, Atlantic and Southern Ocean	2008		<0.004–0.05	50 %		Ahrens et al ., 2010
German Bight	2017		0.44–0.72 (0.53)	LOD= not reported		Joerss et al., 2019

Location	Year	Mean +/- SD	Min-Maks (Median)	n (det. Freq) LOD	Remark	Reference
Baltic Sea			0.15–0.43 (0.24)			
Denmark	2004	0.8	0.30 - 1.09	4 LOD = 0.03		Kallenborn et al., 2004
Farao Islands	2004	0.1	0.05 - 0.20	4 LOD = 0.03		Kallenborn et al., 2004
Finland	2004	0.07	<LOQ - 0.93	3 LOD = 0.03		Kallenborn et al., 2004
Iceland	2004	0.06	0.05 - 0.08	4 LOD = 0.03		Kallenborn et al., 2004
Western Mediterranean Sea	2014	0.01 ± 0.01	nd - 0.02 (0.02)	0.012 (0 %) LOD = 0.01		Brumovský et al., 2016
China	2013	0.66	0.32 - 1.46 (0.49)	(100 %) LOD = 0.12	July	Chen et al., 2016
China	2013	nd	<MLQ - 0.24 (<MLQ)	(19 %) LOD = 0.12	Novemb er	Chen et al., 2016
Pacific Ocean	2007	0.02 ± 0.00		19 (16 %) LOD = Not reported		Wei et al., 2007
Pacific Ocean	2007	0.07 ± 0.01		LOD = Not reported		Wei et al., 2007
South China Sea	2012-2013	0.63 ± 0.43	0.25 - 1.48 (0.45)	17 (100 %) LOD = 0.01		Kwok et al., 2015

PFBS was detected in ocean waters worldwide and the concentrations of PFBS in marine water samples vary in the range of not detected to 6.51 ng/L. The highest concentration was measured at the German coast. Recent investigations of PFAS in coastal areas in the North and Baltic Sea by Joerss et al. (2019) showed a downward trend for levels of PFOS, PFOA and PFBS in seawater in the German Bight in relation to previous studies (Ahrens et al. 2010b), but these trends were not as clear in the Baltic Sea. The authors assumed that this trend is related to the transition from long chain to short chain PFAS in Europe. High concentrations were also detected in China, however at a considerably lower level, ca. 1.5 ng/L. Yamashita et al. (2008) studied the horizontal and vertical distribution of PFAs in ocean waters worldwide. In the Labrador Sea PFBS concentration were low in the subsurface layers at depths of 100–200 m (range 0.01- 0.04 ng/L) but increased below 2000 m depths (range 0.02- 0.07 ng/L). The authors suggested that the increasing concentrations below 2000 m depths were influenced by an independent deep-water current.

3.2.5.6. PFBS in soil and sediment

Concentrations of PFBS found in soil and sediments are compiled in Table 16.

Table 16: Environmental concentrations of PFBS in soil and sediment samples (ng/g dw).

Location	Date	Mean +/- SD	Min-Maks (Median)	n (det. Freq) LOD	Remark	Reference
Soil						
Norway (soil)	2015	<LOD	<LOD - 0.003 (<LOD)	10 (10 %) LOD = 5		Herzke et al., 2016
China, Fuxin (soil)	2017	11.9	<0.2-42 (ng/g fw)	LOD = Not reported	Garden soil near a fluorochemical industry park	Bao et al., 2019
Sediment						
Canada Resolute Lake, local point source (airport)	2003		nd - 0.1	LOD = 0.046	0-3 cm cores	Stock et al., 2007
Canada Char Lake, local point source (airport)	2003	<1.1		LOD = 1.0	0-3 cm cores	Stock et al., 2007
Canada Amituk Lake	2003		0.0 - 0.1	LOD = 0.029	0-3 cm cores	Stock et al., 2007

Location	Date	Mean +/- SD	Min-Maks (Median)	n (det. Freq) LOD	Remark	Reference
Baltic sea (sediment)	2013-2014	0.0002 ± 0.0001		(75 %) LOD = 0.0001		Gebbink et al., 2016a
China (sediment)	2013	0.06	0.0 - 0.2 (0.1)	(100 %) LOD = 0.04	Surface sediments (July)	Chen et al., 2016
China (sediment)	2013	0.06	0.1 - 0.1 (0.1)	(100 %) LOD = 0.04	Surface sediments (November)	Chen et al., 2016
China (sediment)	2015		0.21 to 0.94		Surface sediments	Ding et al., 2018b
Hong Kong (sediment)	2008-2010	<0.026		LOD = 0.03-0.1		Loi et al., 2011
Canada (sediment)	2012-2016	nd		LOD = 0.04	Urban	D'Agostino and Mabury et al., 2017

One study of PFBS in soil in Europe reported 10% frequency of detection of PFBS and a max of 0.003 ng/g dw, which is relatively low (Herzke et al., 2016). In China, a study of PFBS in garden soil in the vicinity of a fluorochemical industrial park found high concentrations of PFBS with a maximum of 42 ng/g dw (Bao et al., 2019).

The range of PFBS in sediments is from < LOD to 0.2 ng/g dw, at the low end. Stock et al. (2007) was able to quantify PFBS in Arctic surface sediments, with a max concentration at 0.1 ng/g dw, however the contamination could be attributed to local emission from an airport. Ding et al. (2018b) found PFBS in surface sediment in Dalian Bay (China) in a range of 0.21 to 0.94 ng/g dw. In general, there is little data on PFBS in soil and sediments in Europe. More data are available from Asia and Ding et al. (2018b) stated that PFBS was, together with PFOA and PFBA, one of the predominant PFAS in surface sediment and two sediment cores in Dailian Bay (China). However, PFBS is not primarily expected to be found in soil and sediments due to the preferred partitioning to water.

3.2.6. **Summary and discussion of environmental distribution**

The high complexity of the PFAS chemistry makes it difficult to predict the sorption of the substances from a single sorbent bulk property. Properties such as pH, identity of the soil/sediment and surface-bound cations interact to determine the binding of PFASs. The sulfonic acids tend to sorb more strongly than carboxylic acids, and the PFAS sorption tends to increase with increasing perfluorocarbon chain length. However, PFBS has been found to be a weakly sorbing substance with a high mobility in the environment. PFBS has a preference for distribution to the aqueous phase and is relatively readily transported when water flows through soil. Log K_{OC} for PFBS has been reported in the range 1.2 to 2.7.

PFBS is a strong acid which is fully deprotonated at environmentally relevant conditions. The corresponding sulfonate anion in solution, as well as dissolved PFBS salts do not volatilise. Preference for the water compartment has been confirmed by distribution modelling and by monitoring data, showing that PFBS is frequently found in surface water, groundwater, drinking water, marine water, effluents from waste water treatment plants and in landfill leachate. The concentrations vary from < LOD to 21200 ng/L in the proximity to known point sources (4030 ng/L as max in Europe). Near point sources, like a PFAS production facility in the Netherlands, PFBS was the dominating PFAS in both river samples (12-27 ng/L) and drinking water (0.5-19 ng/L), Gebbink et al. (2017). In China near a PFAS production facility at Tangxun Lake, the two most dominating PFAS in water were PFBA and PFBS, at means of 4770 and 3660 ng/L, respectively (Zhou et al., 2013). In groundwater a remarkable 24-fold increase in PFBS concentration was recorded in the vicinity of a PFAS production plant in Fuxin, China, from 872 ng/L to 21 200 ng/L from 2009 to 2017 (Bao et al., 2019).

In many cases, PFBS is among the most dominating PFASs in environmental samples where PFBS has been observed to be the third most frequently detected PFAS in bottled water (47%) and tap water (88%) in a global survey (Kaboré et al., 2018). The substance was also frequently detected with 27% in drinking water samples from Brazil, Spain and France (Schwanz et al., 2016). Furthermore, in a recent survey of surface water in Northern Europe, with sampling in 2013, PFBS was found to be the dominating PFAS, contributing with 21% of the sum PFAS (Nguyen et al., 2017). These data confirm the widespread occurrence of PFBS in the environment, not only in Europe but all over the world.

3.3. Removal from the environment, decontamination and purification

Methods for removal of PFBS from environmental media are important in order to have the possibility to purify contaminations and lower environmental and human exposure. As PFBS has a clear preference for the aqueous phase, purification techniques for water are most relevant. PFBS has been detected in surface water and ground water, but also in produced drinking water. Purification techniques suitable for drinking water treatment plants are therefore essential, while the purification of waste water is equally important.

Rahman et al. (2014) reviewed PFAS characteristics, their occurrence in surface water, and their fate in drinking water treatment processes. Occurrence data from full-scale drinking water treatment plants indicated that PFASs, if present in raw water, are not substantially removed by most drinking water treatment processes. Conventional coagulation, flocculation, and sedimentation cannot achieve more than 20% removal of PFAS, nor can rapid granular media filtration. Most PFASs are not substantially removed in oxidation or advanced oxidation processes, typical for drinking water treatment plants. Biodegradation of most PFASs under current drinking water treatment conditions is unlikely. Filtration through granular activated carbon (GAC) may be useful for removing PFASs from drinking water. Longer chain PFASs will sorb better than the shorter chain compounds. However, short-chain PFASs such as PFBA and PFBS may pass through or reach breakthrough very quickly. Ion exchange/non-ion exchange resins may be useful for removing PFASs. However, such equipment is not commonplace in drinking water treatment facilities. Nanofiltration and reverse osmosis membranes may be effective with a high rejection of most PFASs. However, low molecular weight PFASs may be less well rejected by some loose NF membranes. Besides, disposal of concentrate with elevated concentrations of PFASs, will need to be addressed.

Reducing the releases of PFBS in industrial emissions and wastewater also suffers from low efficiency due to the high mobility and low adsorption potential of PFBS. Reemtsma et

al. (2016) point out that surface waters in densely populated areas are the recepients of effluents of WWTPs as well as of runoff from urban surfaces and agricultural land. Wastewater treatment and subsurface barriers are in general ineffective for the removal of persistent and mobile substances, and therefore they may reach the raw waters used for drinking water production.

Ochoa-Herrera and Sierra-Alvarez (2008) investigated the removal of perfluorinated surfactants by sorption onto granular activated carbon, zeolite and sludge. They found that activated carbon adsorption is a promising treatment technique for the removal of PFOS from dilute aqueous streams, while the sorption was weaker for PFOA and PFBS. The estimated concentrations of PFBS sorbed onto GAC was 48 mg/g GAC for PFBS, compared to 57 mg/g GAC for PFOA and 182 mg/g GAC for PFOS. The adsorptive capacity decreased with increasing PFAS concentrations in the water.

Furthermore, the presence of precursor compounds may complicate the water treatment process as the precursors may have considerably different properties and behave differently through the purification steps compared to their degradation products. The precursors may even break down during or after purification which may lead to increased concentrations of the degradation products in the finished water (Rahman et al., 2014).

The behaviour of perfluoroalkyl acids (PFAAs) from raw source water through a drinking water production chain to finished drinking water was examined in a study (Eschauzier et al., 2012). The raw source water was subjected to a series of purification steps, including coagulation, rapid sand filtration, dune passage, aeration, rapid sand filtration, ozonation, pellet softening, granular activated carbon (GAC) filtration, slow sand filtration. It was found that longer chain PFAA were readily removed by the GAC treatment step, while more hydrophilic shorter chain PFAA, especially PFBA and PFBS, were not removed by GAC and their concentrations remained constant through treatment.

In a study, Appleman et al. (2014) measured concentrations of PFASs in 18 raw drinking water sources and 2 treated wastewater effluents and evaluated 15 full-scale treatment systems for the attenuation of PFASs in water treatment utilities throughout the U.S. Water treatment techniques, such as coagulation followed by physical separation processes, and chemical oxidation, aeration and disinfection, were unable to remove PFASs. The levels of several PFASs were reduced by reverse osmosis (RO), granular activated carbon (GAC) and anion exchange (AIX). However, GAC and AIX were less effective at removing the shorter chain PFASs, whereas RO treatment was effective for even the smallest PFAS studied, PFBA. Despite RO's effectiveness, RO would likely be the most costly method for purification.

The low efficiency in most water treatment processes for PFBS is associated with the physicochemical properties of the substance. The high aqueous solubility and the low sorption potential results in a preferred distribution to the aqueous phase and low binding to soil and sediments, as well as adsorbents used in purification. Hence, most conventional purification techniques are ineffective in the removal of PFBS. Some more modern methods are promising and indicate a higher purification effect also for short-chain PFASs like PFBS. However, the equipment is not commonplace in water treatment facilities, and the treatment techniques may be expensive. In addition, it remains to be seen how the methods perform over time in practice with varying influent and the presence of other contaminants that affect the purification efficiency.

Ateia et al. (2018) reviewed the removal techniques from water: current practices and challenges. They concluded that the high solubility of short-chain PFAS in water, low/moderate sorption to soils and sediments and resistance to biological and chemical degradation has resulted in their widespread presence in various aquatic environments. Among the main findings from the review is that conventional treatment plants fail to remove short-chain PFAS, hybrid sorption systems may become alternatives to remove

short-chain PFAS from water, and that destruction methods are promising but new catalysts and approaches are needed. The authors point out that selective, cheap, and scalable treatment alternatives must be developed as countermeasures to increasingly larger concentrations of short-chain PFAS in water environments.

A thorough review of emerging technologies for remediation of PFASs was presented by Ross et al. (2018). This technical overview discusses the practicability of water treatment technologies, considering both the chemistry of PFASs and geological/hydrogeological factors when implementing the remedial techniques. Among the main conclusions in the review is that there are a plethora of technologies evolving to manage PFASs, but development is at an early stage. Many water treatment technologies claim to be able to treat PFASs, but most have not been assessed to treat a broader array of PFAAs or precursor substances. On removal of PFASs by GAC treatment, it is said that GAC becomes progressively less effective for removing shorter chain PFASs such as PFHxA, PFPeA, PFBS and PFBA as the chain length diminishes. A desorption behavior has been observed for PFBS when PFBS had to compete for sorption sites on the GAC with longer chain PFASs and/or natural organic matter. It was further pointed out that a recent theoretical study suggests that some precursors are unlikely to be effectively removed by GAC (Xiao et al., 2017). However, there are immense opportunities to develop more effective and sustainable remediation solutions for PFASs.

Li et al. (2020) arrived at similar findings in their recent review: 1) Short-chain PFAS are more widely detected, more persistent and mobile in aquatic systems, and thus may pose more risks on the human and ecosystem health; 2) conventional adsorption, ion-exchange, and membrane filtration can remove short-chain PFAS, but less effectively than the long-chain homologues, and the methods are challenged with poor material regeneration efficiency and disposal of process waste residual; 3) advanced oxidation such as thermolysis and sonolysis can achieve complete mineralisation, but come with a high process cost; and 4) direct photolysis, oxidation/reduction, photocatalysis, and electrochemical reaction may degrade short-chain PFAS following similar degradation pathways as long-chain PFAS, but at a slower rate, and photocatalytic processes appear most promising.

In summary, due to the high aqueous solubility and the low sorption potential, resulting in a preferred distribution to the aqueous phase, PFBS is not efficiently removed with conventional purification techniques. Both wastewater purification, drinking water production and removal of industrial emissions may suffer from the low purification efficiency for PFBS. The presence of PFBS precursors may complicate the water treatment process as the precursors may behave differently through the purification steps and may break down to PFBS either during or after purification. Efficient, practically and economically available methods do not exist today to remove PFBS contaminations and purify waste water and drinking water and lower environmental or human exposure. However, some more modern methods have promising removal efficiencies for PFBS and may be developed into practically available treatment techniques in the future.

3.4. Data indicating potential for long-range transport

3.4.1. **Mobility and the potential for long-range transport**

The hazards of short-chain perfluoroalkyl acids and other fluorinated alternatives to long-chain PFASs were assessed and compared to their long-chain homologues by Wang et al. (2015). It was concluded that short-chain PFAAs, due to their higher solubility in water and lower sorption to solids, are more mobile in soil and sediment. This leads to higher mobility in the environment. In combination with the high environmental stability, the high mobility implies that the short-chain PFAAs have a high global contamination potential.

This is supported by the findings in Sections 3.2.1 and 3.2.2 on adsorption/desorption and Section 3.2.4 on the modelling of distribution, wherein physical-chemical properties and model estimates suggest that PFBS is a highly soluble, weakly sorbing substance, with a high mobility in the environment and a preference for distribution to the aqueous phase. PFBS maintains in the water phase and is subject to long-range transportation first of all via sea currents. Yamashita et al. (2008) found increased PFBS concentration in the Labrador Sea at depth below 2000 m compared to subsurface concentrations and attributed this to influences by an independent deep-water current.

Wania (2003) looked at the partitioning properties that favour enrichment in the Arctic ecosystems and found that the long-term Arctic contamination potential of a perfectly persistent organic compound tends to be high for relatively volatile and water-soluble substances, and semivolatile and relatively hydrophobic substances with log K_{oa} 6.5 – 10 and log K_{AW} > -3. Arp and Slinde (2018) refined the assessment and explaind that for ionic substances like PFAS, one should use the D_{aw} rather than K_{aw}, and $K_{oc,a}$ rather than K_{oa}. Recalling from Table 9 the log $K_{OC,a}$ values for PFBS (4.8) is very similar to PFHxS (5.0) and PFOS (4.7). The log D_{aw} values at pH 8 for PFBS (-14.5) are considered extremely low, similar to PFHxS (-13.8) and PFOS (-13.1), such that volatilisation from water to air for all three substances is negligible. This combination of physical-chemical properties implies that PFBS strongly will prefer the water phase, will be transported with sea currents, and will reach and accumulate in the Arctic if emissions are allowed to continue. A similar behaviour has been observed for PFOS and PFHxS.

Ding et al. (2018a) measured the partitioning behaviour of PFASs between the dissolved phase, surface sediment and suspended particulate matter in the Dalian Bay, China. PFOA, PFBA, and PFBS were the predominant PFASs in the water dissolved phase, while PFBS, PFOS and PFOA were the most prevalent compounds in suspended particulate matter. A log K_d for PFBS of 3.4 was reported, and it was concluded that PFSAs (including PFBS) and the long-chain PFCAs were more inclined to prefer the suspended particulate matter phase. A more recent study by Liu et al. (2019) observed a somewhat different behaviour when similar measurements were performed around coastal areas of Bohai Bay, China. They found that PFBS and PFHxS were entirely preserved in the water-dissolved phase, while PFDS tended to stay in the suspended particulate matter section. A log K_d of 1.3 can be calculated from the concentration data in the study. However, the authors pointed out that in both water-SPM and water-sediment systems, K_d values can vary widely and even exceed 2 log units if steady state has not been reached. Hence, the two log K_d values from these two studies are not given too much weight. The differences between the two studies can also in parts indicate that the tendency of PFBS to bind to particles in water may depend upon the specific properties of the particles, but this may even depend on the concentrations of other PFASs in the samples.

A study of the role of sea spray aerosols in the long-range transport of perfluoroalkyl acids was performed by Johansson et al. (2018). It was measured from 200 to 62000 times higher concentrations of PFOS in the aerosols compared to the water concentrations, depending on the aerosol size. No specific values were given for PFBS, but the study indicated enrichment factors approximately one order of magnitude lower for PFBS than for PFOS. The results demonstrated that sea spray aerosols have the capacity to circulate significant amounts of PFAAs between the oceans and the atmosphere. A portion of the mass emitted from the oceans will deposit on land, thus re-entering the terrestrial system. This suggests that human exposure to PFAAs will continue even if strict global emission controls are implemented.

Reemtsma et al. (2016) pointed out that persistent and mobile organic compounds may be of concern for water quality because they are persistent in the environment and are not removed from water by sorption processes due to their high polarity and excellent water solubility. They may end up in drinking water, posing a potential risk to human health. However, there is no commonly accepted quantitative definition for a compound's

mobility in regulations or elsewhere. Two possible quantifiers of mobility in water are water solubility and sorption tendency. Short-chain perfluoroalkyl carboxylates and sulfonates (especially C2 and C3) were mentioned as a group of substances with properties of persistent and mobile compounds. Brendel et al. (2018) concluded that short-chain PFAAs, including PFBS, PFBA and PFHxA, have physicochemical properties that make them very mobile and prone to end up in the aqueous compartment. Due to the low adsorption potential, short-chain PFAAs will not bind to particles and stay mainly dissolved in the water phase. Furthermore, the short-chain PFAAs will have a higher potential for long-range transport compared to the long-chain homologues because of the high mobility and low adsorption potential.

As a highly mobile substance, there are no local or intermittent sinks for the pollution stock of PFBS, and therefore the substance has high potential for continuously increasing environmental concentrations and exposure of wildlife.

The ionic PFASs have high water solubility and low pKa values and are therefore almost completely dissociated at environmentally relevant pH values. Oceanwater is important as a sink and for transport of these compounds. The occurrence of high concentrations of PFASs in coastal waters could possibly be problematic, because the substances will be bioavailable and can accumulate in the marine food chain (Cai et al., 2012b).

3.4.2. **Estimation of long-range transport potential**

An atmospheric half-life of 76.4 days was estimated for PFBS with the Atmospheric Oxidation Program (AOPwin, v1.92) for EPISuite models, see Section 3.1.1.2. This value exceeds the threshold of two days which indicates a long-range transport potential for a substance.

The long-range transport potential (LRTP) of PFBS was modelled using the OECD P_{ov} & LRTP Screening Tool, a tool for estimation of the Long-Range Transport Potential (LRTP-Tool). Inputs to the model are half-lives in air, water and soil, as well as log K_{aw} and log K_{OW}. The half-lives were estimated at $t_{1/2}$(air) = 1830 h, $t_{1/2}$(water) = 4320 h and $t_{1/2}$(soil) = 8640 h (Level III Fugacity Model, as present in OECD QSAR toolbox v4.2, February 2018). Air-water partitioning for an ionic substance like PFBS is pH-dependent. Log K_{aw} represents the neutral form, while log D_{aw} varies with pH and shows the pH-dependence of the air-water partitioning. Values and ranges for log K_{aw} and log D_{aw} are presented in Table 5. In the present dossier, the estimate based on log K_{aw} for the neutral form (log Kaw = -2,59, Table 5) is presented, as this allows for comparison with similar ionic substances for which estimates based on the neutral form exist. A substance with a lower log K_{aw} (e.g. PFBS at neutral pH) would be more influenced by the persistence in water, which for PFBS in this model is lower, compared to air. The transport rate is lower in water compared to air, and the distance travelled over a given time will be shorter. Log K_{OW} is also pH-dependent and in Table 5 different values are reported for different pH-values. At pH 7-8, log K_{OW} is reported in the interval -8.0 to -3.0. The LRTP-Tool returns the same results for both extremes -8.0 and -3.0. Hence, log K_{OW} = -3.0 was selected for the estimation.

The LRTP-Tool estimated for PFBS a characteristic travel distance (CTD) = 17616 km and overall environmental persistence, P_{OV} = 220 days. CTD indicates the distance from a point source at which the chemical's concentration has dropped to 38% of its initial concentration. The results for PFBS indicate a high potential for long-range transport. The results are presented graphically in Figure 4. However, the present estimates represent screening estimates and are influenced by the physico-chemical input parametres. The results are in support of other evidence of transport of PFBS to remote areas. Carrying out the modelling based on input for the ionized form from Table 5 and increasing the half-life in water based on the reasoning in Section 3.1 ($t_{1/2}$(water) = 10 years; log D_{OW} = -8.0; log D_{aw} = -14.5), the model returns P_{OV} = 5194 days and CTD = 7858 km.

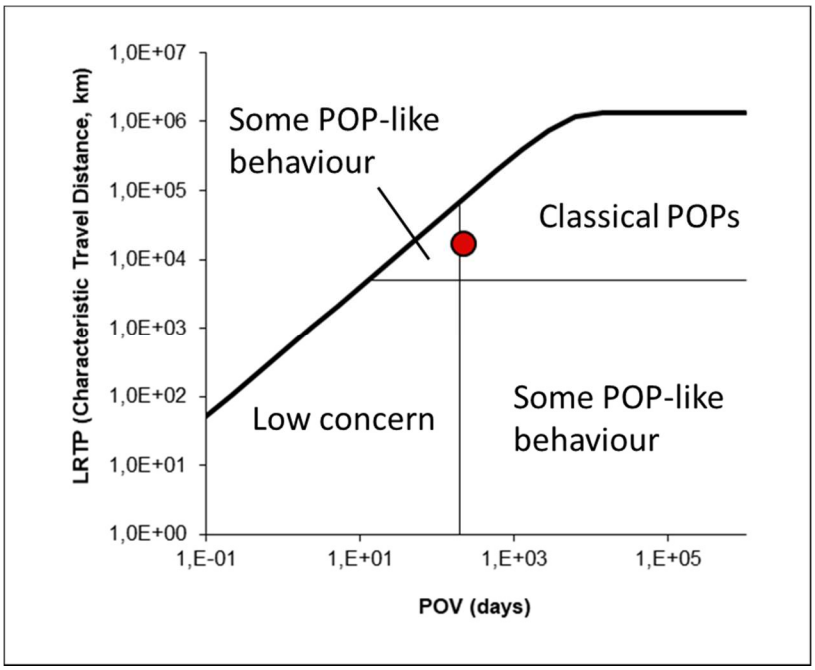


Figure 4: Graphical output for the calculated CTD = 17616 km and POV = 220 days for PFBS (red dot). The lines in the figure represent the border for POP-like substances with high POV and high LRTP. The results indicate a high potential for long-range transport for PFBS.

According to Crookes and Fisk (2018), the overall CTD for substances with log K_{aw} down to around -3 is governed mainly by transport via the atmosphere, but at lower log K_{aw} values, the overall CTD is increasingly governed by transport via the water phase.

3.4.3. **Occurrence of PFBS in remote areas**

Butt et al. (2010) reviewed the levels and trends of PFASs in the Arctic environment and concluded that PFASs are ubiquitous in the Arctic. PFBS is reported at low levels in Arctic biota, and this was explained by a low tendency to bioaccumulate due to the short biological half-life. PFBS was a more dominant contributor to the overall PFAS levels in Arctic water samples.

Furthermore, Butt et al. (2010) discussed the relative importance of the two dominant transport pathways of PFASs to the Arctic: Atmospheric transport of precursors and degradation, versus direct transport of PFASs via ocean currents. However, there was not enough evidence to draw any final conclusions. Local sources cannot be totally excluded in remote areas, like at local research stations in polar regions (Wild et al., 2015), but their local input will not significantly influence regional concentrations. The widespread presence of PFBS in environmental samples in remote areas is of concern, showing that PFBS has a potential for long-range transport.

Zhao et al. (2012) investigated the global distribution and long-range transport of PFASs using seawater samples collected from the Greenland Sea, East Atlantic Ocean and the Southern Ocean in 2009-2010. Relatively high concentrations of PFASs were detected near the European continent. In the Greenland Sea, the five most frequently detected compounds were PFOA, PFHxS, PFHxA, PFOS and PFBS.

In the following sections PFBS levels in different compartments in remote regions are compiled.

3.4.3.1. PFBS in remote areas – fresh water, air, snow and ice

Measurements of PFBS in samples of fresh water, air, snow and ice in remote regions are collected in Table 17.

Table 17: Environmental concentrations of PFBS in remote areas; fresh water, air, snow and ice samples (ng/L).

Location	Year	Mean +/- SD	Min-Maks (Median)	n (det. Freq) LOD	Remark	Reference
Arctic Ocean	2010	0.17 ± 0.42	<0.017 - 1.50 (0.02)	12 (50 %) LOD = 0.017	Snow/Ice	Cai et al., 2012a
Canada	2007 - 2008		0.01 - 0.02	11 LOD = Not reported	Lake	Veillette et al., 2012
Greenland	2015	0.0068		LOD = 0.00091	Ice cap (19 cm = 2015)	Pickard et al., 2018
Greenland	2015	0.0042		LOD = 0.00091	Ice cap (134 cm = 2013)	Pickard et al., 2018
Northern Sweden	2009	0.099	nd - 2.16	24 LOD = 0.021	Snow/Ice	Codling et al., 2014
Svalbard, Longyearbyen	2015	2.38		LOD = 0.003	Run-off water	Skaar et al., 2019
Svalbard, Longyearbyen	2015	2.41		LOD = 0.003	Waste water effluent	Skaar et al., 2019
Svalbard, Longyearbyen	2015	2.33		LOD = 0.003	Local firefighting training site (run-off)	Skaar et al., 2019

Location	**Year**	**Mean +/- SD**	**Min-Maks (Median)**	**n (det. Freq) LOD**	**Remark**	**Reference**
Svalbard, Ny-Ålesund	2015	22.99		LOD = 0.003	Freshwater, contaminated location	Skaar et al., 2019
Antarctica (Lake)	2011	0.038	<0.0083 - 0.05	4 LOD = 0.017	Lake (King George Island)	Cai et al., 2012
Antarctica	2011	0.017	<0.0083 - 0.02	4 LOD = 0.017	Snow/Ice (King George Island)	Cai et al., 2012
Alert, Greenland	2006-2015	0.1 ± 0.2	BDL - 1.5 (0.0)	169 (66 %) LOD = 0.008-1.8	Air, pg/m^3	Wong et al., 2018
Zeppelin, Svalbard	2006-2015	nd		383 LOD = 0.008-1.8	Air, pg/m^3	Wong et al., 2018

The PFBS levels measured in air, fresh water, snow and ice in remote regions vary between not detected and 2.16 ng/L, when not including samples with known contamination. The concentrations are generally low, but the frequent detection shows that PFBS has spread via long-range transport to the Arctic, as well as the Antarctic. Local emission sources like waste water and runoff samples from Svalbard contained PFBS at concentrations in the range 2.33 – 22.99 ng/L.

3.4.3.2. PFBS in remote areas – marine water

PFBS has been reported in marine water throughout the world. Measurements from marine water collected in remote regions are shown in Table 18.

Table 18: Environmental concentrations of PFBS in marine water samples from remote regions (ng/L).

Location	Year	Mean +/- SD	Min-Max (Median)	n (det. Freq) LOD	Reference
Arctic Ocean	2010	0.03 ± 0.03	<0.017 - 0.08 (<0.017)	13 (38 %) LOD = 0.017	Cai et al., 2012
Central Arctic	2012	0.01 ± 0.01	0.04	LOD = 0.005	Yeung et al., 2017
Faroe Islands	2004		0.05 - 0.11	LOD = 0.03	Kallenborn et al., 2004
Iceland	2004		0.05 - 0.08	LOD = 0.03	Kallenborn et al., 2004
North Atlantic	2009-2010	nd	0.045	62 LOD = 0.0059-0.051	Zhao et al., 2012
Northwest Pacific Ocean	2010	0.02 ± 0.03	<0.017 - 0.10 (<0.017)	9 (33 %) LOD = 0.017	Cai et al., 2012
Russian Arctic Baydaratskaya Bay	2007	0.0132		LOD = Not reported	Saez et al., 2008
Antarctica	2007	0.0029		LOD = Not reported	Wei et al., 2007
South Atlantic	2009-2010	nd	nd-0.013	39 LOD = 0.0059-0.051	Zhao et al., 2012
Mid-Atlantic	2009-2010	nd	nd-0.017	20 LOD = 0.0059-0.051	Zhao et al., 2012
Svalbard, Longyearbyen	2015	0.105		0.003	Skaar et al., 2019
Svalbard, Ny-Ålesund	2015	<0.001		0.001	Skaar et al., 2019

PFBS has been detected in marine water from remote regions in the range not detected to 0.11 ng/L in a sample from the Faroe Islands (Kallenborn et al., 2004). The ubiquitous

presence of PFBS in marine waters over a very large area is an evidence of long-range transport of PFBS.

3.4.3.3. PFBS in remote areas – biota

According to Arp and Slinde (2018), prior to 2012, the majority of studies on Arctic marine biota did not report PFBS above the detection limits. In more recent studies, it is starting to appear more frequently. Routti et al. (2016) did not detect PFBS in any blood plasma samples in ringed seals from Svalbard in the period of 1990 to 2010. In a study by Gebbink et al. (2016) in East Greenland, PFBS was detected in all polar bear livers examined (8/8) at 0.032 ± 0.008 ng/g and in 67% of the killer whale livers at 0.0052 ± 0.0017 ng/g. However, PFBS was below detection limit in the ringed seal livers. Total PFSA in the three species was in the range 94-1825 ng/g, with PFOS being the predominant single substance. PFBS was measured in livers of both mothers and foetus of killer whale with a transfer rate of 2.3% (4.7% for PFOS). Routti et al. (2017) detected PFBS in polar bear plasma at Svalbard. In a study of temporal trends of perfluoroalkyl compounds in the period 1975-2011, Braune and Letcher (2013) detected PFBS in eggs of thick-billed murres (*Uria lomvia*) and northern fulmars (*Fulmarus glacialis*) from the Canadian Arctic in 2010 and 2011. Concentrations of PFBS measured in marine biota in the Arctic is listed in Table 19.

Table 19: Environmental concentrations of PFBS in Arctic biota (ng/g ww).

Location	**Date**	**Mean +/- SD**	**Min – Max** **(Median)**	**n (df)** **LOD**	**Remark** [a)]	**Reference**
Greenland	2012-2013	<0.002		10 LOD = 0.002	Ringed seal liver (*Pusa hispida*)	Gebbink et al., 2016
Greenland	2012-2013	0.03 ± 0.01		8 (100 %) LOD = 0.002	Polar bear liver (*Ursus maritimus*)	Gebbink et al., 2016
Greenland	2012-2013	0.01 ± 0.00		6 (66 %) LOD = 0.002	Killer whale liver (*Orcinus orca*)	Gebbink et al., 2016
Canadian Arctic	1975-2011	0.57 ± 0.46		12 (4 pools of 3 eggs) detected in 2011 LOD = 0.1	Northern fulmars (*Fulmarus glacialis*)	Braune and Letcher (2013)
Canadian Arctic	1975 - 2011	0.04 ±0.02 (2010) 0.07 ± 0.05 (2011)		15 (5 pools of 3 eggs) LOD = 0.1	Thick-billed murres (*Uria lomvia*)	Braune and Letcher (2013)
Nunavut, Canada	1972-2005	nd		184 LOD = Not reported	Ringed Seal liver	Butt et al., 2008

Location	Date	Mean +/- SD	Min – Max (Median)	n (df) LOD	Remark [a)]	Reference
Svalbard	1990-2010	<0.07		LOD = Not reported	Ringed seal plasma	Routti et al., 2016
Svalbard	2000-2014	0.27	0.08 - 0.69 (0.27)	70 (100 %) LOD = Not reported	Polar bear plasma	Routti et al., 2017
Svalbard	2007	nd		LOD = 0.06-0.11	Black guillemots liver (*Cepphus grylle*	Axelson et al., 2014
Svalbard	2007	nd		LOD = 0.06-0.11	Glaucus gull liver (*Larus hyperboreus*)	Axelson et al., 2014
Svalbard	2016	nd		LOD = Not reported	Ringed seal liver	Schlabach et al., 2018
Svalbard	2017	<0.05		10 LOD = Not reported	Common eider eggs (*Somateria mollissima)*	Schlabach et al., 2018
Svalbard	2017	<0.07		5 LOD = Not reported	Black-legged Kittiwake eggs (*Rissa tridactyla*)	Schlabach et al., 2018
Svalbard	2017	<0.08		5 LOD = Not reported	Glaucus gull eggs	Schlabach et al., 2018
Svalbard	2017	0.04 ± 0.02	0.02 - 0.08 (0.04)	10 (60 %) LOD = Not reported	Polar bear plasma	Schlabach et al., 2018

The PFBS concentrations measured in Arctic biota vary between not detected and 0.69 ng/g ww. In most of the samples, concentrations were low. However, in a study of polar bear (*Ursus maritimus*) higher levels (0.08 – 0.69 ng/g ww, median 0.27 ng/g ww) were found in plasma (Routti et al., 2017). Low levels were also found in killer whales (*Orcinus orca*) from Greenland in both mothers and the foetus (Gebbink et al., 2016). PFBS is also found in eggs of northern fulmars and thick-billed murres in the Canadian Arctic.

3.4.4. **Long-range transport of precursors and degradation to PFBS**

In some cases, PFBS-related substances may also be efficiently transported over long distances or transported via other routes than PFBS itself, e.g. via atmospheric transport. Subsequent degradation with formation of PFBS will then contribute to the wide distribution of PFBS all over the globe. Due to the negative charge of the PFBS sulfonate anion, PFBS will have a low tendency to evaporate and for transport via air, while neutral precursors in many cases will have a higher vapour pressure and a stronger tendency to spread via air (Armitage et al., 2009). Another route of long-range transport is via distribution of PFBS-containing products. PFBS-related substances, including polymers, are in use in various products, like textiles, as a surface treatment agent (Chu and Letcher, 2014). Such products may be distributed world-wide for sale and use, and degradation of the precursors to PFBS during the use phase or in the waste stage will contribute to the increasing PFBS levels over a wide area.

A review of the microbial degradation of polyfluoroalkyl chemicals in the environment points out that perfluoroalkane sulfonamido derivatives may undergo aerobic biodegradation, via the relatively stable intermediate sulfonamides, to the sulfonic acids (e.g. PFBS) as the final degradation products (Liu and Avendaño, 2013). A comprehensive discussion of abiotic degradation of PFBS-precursors to PFBS may be found in the study by Nielsen (2017), and a summary in Section 1.3.3.

Monitoring data show that transport via air of neutral and volatile PFASs, like various substituted fluoroalkane sulfonamides, including the PFBS-precursors MeFBSE and MeFBSA, is actually happening (Lai et al., 2016). However, the dominant neutral PFAS-group detected in the study was the fluorotelomer alcohols (FTOHs). D'eon et al. (2006) showed that MeFBSE can degrade in reaction with OH radicals in the atmosphere to produce MeFBSA, in addition to PFBS and PFCAs as final degradation products. The atmospheric lifetime of MeFBSA was estimated at >20 days which suggests that the substance is long-lived enough to account for distribution throughout the North American troposphere, linking the production of MeFBSE to the ubiquity of this class of compounds in the remote environment (D'eon et al., 2006).

3.4.5. **Summary of data indicating potential for long-range transport**

Due to the high solubility in water and low adsorption to solids, PFBS has a preference for the aqueous compartment and is highly mobile in the environment. In combination with the high environmental stability, the high mobility implies that PFBS has a high global contamination potential. The long-range transport of PFBS is mainly via the water phase and sea currents, while sea spray aerosols have the capacity to circulate significant amounts of PFAAs between the oceans and the atmosphere. A portion of the mass emitted from the oceans will deposit on land, thus re-entering the terrestrial system.

An atmospheric half-life of 76.4 days was estimated for PFBS with the Atmospheric Oxidation Program, indicating a potential for long-range transport. This was confirmed by modelling with the OECD LRTP-Tool which concluded that PFBS has a characteristic travel distance of 17616 km and a high potential for long-range transport.

In addition to the long-range transport of PFBS itself, long-range transport of PFBS-related substances and degradation to PFBS may take place. The related substances have different properties than PFBS and may be transported via other routes, like atmospheric transport.

PFBS has been detected in samples of marine water, surface water, snow, ice, air and biota from remote areas, demonstrating that long-range transport has taken place. However, the measured concentrations of PFBS are generally low.

3.5. Bioaccumulation

REACH Annex XIII, Sections 1.1.2 and 3.2.2(a), defines the numerical criterion for bioaccumulation when assessing biaccumulation data from fish or other aquatic species. However, due to their water solubility, short-chain PFASs are expected to be quickly excreted via gill permeation in water breathing animals. This reduces the bioaccumulation potential in such species.

Annex XIII (Section 3.2.2) further defines information which should be taken into account when the numerical criterion is not applicable, i.e. data on the bioaccumulation potential in terrestrial species and detection of elevated levels in biota, in particular in endangered species or in vulnerable populations, compared to levels in their surrounding environment. Annex XIII (Section 3.2.2 (b)) also allows taking data from human body fluids or tissues and the toxicokinetic behavior of a substance into account. In accordance with this, elimination half-lives have recently been used for certain PFAAs as a metric to estimate the bioaccumulation potential in air-breathing organisms. For example, the elimination half-life for PFOA in humans was important in the identification of PFOA (half-life 2 – 4 years) as a substance fulfilling the B criteria in Annex XIII (ECHA, 2013). PFHxS with a half-life in humans of ca 7-8 years (or longer) was agreed to fulfil the vB criteria (ECHA, 2017).

3.5.1. **Laboratory studies on bioaccumulation**

3.5.1.1. Bioaccumulation in aquatic organisms

In a study by Martin et al. (2003a) in rainbow trout (*Oncorhynchus mykiss*), fish were exposed simultaneously to a homologous series of perfluoroalkyl carboxylates and sulfonates (including potassium PFBS) for 12 days, followed by 33 days of depuration in clean water in a flow-through system to determine compound-specific tissue distribution and bioconcentration parameters for perfluoroalkyl acids (PFAAs). In general, PFAAs accumulated to the greatest extent in blood > kidney > liver > gall bladder. PFBS was not detected in blood, liver and carcass at any sampling times, although as pointed out by the authors, a half-life of PFBS in liver of rainbow trout of 3.3 days is measured in a dietary accumulation study (see next paragraph). The study is well reported and regarded as reliable with a Klimisch reliability of 2 as the limit of detection in the different tissues is not reported.

In another study by Martin et al. (2003b) juvenile rainbow trout were exposed to a homologous series of perfluoroalkyl carboxylates and sulfonates (including potassium PFBS) in the diet for 34 days, followed by a 41 days depuration period. On six occasions during both the uptake period and the depuration period, fish were removed for analysis of carcass and liver concentrations. Concentrations were determined by LC-MS, and kinetic rates were calculated to determine compound-specific bioaccumulation parameters. Spiked food concentration was 0.32 µg/g for PFBS. PFBS was only detectable at the last three uptake sampling intervals and at the first sampling time of the depuration phase and was apparently only detected in liver. Limits of detection are not reported. A depuration half-life for PFBS for liver of 3.3 days is reported. The depuration half-lives for liver for PFHxS and PFOS were reported to be 13 days and 20 days, respectively. In general sulfonates bioaccumulated to a greater extent than carboxylates of equivalent perfluoroalkyl chain length. The recovery for PFBS in carcass and liver was low; around 60% or less. Otherwise, the study is regarded as reliable with a Klimisch reliability of 2. For PFBS a BAF could not be calculated. According to the authors, dietary exposure will not result in biomagnification of perfluoroalkyl acids in juvenile trout, but extrapolation to larger fish and homeothermic organisms should not be performed.

In a feeding study with adult rainbow trout the biomagnification potential and tissue-specific distribution of several perfluoroalkyl acids (PFAAs) were investigated (Goeritz et al., 2013). Tetrabutylammonium nonafluorobutane sulfonate at a chemical purity of sulfonic acid ≥ 98% was used. Biomagnification factors (BMFs) were determined based on a kinetic approach. Distribution factors were calculated for each test compound to illustrate the disposition of PFASs in rainbow trout after 28 d of exposure. Mean concentration of PFBS in the feed was 185 ± 26 µg/kg feed. BMF values of 0.02 for PFBS, 0.42 for PFOS and 0.18 for PFHxS were calculated. Liver, blood, kidney, and skin were identified as the main target tissues. Muscle tissue (37.6% of total body wt) comprised 41%, 41% and 26 % of the whole-body burden of PFBS, PFOS and PFHxS, respectively, on day 28 of the exposure period. The accumulation phase was followed by a 28-d depuration phase, in which the test animals were fed with non-spiked feed. The depuration half-life for whole fish was calculated to be 10.8 days for PFBS, 8.75 days for PFHxS and 16.1 days for PFOS. The authors pointed out that despite relatively low PFAS contamination in muscle and skin, the edible parts of the fish can significantly contribute to the whole-body burden.

In a follow up publication (Falk et al., 2015), based on data collected in the Goeritz study with rainbow trout described above, tissue specific uptake and elimination of the perfluoroalkyl acids (PFAAs) were further investigated. Muscle, liver, kidneys, gills, blood, skin and carcass were examined individually. At the end of the accumulation phase 0.89% (PFBS), 3.79% (PFHxS) and 17.5% (PFOS) of the absolute, applied quantity of the PFSAs was recovered in the whole fish. The main target organ was the liver with recovery rates between 0.11% (PFBS) and 4.01% (PFOS) of the total amount of PFSAs supplied. For PFBS a maximum concentration of 110 µg/kg was found in the liver at the end of the accumulation phase (day 28), and 80% of the maximum was reached by day 7. Perfluoroalkyl sulfonic acids were taken up more readily and had longer estimated elimination half-lives than perfluoroalkyl carboxylic acids of the same chain length. The longest estimated tissue specific elimination half-life was found to be for PFOS and was 20.4 days in the liver. For PFBS the longest tissue specific elimination half-life was for liver and was 6.5 days.

KPFBS bioconcentration was examined in Bluegill sunfish (*Lepomis macrochirus*) in a test conducted under OECD 305 guidelines. Fish were exposed for 28 days with a 14-day depuration period. Average measured test substance concentrations were 0.53 mg/L and 5.2 mg/L. Steady-state BCFs (whole fish) was 0.3 and were attained in ≤ 7 days. Kinetic BCF values were 0.36 at 5.2 mg/L and 1.1 at 0.53 mg/L. The study was assigned a Klimisch reliability 1 (ECHA dissemination website).

In a study by Chen et al. (2016a) adult female zebrafish (*Danio rerio*) were exposed for 24 days to an aqueous mixture of multiple PFASs (including free acid form of PFBS), followed by a 24 days depuration phase. The test was performed according to OECD Guideline 305 (Bioconcentration: Flow-through Fish Test). The water concentration of PFBS was 2.15 ± 0.28 µg/L in the low dose group and 17.8 ± 1.28 µg/L in the high dose group. PFBS was detected in all tissues except for muscle tissue of fish in the low-dose exposure group. Growth-corrected concentrations were calculated using the exponential growth model. Estimated BCF values (whole fish) for PFBS were from 19.5 – 27.5. The body burden was calculated as the sum of tissue concentrations measured (plasma, liver, muscle, ovary) times the wet weight of the respective tissues, other tissues not included in estimating the whole-body burden. → *Klimisch reliability 2-well documented Guideline study, acceptable for assessment.*

Accumulation of PFAAs in the oligochaete *Lumbriculus variegatus* was investigated in a 28-days exposure study using sediments from contaminated areas (Lasier et al., 2011). The study is well documented, reliability 2 (ECHA dissemination website). Concentrations of PFAAs in oligochaete tissues revealed patterns similar to those observed in the respective sediments. Bioaccumulation of PFSAs and PFCAs by *L. variegatus* appeared to be primarily related to sediment concentrations. The tendency to bioaccumulate increased with PFAA

chain length and the presence of the sulfonate moiety. With respect to PFBS a biota-sediment accumulation factor (BSAF) of 0.31 was determined that exceeded the BSAF values of PFOA (0.07), PFNA (0.20), PFDA (0.25), and PFUnDA (0.29). The authors concluded that sulfonates with four to seven carbons may be equally likely to bioaccumulate as PFOS in this species.

3.5.1.2. Bioaccumulation in earthworms

Earthworms (*Eisenia fetida*) were exposed to artificially contaminated soils with ten perfluoroalkyl substances (Zhao et al., 2013). A loamy surface soil (0 - 10 cm) without detectable PFASs was collected from a farm about 20 km southwest of Tianjin, China. The typical physicochemical properties of the soil were characterised as follows: pH 7.67; organic matter 4.88%; cation exchange capacity 38.47 cmol kg_1; moisture content 1.03% for airdried soil; clay 24%, silt 64%, and sand 12%. The soil was air-dried for 2 weeks, ground, and sifted through a 2 mm mesh. A small portion of pre-weighed soil was spiked with one mL of the mixed solution of 10 PFASs, dissolved in methanol and mixed thoroughly. The spiked soil was placed in a fume hood to allow the solvent to evaporate for 24 h. An aliquot of the untreated soil was added to the spiked soil and then mixed thoroughly. This step was repeated until all the pre-weighed soil was mixed. Three spiked soils were prepared and the concentration of each PFAS compound, including PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoA, PFBS, PFHxS and PFOS, was at 100, 200, and 500 ng/g, respectively. The recoveries of PFASs were 76 - 105% for earthworms. The PFASs were not detected in the controls. The Method Detection Limits of PFASs were in the range of 0.5 - 1.0 ng/g dry weight (dw) for the target compounds. According to the authors, all the studied PFASs, including those with seven or less perfluorinated carbons, were bioaccumulated in the earthworms, and the biota-to-soil accumulation factors (BSAFs) increased with increasing perfluoroalkyl chain length and were greater for sulfonic acids than for the carboxylic acids of equal perfluoroalkyl chain length. The BSAFs were found to be dependent on the concentrations of PFASs in soil and decreased as the level of PFASs in soil increased. For PFBS, a BSAF value (kinetic) of 0.048 (0.768 for PFOS) was measured. The depuration half-life for PFBS was 9.6 days (18 days for PFOS). The BSAF measured based on wet weight of the organism, was 0.021 for PFBS at an exposure concentration of 100 ng/g (0.145 for PFOS).

In a study by Navarro et al. (2016) earthworms (*Eisenia andrei*) were exposed to sand soil spiked with a technical mixture of PFOS that contained PFBS. Worms were exposed for 21 days and allowed to depurate for 24 hours. PFBS was not detected in earthworms at the end of the depuration period although concentration in soil in some case (0.19 ng/g d.w.) reached levels similar to PFOS (0.21 ng/g d.w.). In another study by Navarro et al. (2017) the bioaccumulation behavior of perfluoroalkyl substances (PFASs) and halogenated flame retardants (HFRs) were examined in three horticultural crops and earthworms. Two species, spinach (*Spinacia oleracea*) and tomato (*Solanum lycopersicum L.*), were grown in field soil amended with a single application of biosolids (at agronomic rate for nitrogen), to represent the scenario using commercial biosolids as fertiliser. The third crop, corn (*Zea mays*), was grown in spiked soil with ~50 mg PFOS/kg soil (treatment 1), ~5 mg Deca-BDE/kg soil (treatment 2) and a mixture of both, ~50 mg PFOS and ~5 mg Deca-BDE/kg soil to represent a worst-case scenario (treatment 3). PFASs from wastes and soils (at the beginning t=0, and the end t=final; of the experiments) were extracted and purified. To examine the bioaccumulation in soil invertebrates, earthworms were exposed to the spiked soil after removal of the plants where corn had been grown. Worms were exposed for 28 days and allowed to depurate for 24 hours to avoid the presence of soil particulates that could interfere with the bioaccumulation study. Levels (treatment 1 and 3) found in earthworms were: PFBS 0.11 - 0.07 µg/g d.w., PFHxS 3.18 – 2.93 µg/g d.w. and PFOS 74.3 – 84.7 µg/g d.w. BAFs (treatment 1 and 3) for individual substances, calculated based on levels in earthworms and levels in soil at t=final, were as follows: 3.89 – 6.09 (PFOS), 3.38 – 3.53 (PFHxS), 2.75 – 2.33 (PFBS).

3.5.1.3. Bioaccumulation in birds

In an acute and chronic effects (reproduction) study, adult Northern bobwhite quail (*Colinus virginianus*) was exposed to nominal dietary concentrations of 100, 300, or 900 mg PFBS/kg, ww feed for up to 21 weeks (Newsted et al., 2008 and registration dossier). The chronic study was performed according to OECD Guideline 206 (Avian Reproduction Test, see Registration dossier for KPFBS). PFBS was present in liver and blood serum of adult quails in a dose-dependent manner. The ratio between mean PFBS concentrations in blood serum and liver for both adult male and female quail ranged from 3.4 to 5.1 with a mean value of approximately 4. Concentrations of PFBS in blood serum and liver were approximately 11- and 41-fold less than those in the diet, respectively, indicating that it is not bioaccumulating in the serum or liver of adult quails.

Concentrations of PFBS in eggs were directly proportional to dietary concentrations and were also dependent on when the eggs were laid during the study. Concentrations of PFBS in eggs laid during the seventh week were approximately 1.6-fold greater than that measured in eggs laid during the second week. Based on PFBS concentrations measured in adult females, the egg-to-serum ratio was approximately 1.0, while the egg-to-liver ratio was approximately 3.4 for all treatment groups. In 14-day-old offspring, PFBS concentrations in blood serum and liver were proportional but less than that measured in adult female quail or eggs from the same treatment group. PFBS concentrations in the tissues of offspring were at least 480-fold less than those measured in eggs from the seventh week for all treatment groups. The authors suggested that the contribution from eggs to chicks would not be expected to persist for a significant period of time due to either rapid loss from the tissues through elimination processes or through growth dilution.

The study is a guideline study and considered reliable wihout restriction – Klimisch score 1.

3.5.1.4. Bioaccumulation in mammals

A study by Numata et al. (2014) examined the transfer of a mixture of perfluoroalkyl acids (PFAAs) from contaminated feed into the edible tissues of 24 fattening pigs (*Sus scrofa domesticus*). The feed, PFAA contaminated hay and barley, had been cultivated and harvested from a PFAA-contaminated region in Lower Saxony, Germany. The agricultural patch was polluted from the use of a soil improver provided by a recycling company that laced it with industrial waste containing high concentrations of PFAS. Mean concentration of PFBS in the feed was 132 ± 11 µg/kg on a dry weight basis. This study showed a tissue distribution of PFBS to blood plasma, liver, kidney, muscle tissue and fat. PFBS was eliminated through feces and urine. The geometric average elimination half-lives (up to 24 pigs) reflecting both plasma and edible tissue, was estimated to be 43 days. The 95% variability elimination half-life intervals was 13-135 days for PFBS taken from data analysis of the Numata article in the Support Document for PFHxS ("as measured from figure 4 in the article") ECHA, 2017. The elimination half-lives for PFOS was 634 days (194-1970 days), for PFHxS 713 days (249-1970 days) and for PFOA 236 days (46-1074 days). Consequently, the individual variability was quite large, the highest values for PFBS are overlapping with the lower values for PFOA. BMF values reported for PFBS were 1.2 (whole pig), 0.8 (meat = mean of dorsal muscle, ventral muscle, and fat), 6.4 (liver), 14 (blood plasma), 2.2 (kidney) and 0.9 (fat). For comparison BMF values for PFOS were 17.9 (whole pig), 9.7 (meat), 503 (liver), 97 (blood plasma), 139 (kidney) and 8.3 (fat). The behavior of PFAAs in pigs is of interest because pigs are considered to be a biomedical model of human physiology (Numata et al., 2014).

Although measurement during the depuration period is recommended in TG OECD 417 for rats, when using a mathematical (differential equation) based toxicokinetic method as developed by Numata et al. (2014), the inclusion of a long depuration period may not be necessary to obtain the plasma half-life (T1/2) and other parameters. Repeated plasma

measurements were performed during the 22-day exposure period, but sacrifice took place already 20 h after the last administration. The average recovery for all 7 compounds was 97% in the analyzed compartments.

In general, for chemicals with short plasma T1/2, it may be possible to obtain a plasma steady state concentration towards the end of the repeated exposure period (e.g. often 14 days following TG OECD 417). However, for chemicals having a long plasma T1/2 (as here), a steady state is often not achieved within the dosing period. This is in any case not a requirement in OECD 417 which states that "Single dose toxicokinetic and tissue distribution data may be adequate to determine the potential for accumulation and/or persistence", thus not at steady state. The pigs were given a low dose polluted feed corresponding to a realistic situation, and the authors estimate a 'Time to 95% completion of the steady state' for PFBS to be 217 +/-120 days, Table S5, and even longer for most of the other chemicals measured, which is not a realistic exposure period. A steady state may likely be reached faster at higher doses but would then not have reflected the environmentally realistic contamination situation.

The authors do explain how they have calculated the BMF value, but not all data seem to be reported, some estimates have been made (e.g. urine output), and it is in general difficult to follow in silico calculations of this kind as well as checking that they were performed correctly. The authors do not verify their model which could have been done with inclusion of a depuration period for some animals. Regardless of some limitations, we observe that the results comply well with the general picture to be expected from other data sources, e.g. showing half-lives for different substances in the order PFHxS > PFOS > PFOA > PFBS

In a study dairy cows (*Bos taurus*) were given feed that was contaminated with PFAAs, that is hay and grass silage which had been grown on a PFAA-contaminated farmland in Lower Saxony, Germany (Kowalczyk et al., 2013). Average concentration of PFBS was 68.4 ± 23.1 µg/kg in grass silage and 993.6 ± 224.4 µg/kg in hay. After the PFAA-feeding period of 28 days, three cows were slaughtered while the other 3 were fed PFAA-free feed for another 21 days (depuration period). The average daily intake of PFBS was 3.4 ± 0.7 µg/kg body weight, (for PFHxS, PFOS, and PFOA the average daily intake was 4.6 ± 1.0, 7.6 ± 3.7 and 2.0 ± 1.2 µg/kg body weight (bw) respectively). During the PFAA-feeding period, PFBS concentrations above the detection limit (LOD of 0.1 µg/L) were detected only in milk samples (n = 11) of one cow with a mean concentration of 0.12 ± 0.02 µg/L and was not detected in milk during the PFAA-free feeding period. 0.005 and 0.003% of the ingested dose of PFBS were quantified in liver and kidney, respectively, of cows slaughtered directly after the PFAA-feeding period. The concentration of PFBS in liver after the feeding period was 0.3 ± 0.3 µg/kg, detectable in 2 of 3 cows (LOD = 0.2 µg/kg) and in kidney 1.0 ± 0.3 µg/kg. The concentration of PFBS in plasma was 1.8 ± 0.8 µg/L between the 1st and 28th days and PFBS was not detectable in plasma 4 days after the end of the PFAA-feeding period (day 32). For comparison, PFOA plasma concentrations were 8.6 ± 4.2 µg/L during the feeding of PFAA-contaminated feed and decreased rapidly after the end of the PFAA-feeding period, reaching the LOD (0.2 µg/L) on day 41 of the PFAA-free feeding period. Regarding secretion into milk the kinetics of PFOA in cows were similar to those of PFBS and substantially differed from those of PFHxS and PFOS (Kowalczyk et al., 2013).

More information on mammalian toxicokinetics and elimination half-lives is provided in Sections 3.5.3.2 and 4.1.

3.5.2. **Persistence and mobility in relation to bioaccumulation**

The Peter Fisk Associates consultancy company evaluated the use of persistence and mobility of chemicals in the environment to fulfil the bioaccumulation criteria of the Stockholm Convention (Crookes and Fisk, 2018). They found that substances that have a

low bioaccumulation potential could potentially reach similar levels in biota to substances that are known to bioaccumulate, provided that they are sufficiently persistent and mobile in the environment. Persistence in this model relates to the life-time in the relevant compartment, water, and degradation as well as sedimentation and other processes that remove the substance from the compartment. For example, calculations in the study show that a substance with a half-life of 365 days and a BCF of 800 l/kg may reach comparable concentrations in a system as a substance with a half-life of 60 days and a BCF of 5000 l/kg, if time allows for steady-state to be reached (Figure 3 in reference). Based on their findings, the authors proposed screening criteria under the Stockholm Convention for substances that may be of concern in relation to their mobility.

In their report, Crookes and Fisk (2018) point out that substances with log K_{OW} <5 or log K_{OC} <5 and log K_{aw} in the range <-3 tend to be mobile in water. Furthermore, they found that the overall CTD for substances with log K_{aw} down to around -3 is governed mainly by transport via the atmosphere, but at lower log K_{aw} values the overall CTD is increasingly governed by transport via the water phase.

The Crookes and Fisk model was applied to PFBS, See Annex II. The calulations show that with an assumed half-life in water of 10 years and a reported BAF in crab of 110 (reported as field-based bioconcentration factors (BCFs) by the authors), the concentrations of PFBS in aquatic biota may be expected to exceed the biota concentrations for a hypothetical compound just exceeding the P and B criteria over time. The half-life of 10 years for PFBS was chosen as a best-guess estimate in the absence of any measured half-life.

3.5.3. **Field studies**

In a study by Zhou et al. (2013) BAFs for short-chain PFASs in fish muscle from crucian carp (*Caracissius caracissius*) and fish sharpbelly (*Hemiculter leusisculus*), based on environmental measurements, were < 1. The samples were collected from Tangxun lake which is located near a production base of the flurochemical insustry in Wuhan, China.

Naile et al. (2013) investigated the distribution and bioconcentration characteristics of PFASs in environmental samples collected from the west coast of Korea. During May of 2009, the concentrations of PFASs were determined in water (n = 15), sediment (n = 12), soil (n = 13), and biota (n = 74) from estuarine and coastal areas along the west coast of Korea. Water samples (1 L) were collected by dipping a clean, 1 L polypropylene (PP) bottle, which had been rinsed with methanol, just under the surface of the water. Samples of biota were collected by hand in coastal tidal pools and along the shore of inland bodies of water, and were transferred to and stored in clean PP bags. Duplicate samples and field blanks were collected daily, and were analyzed along with laboratory and procedural blanks. All samples of biota were pooled, homogenised, and freeze-dried. Compound-specific, bioaccumulation factors (BAFs) (reported as field-based bioconcentration factors (BCFs) by the authors) were calculated for aquatic organisms based on site-specific concentrations of PFASs in water samples. BAF for crab was reported as a mean for five different crab species: *Acanthogobius flavimanus, Sebastes schlegeli, Tridentiger obscurus, Hexagrammos otakii, and Mugil cephalus*.

Relatively high concentrations of PFBS were measured in crab, as compared to fish, gastropod and bivalve with a mean concentration of 0.25 ng/g ww in 44 samples. It was further found that in crab the greatest BAF values were measured for PFBS and PFOS, while in fish PFOS and PFDA had the greatest BCF values, and in gastropods and bivalves PFHxS. Whole body BAF as a mean for the five crab species was reported as: log BAF =

2.04 ± 0.70, corresponding to a BAF (crab[7]) of 110. In comparison, the mean BAFs for PFHxS, PFOS and PFOA in crab were 575, 257 and 30, respectively. Mean BAFs were also reported for fish[8] (69), gastropod[9] (107) and bivalve[10] (12). Scatter plots of concentrations of PFASs in crab as a function of those in water, demonstrated a linear relationship. In general, the measured BAFs for PFASs were greater for fish and comparable for crab to those previously observed in Korea. The study would have benefitted from measuring of BAF for each individual crab species. However, BAF reported as a mean for five different crab species is also useful. The study is considered reliable with limitations, Klimisch score 2.

Similar results to the previsous study were found by Hong et al. (2015) when they investigated the bioaccumulation characteristics of perfluoroalkyl acids (PFAAs) in coastal organisms from the west coast of South Korea. Twelve individual PFAAs in samples of water (n = 43) and biota (n = 59) were quantified by use of HPLC–MS/MS after solid phase extraction. Bioaccumulation of PFAAs in various organisms including fishes, bivalves, crabs, gastropods, shrimps, starfish, and polychaetes were determined. Samples of water and biota were collected from the same locations and according to the same method as in the previous study by Naile et al. (2013). The compositions of PFAAs accumulated in samples were slightly different among species. For example, PFOS was the predominant PFAA in fish and shrimp, while PFBS, PFPeA, and PFOA were dominant in bivalve, crab, and gastropod. The authors suggested that bioaccumulation of PFAAs in aquatic organisms is strongly dependent on the concentrations of PFAAs in water regardless of species. Field-based bioaccumulation factors (BAFs) of PFAAs in various aquatic organisms were calculated based on concentrations in water and biota (wet mass basis). For PFBS the following log BAFs were reported: 2.0 ± 0.46 (fish, n=2), 1.5 ± 0.53 (bivalve, n=8), 2.3 ±0.57 (crab, n=13), 2.3 ± 0.79 (gastropod, n=14) and 1.6 ± 0.18 (shrimp, n=7). This corresponds to a BAF (crab) = 200, which is in the same range as the values reported by Naile et al. (2013). The study is considered a reliable, non-guideline study with some limitations in the documentation, Klimisch score 2.

Shi et al. (2018) investigated the processes governing uptake, distribution and elimination of PFASs in a study of the differential tissue distribution and bioaccumulation behavior of 25 PFASs in crucian carp from two field sites impacted by point sources. The highest median tissue-blood ratios for PFBS were observed in bile, gonad, heart, kidney and liver. However, when considering the relative body burdens, blood, gonads, and muscle together accounted for >90% of the amount in the organisms.

When comparing different PFSAs and PFCAs the authors concluded that the trends in tissue blood ratios, relative body burdens and BAFs were most consistent with specific protein interactions as the governing mechanism for uptake and accumulation of PFASs although phospholipid partitioning may contribute to the accumulation of long-chain PFASs in specific tissues. Median tissue/blood ratios (TBRs) were consistently <1 for all PFASs and tissues except bile which displayed a distinct distribution pattern and enrichment of several perfluoroalkyl sulfonic acids.

Bioaccumulation of PFASs was quantified in eel (*Anguila anguila*) from several locations in the Netherlands (Kwadijk et al., 2010). Water and eels were collected at 21 different locations between May 2007 and August 2007. Fillets from 30 individual eels per location

[7] Mean concentrations including Acanthogobius flavimanus, Sebastes schlegeli, Tridentiger obscurus, Hexagrammos otakii, and Mugil cephalus

[8] Mean concentrations including Hemigrapsus sanguineus, Sesarma pictum, Hemigrapsus penicillatus, Helice tridens tridens, and Philyra pisum

[9] Mean concentrations including Littorina brevicula, Monodonta labio, Umbonium thomasi, Glossaulax didyma, and Monodonta labio

[10] Mean concentrations including Mytilus edulis, Mactra veneriformis, Nuttallia olivacea, and Sinonovacula constricta

were randomly selected and homogenised prior to extraction and analysis. Historical eel tissue samples collected at three different locations from 1978 and onward for monitoring purposes, were also analysed for PFASs. Average BAF for PFBS in eel was 18 ± 4 (n=9). The BAF for PFBS was similar to that of L-PFOA (13) and PFOA (12), but lower than BAFs for the other measured PFASs.

In biota samples from Llobregat river in Catalaonia, Spain, the highest BAF calculated for PFBS was 1736 (Campo et al., 2015). However, this BAF was calculated by the authors as highest fish concentration divided by highest water concentration (L/kg, fish not paired with water sample from sampling location). The biota sample size was low, and PFBS was detected in 2 of 14 water samples only. Three fish species were sampled from five different locations along the river, *Barbus graellsii*, *Cyprinus carpio* and *Micropterus salmoides*. PFBS was detected in 8/12 fish samples. *→ Klimisch reliability 3 – not reliable, limitations in field-based estimations.*

3.5.3.1. Measured levels of PFBS in biota (other than remote arctic regions)

Measured levels of PFBS in biota in remote regions are reported in Section 3.4.3.3.

3.5.3.1.1. Marine biota

PFBS has been reported in several marine biota samples throughout the world. The largest concentration was found in shark liver (*Sphyrna tiburo* or *Rhizoprionodon terraenovae*) at a mean of 0.7 ng/g ww and max of 2.1 ng/g ww (Green et al., 2016). Van de Vijver et al. (2005) detected PFBS in the spleen of harbour seals from the Dutch Waden Sea. Ahrens et al. (2009b) investigated temporal trends of PFBS in the liver of harbour seals from the Germany Bight between 1999 to 2008. Average concentrations in seals older than seven month rangend from 0.2-0.8 ng/ww and 0.2-0.6 ng/ww respectively for seals up to seven month age. No significant temporal trend could be observed for the whole dataset. If the linear regressions was carried out from 2000 to 2008, the PFBS concentration decreased statistically significant by 59 %. Keller et al. (2012) detected PFBS in blood plasma from several species of turtles in the US, see Table 20. PFBS was detected in only a few samples from each species, with the exception of leatherbacks, for which it was nondetectable. Most of the detectable values of PFBS (0.02–0.14 ng/g) ranged from just above the reporting limit to three times the reporting limit, except for one green turtle with a PFBS concentration of 0.85 ng/g. This outlier resulted in green turtles having the highest average PFBS concentration compared with the other species; however, the median was lower than that of the Kemp's ridley as expected. The green turtle is an endangered species according to International Union for Conservation of Nature (IUCN).

O'Connell et al. (2010) investigated temporal trends as well as large-scale spatial trends of PFAS concentrations in threatened juvenile loggerhead sea turtles near or from Florida Bay, Cape Canaveral, Charleston, Core Sound, and Chesapeake Bay. They found mean plasma/serum concentrations in the range 0.024 to 0.026 ng/g in turtles from the Florida Bay and Core Sound, while PFBS was below the detection limit at the other locations.

Lam et al. (2016) measured perfluoroalkane sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs), in liver samples of Indo-Pacific humpback dolphins (*Sousa chinensis*) and finless porpoises (*Neophocaena phocaenoides*) from the South China Sea between 2002 and 2014. Levels of total perfluoroalkyl substances (PFASs) in samples ranged from 136–15,300 and 30.5–2,720 ng/g dw for dolphin and porpoise, respectively. PFBS concentrations in liver of dolphins and porpoises was from non-detect (<0.100 ng/g dw) to 12.3 ng/g dw and from non detect (<0.100 ng/g dw) to 1.76 ng/g dw, respectively. PFBS concentrations showed a significant increasing trend in the liver samples of dolphins from 2002 to 2014. The authors extrapolated, through regression analysis, that the levels of PFBS in dolphins were likely to increase 10-fold within 2024. Also, a significant increase

in the ratio of PFBS to PFOS against time was found for the dolphin samples. No significant temporal trends of ΣPFASs appeared over the sampling period (Lam et al., 2016).

PFBS has even been detected in Antarctic penguin dung, however at low levels compared to PFOS, Llorca et al. (2012).

Table 20: Environmental concentrations of PFBS in marine biota from other regions than remote arctic areas (ng/g ww).

Location	Date	Mean +/- SD	Min – max (Median)	n (df) LOD	Remark	Reference
Baltic sea	2013-2014	<0.002		LOD = 0.002	Zooplankton	Gebbink et al., 2016a
Baltic sea	2013-2014	<0.01		LOD = 0.01	Herring whole fish	Gebbink et al., 2016a
Baltic sea	2013-2014	<0.06		LOD = 0.06	Sprat whole fish	Gebbink et al., 2016a
Baltic sea	2013-2014	<0.004		(100 %) LOD = 0.0004	Guillemot egg	Gebbink et al., 2016a
Black Sea	1997-1998	Nd		LOD = 1.4-3.2	Harbor porpoise organs	Van de Vijver et al., 2007
Wadden sea, Netherlands	2002	2.34 ± 0.68	1.74 – 3.28 (2.17)	4 LOD not available	Harbor seals (spleen)	Van de Vijver et al., 2005
German Bight, Germany	1999-2008	0.2-0.8 seal> 7 month 0.2-0.6 seals > 7 month		55%	Harbor seals (liver)	Ahrens et al., 2009b
Faroe Islands	1986-2013	<0.01		LOD = 0.01	Pilot whale muscle	Dassuncao et al., 2017
Norway	2015	<0.1	(0.10)	128 LOD = 0.1	Cod liver	Green et al., 2016
Spain	2009	ND		LOD = Not reported	Fish and shellfish (composite samples)	Domingo et al., 2012
Minnesota/ Mississippi R.	2007	ND		LOD = 0.01-1.89	Bluegill, Black crappie &	Delinsky et al., 2010

Location	Date	Mean +/- SD	Min – max (Median)	n (df) LOD	Remark	Reference
					Pumpkinseed fillet	
USA, Georgia	2006	<0.3	0.3	LOD = Not reported	Shark soft tissue	Kumar et al., 2009
USA, Georgia	2006	0.7	<0.1 - 2.10	LOD = Not reported	Shark liver	Kumar et al., 2009
USA, Georgia	2006	0.4	<0.1 - 0.80	LOD = Not reported	Shark muscle	Kumar et al., 2009
USA	2007	ND		7 LOD = 0.02	Leatherback turtle plasma	Keller et al., 2012
USA	2007	0.02 ± 0.04	<0.01 - 0.126 (0.0019)	15 (20 %) LOD = 0.02	Loggerhead turtle plasma	Keller et al., 2012
USA	2007	0.02 ± 0.02	<0.01 - 0.0558 (0.0177)	10 (30 %) LOD = 0.02	Kemp's ridley turtle plasma	Keller et al., 2012
USA	2007	0.04 ± 0.06	<0.02 - 0.139 (0.008)	5 (40 %) LOD = 0.02	Hawksbill turtle plasma	Keller et al., 2012
USA	2007	0.09 ± 0.27	<0.02 - 0.846 (0,0000547)	10 (20 %) LOD = 0.02	Green turtle plasma	Keller et al., 2012
USA, Florida Bay	2010	0.026 ± 0.025	(0.016)	10 (91%)	Loggerhead trutle, plasma/serum	O'Connell et al., 2010
USA, Core Sound	2010	0.024 ±0.042	(0.006)	15 (20%)	Loggerhead turtle, plasma/serum	O'Connell et al., 2010
USA, Cape Canaveral	2010	< 0.117		10 (0)	Loggerhead turtle, plasma/serum	O'Connell et al., 2010
USA, Charleston	2010	< 0.117		9 (0)	Loggerhead turtle, plasma/serum	O'Connell et al., 2010
USA, Chesapeake Bay	2010	< 0.389		14 (0)	Loggerhead turtle, plasma/serum	O'Connell et al., 2010

Location	Date	Mean +/- SD	Min – max (Median)	n (df) LOD	Remark	Reference
South China Sea	2002 - 2014		< 0.100 – 12.3 (ng/g dw)	17 (71 %) LOD = 0.1	Indo-Pacific humpback dolphins' liver	Lam et al., 2016
South China Sea	2002 - 2014		<0.100 – 1.76 (ng/g dw)	50 (8%) LOD = 0.1	Finless porpoises' liver	Lam et al., 2016
Røst, Norway	1983	<0.009		5 LOD = 0.009	Herring Gull egg	Verreault et al., 2007
Røst, Norway	1993	<0.009		5 LOD = 0.009	Herring Gull egg	Verreault et al., 2007
Røst, Norway	2003	<0.009		5 LOD = 0.009	Herring Gull egg	Verreault et al., 2007
Røst, Norway	2017	<0.06		5 LOD = Not reported	European shag eggs (*Phalacrocorax aristotelis*)	Schlabach et al., 2018
Hornøya	1983	<0.009		5 LOD = 0.009	Herring Gull egg (*Larus argentatus*)	Verreault et al., 2007
Hornøya	1993	<0.009		5 LOD = 0.009	Herring Gull egg	Verreault et al., 2007
Hornøya	2003	<0.009		5 LOD = 0.009	Herring Gull egg	Verreault et al., 2007
Tromsøya, Norway	2017	0.03 ± 0.03	0.01 - 0.07 (0.02)	5 (80 %) LOD = Not reported	Common gull egg (*Larus canus*)	Schlabach et al., 2018

3.5.3.1.2. Freshwater biota near point sources

Measurements in freshwater biota were primarily from Taihu Lake, China (Fang et al., 2014) and a national survey of river basins in Vietnam (Lam et al., 2017), see Table 21. The highest concentration in Taihu Lake was associated with zooplankton (1.68 ng/g ww) and in Vietnam with Tilapia liver (1.34 ng/g ww). In samples from Taihu Lake, PFBS was not detected in phytoplankton, invertebrates (mussels) and in 4/9 sampled fish species (muscle tissue), Fang et al. (2014). PFBS was among the PFASs detected in all aquatic

biota species sampled (shrimp, fish and mallard (*Anas platyrhynchos*)) from a lake in China (Zhou et al., 2014).

Table 21: Environmental concentrations of PFBS in freshwater biota (ng/g ww).

Location	**Date**	**Mean +/- SD**	**Min – max** **(median)**	**n** **LOD**	**Remark**	**Reference**
China, Taihu Lake	2012	1.68 ± 0.04		3 LOD = 0.054	Zooplankton	Fang et al., 2014
China, Taihu Lake	2012	0.10 ± 0.00		3 LOD = 0.054	Crucian carp muscle (*Cyprinus carp)*	Fang et al., 2014
China, Taihu Lake	2012	0.08 ± 0.00		3 LOD = 0.054	Lake saury muscle (*Cololabis adocetus*)	Fang et al., 2014
China, Taihu Lake	2012	0.54 ± 0.01		20 LOD = 0.054	Mongolian culter muscle (*Culter mongolicus)*	Fang et al., 2014
China, Taihu Lake	2012	0.24 ± 0.01		10 LOD = 0.054	Mudfish, muscle (*Oriental weatherfish*)	Fang et al., 2014
China, Taihu Lake	2012	1.20 ± 0.03		3 LOD = 0.054	Whitebait, muscle (*Reganisalanx brachyrostralis*)	Fang et al., 2014
Vietnam	2013-2015	0.11 ± 0.54	1.34	149 LOD = 0.03-0.48	Fish and shellfish tissue (average of 5 fish species, 2 crustaceans (paddle crab, giant prawn), 1 gastropod (golden apple snail), 1 bivalve (golden freshwater clam)	Lam et al., 2017

3.5.3.1.3. Freshwater biota

Zafeiraki et al. (2019) detected PFBS in European eels caught between 2010-2016 in several Dutch waters, with tissue sample concentrations ranging from <0.3 – 7.1 ng/g ww, often in presence with other PFASs. PFBS was detected in several samples with a detection frequency of less than 40%.

3.5.3.1.4. Other biota samples

PFBS in the interval 0.07 - 0.33 ng/g ww were detected in the liver of mink (*Neovison vison*) from Norway (Schlabach et al., 2018). In a monitoring study on levels of PFAS in the terrestrial environment in Norway (Herzke et al., 2016) PFBS was detected in 5 out of 6 earthworms *(Lumbricidae*), levels ranged from 0.13 to 1.10 ng/g ww, see Table 22.

Table 22: Environmental concentrations in other biota samples (ng/g ww)

Location	**Date**	**Mean +/- SD**	**Min – max.** **(median)**	**n** **LOD**	**Remark**	**Reference**
Troms, Norway	2013-2014	0.19 ± 0.11	0.07 - 0.33 (0.19)	10 (100 %) LOD = Not reported	Mink liver (*Neovison vison*)	Schlabach et al., 2018
Norway, Oslo	2015	0.75	0.13 - 1.10 (0.94)	5/6 (83%) LOD = Not reported	Earthworms *(Lumbricidae*)	Herzke et al., 2016

3.5.3.2. Measured half-lives and levels in humans

Toxicokinetic data show that PFBS is found in many different tissues as it binds to the serum protein, albumin, and several other tissue proteins. The major route of elimination is through urine, although PFBS is also found in faeces and has been detected in milk of dairy cows.

The half-lives of PFBS in mice and rats are only a few hours, with a shorter half-life in female than male rodents. In monkeys, a half-life of up to a few days has been determined. Half-lives in humans have been measured in only 6 workers. The one female employee had the longest serum elimination half-life of 45.7 days, while the mean serum half-life of elimination in men was 24.1 days. A relatively large individual half-life variability for different other PFAAs was also measured using data from up to 66 individuals in a study by Zhang et al. (2013b). The half-life in pigs (43 days) seems to be similar to humans. See Section 4.1 and Table 27 for more details.

Comparing the measured half-lives of PFBS in rodents and humans shows that the half-life in humans is considerably longer than the half-life in rodents, although PFBS has a relatively short half-life in the human body compared to e.g. PFOA (2 – 4 years).

PFBS has been detected in human blood, breast mik, lung, bone, kidney, urine and hair. Concentrations of PFBS in human blood have been measured in a number of studies from Europe, US and Asia. The PFBS level is often below the quantification limit, with a concentration frequently below 0.5 ng/mL. However, in a few studies concentrations up to 5 ng/mL were observed. In persons with known elevated exposure to PFBS, e.g. from drinking water or in occupational settings, also higher levels have been measured.

Furthermore, PFBS has been detected in human cord blood with a mean concentration of 0.053 ng/mL, while the highest concentration was 0.46 ng/mL, see Section 4.3 and Table 28 for more information.

3.5.4. **Summary of bioaccumulation**

Due to being both hydrophobic and lipophobic, PFAAs do not bioaccumulate via distribution to fatty tissues like the legacy persistent pollutants (POPs) do. Due to their high water solubility, short-chain PFAAs are expected to be quickly excreted via gill permeation in water breathing animals. This reduces the bioaccumulation potential in such species.

Measured BCF values in fish are in the range 0.36 to 27.5. The highest fish BAF measured (field study) is 1736, however, the study had limitations; not reliable. In another field study a mean BAF for fish of 69 was reported while for crab a mean BAF of 110 was measured.

It has been estimated that substances with a low bioaccumulation potential could potentially reach similar levels in biota as substances that are known to bioaccumulate, provided that they are sufficiently persistent and mobile in the environment and when considering a longer time-scale.

PFBS has been detected in blood and different organs of animals and humans and is therefore bioavailable. PFBS has been shown to be able to bind to different proteins (transport proteins in blood, receptor proteins, enzymes and organic anion transporters), although the binding potential is lower compared to the longer chain PFAAs. According to Annex XIII also other information on the bioaccumulation potential can be considered in the bioaccumulation assessment, among others data from human body fluids or tissues, toxicokinetic data, results from bioaccumulation studies in terrestrial species and detection of elevated levels in biota, in particular in endangered species or in vulnerable populations.

PFBS has been detected in human blood, breast milk, lung, bone, kidney, urine and hair. It has also been detected in human cord blood. Hence, bioavailability has been demonstrated. A serum elimination half-life of around one month (up to 46 days) has been measured in humans, which is considerably longer than the half-lives measured for rodents. The average measured half-life in pigs, 43 days, is similar to that in humans. The individual variability in the study with pigs was large and the higher half-life values for PFBS were overlapping with the lower values for PFOA. It should be remembered that data on human half-life is scarce and that data measuring half-lives of other PFAAs in humans has also shown a great individual variability. No cut-off values for human elimination half-lives for fulfilling the B or vB criteria have been defined. Based on the lower half-life reported for PFBS in human blood compared to the half-lives of PFOA, 2 – 4 years (B), and PFHxS, 7 - 8 years (vB), it is concluded that PFBS is less bioaccumulative. It should be recognised that even though blood is used as a proxy for the entire body, the half-life in human blood is not necessarily identical with the whole body elimination half-life in humans (ECHA, 2017). The available information indicates at least a moderate bioaccumulation potential in humans. BMF values > 1 (1.2 (whole pig), 0.8 (meat), 6.4 (liver), 14 (blood plasma), 2.2 (kidney) and 0.9 (fat)) have been reported in pigs.

PFBS has been detected in different species of wild life including green turtles and polar bears, both are endangered or vulnerable populations. PFBS has also been detected in other marine mammals, like dolphins and whales. In dolphins PFBS concentrations showed a significant increasing trend in the liver samples from 2002 to 2014. Also, a significant increase in the ratio of PFBS to PFOS against time was found for the dolphin samples. In the whale it was shown that PFBS transfers from mother to foetus. Additionally, PFBS was

shown to transfer from mother to the eggs of birds. The findings of PFBS in sensitive life stages and in endangered species are of special concern.

3.6. Protein binding

PFAAs bind to proteins in the blood and tissues, and protein binding affects tissue distribution of PFAAs (Ng and Hungerbühler, 2014; Kerstner-Wood et al., 2003; Liu et al., 2017; Zhang et al., 2013a; Zhang et al., 2014). In blood, PFBS has been found to bind mainly to serum albumin, whereas PFOS, PFOA, and PFHxS have the potential to bind also to other human serum binding proteins, including plasma gamma-globulin, alpha-globulin, alpha-2-macroglobulin, transferrin, and beta-lipoproteins (Kerstner-Wood et al., 2003). The binding affinity to serum albumin seems to decrease in the order PFHxS > PFOS > PFBS (Bischel et al., 2011; Ng and Hungerbühler, 2014). A study by Liu et al. (2017) supports that the binding affinity to human serum albumin is lower for PFBS than for PFHxS and PFOS. PFBS binding did not cause apparent conformational changes to human serum albumin, but it induced functional changes as suggested by a decrease in esterase like activity of albumin at high concentrations (IC_{50} 0.13 mM).

Perfluoroalkyl acids (PFAAs) are known to accumulate in the liver and induce hepatotoxicity in experimental animals. They resemble fatty acids in structure and concerns have been raised that PFAAs may disrupt fatty acid binding to transport proteins or receptors. Binding of PFAAs to L-FABP (liver-fatty acid binding protein) has been shown *in vitro* (Luebker et al., 2002; Zhang et al., 2013a). This binding may explain the high concentration in liver tissues or in kidneys were L-FABP is also expressed. Binding affinity of perfluoroalkyl acids to human L-FABP was reported to increase significantly with chain length from C4 to C11 and decrease with chain length above 11. PFBS was shown to bind human L-FABP, with the lowest affinity of the three tested PFSAs: PFBS, PFHxS and PFOS (Zhang et al., 2013a). No binding was detected for the two fluorotelomer alcohols (6:2 FTOH and 8:2 FTOH).

Moreover, PFAAs may affect lipid metabolism through activation of peroxisome proliferator-activated receptors (PPARs). COS-1 cells were transfected with mouse or human PPARα receptor-luciferase reporter plasmid to investigate the effects of different PFAAs on PPARα activation. PFNA (C20max mouse 5μM; human 11 μM) and PFOA were predicted to have the highest PPARα activating potencies of the tested PFAAs whereas PFBS had lower predicted PPARα activation potencies (PFBS C20max mouse, 317 μM; human, 206 μM) (Wolf et al., 2008). As shown for PPARα, PFAAs also bind to the human PPARγ (Zhang et al., 2014). Similar to PPARα, the binding affinity for the different PFASs to human PPARγ *in vitro* increased with chain length from C4 to C11, and then decreased slightly with carbon chain length above 11 (Zhang et al., 2014). PFAS containing sulfonic acid groups bound more strongly than their carboxylic acid counterparts. Binding of PFBS to the ligand-binding domain of PPARγ was not detected under the conditions used in this *in vitro* binding affinity assay. However, in the activity assay of the receptor, a small, but significant increase in activity was detected, albeit at higher concentrations of PFBS (150μM) compared to PFOS (10 μM) (Zhang et al., 2014).

A recent publication by Rosenmai et al. (2018) found that cellular uptake of PFASs *in vitro* in HepG2-cells was in general low (below 1%). The sulfonates tested had lower uptake than the acids, although sufficient to activate PPARα. Uptake varied also with chain length. For the sulfonates, the shorter chain PFBS showed higher intracellular uptake than PFOS (in contrast to the acids). PFBS showed also higher potency for PPARα-activation at the lowest intracellular concentration. These data indicate that the potency of the short chain PFBS for activating PPARα may be comparable to, or even higher than, its' longer counterparts (PFHxS and PFOS) (Rosenmai et al., 2018).

The capacity of several PFASs, including PFBS, to compete with thyroxine (T4) for binding to the human thyroid hormone transport protein transthyretin (TTR) was examined by Weiss et al. (2009). The binding potency decreased in the order: PFHxS > PFOS/PFOA > PFHpA (perfluoroheptanoic acid) > PFOSi (sodium perfluorooctane sulfinate) > PFNA (perfluorononanoic acid), with TTR binding potencies 12.5–50 times lower than the natural ligand T4. PFBS was determined to have more than 100 times lower potency than T4 (IC_{50} of 19,460 nM that is approximately 20 times lower than the IC_{50} determined for PFOS). Similar values for TTR binding potency for PFBS were found in a study by Ren et al. (2016).

Occurrence of perfluoroalkyl acids in animal protein feeds

In a recently published study by Li et al. (2019) the most prevalent animal protein supplement feeds (APF), which are blood meal, meat meal, feather meal, soybean meal and distillers dried grains with solubles (DDGS), were analysed for 16 PFAAs. The animal-derived APFs possessed higher ΣPFAAs with a mean of 10.9 ng/g dw compared to plant-derived APFs, mean 0.75 ng/g dw. The short chain PFAAs (PFBA, PFBS and PFHxS) were primarily found in blood meal, meat meal, soybean meal and DDGS, while the long-chain counterparts (PFOS) dominated in feather meal. In animal derived supplement feeds PFBS contributed with 17%, 13.3%, 9.2% of the total PFAAs in blood meal, feather meal, meat meal, respectively. In plant derived supplement feeds PFBS contributed with 30% in DDGS and 13% in soybean meal. The wide occurrence of short-chain PFAAs (PFBA, PFBS) in fish meal were found for the first time by Li et al. (2019b). In fish meal, which is the most important animal-derived feed in global husbandry, PFAAs were analysed and the average for Σ16 PFAAs was 18.2 ng/g, (12% moisture). Short-chain PFAAs (PFBA, PFBS and PFHxS) were frequently detected and jointly contributed 15.7% of Σ10 PFAAs in fish meal.

Increasing PFBS concentrations in the environment, its ability for protein binding and enrichment in plants results in increasing occurrence of PFBS in animal and plant derived protein supplement feeds. Since these supplemental feeds might be the main feed source for livestock and poultry, human exposure through consumption of livestock and poultry together with PFBS-contaminated drinking water and plant-derived food, might lead to long-term continuous human exposure.

In summary, PFBS has been shown to bind to serum albumin and several other proteins, e.g. peroxisome proliferator-activated receptors (PPARs), liver-fatty acid binding protein (L-FABP) and thyroid hormone transport protein transthyretin (TTR). The ability to bind to proteins affects tissue distribution/accumulation and may be of toxicological significance. In general, the studies show a PFAA-protein binding affinity that depends on chain length. A carbon chain-length of 4 tends to have a lower binding affinity compared with a carbon chain length of 6-8. PFAAs containing the sulfonic acid group tend to bind more strongly to proteins than the corresponding carboxylic acids. Even though the binding affinity of PFBS to different transport proteins and receptors is lower than for its longer counterparts in vitro, the intracellular uptake and activation potencies may be different as indicated by Rosenmai et al. (2018).

The occurrence of PFAAs in animal protein supplement feeds (APFs) has been reported. In animal-derived supplement feeds PFBS contributed with 17%, 13.3% and 9.2% of the total PFAAs in blood meal, feather meal and meat meal, respectively. In plant derived supplement feeds PFBS contributed with 30% in distillers dried grains with solubles and 13% in soybean meal of the total PFAAs.

3.7. Enrichment in plants

Several studies investigated the uptake of PFBS and other PFASs from the surrounding environment into plants, Table 23. The studies were conducted under strictly controlled laboratory conditions, under semi-natural conditions, as well as field studies under environmental conditions, and monitoring data are compiled.

Krippner et al. (2015) examined PFAS accumulation in straw and grains of maize plants by looking at ten C4 to C10 substances containing the carboxylic (PFCAs) or sulfonic (PFSAs) functional groups, all individually spiked at 0.25 and 1 mg/kg soil. They confirmed the direct correlation between soil and plant tissue concentrations: the higher the soil PFAS content, the more the plants accumulate. They suggest that shorter-chain PFAS are more water soluble and therefore more mobile in soil and preferentially taken up by plants than longer-chain PFAS. Moreover, they observed lower translocation of long-chain PFAS within the plant than for short-chain PFAS, where long-chain PFAS largely remains in the roots and storage organs and short-chain PFAS transfers and accumulates in the above-ground plant parts. Müller et al. (2016) observed that tissue concentrations of PFAAs in hydroponic model plant system (*Arabidopsis thaliana*) during depuration rapidly declined in roots but remained constant in shoots, demonstrating irreversibility of the translocation process. Differences in accumulation levels of compounds in plants depends on the chain length, with the longer-chained compounds generally having lower accumulation rates, and on the associated functional groups, with PFSAs generally having lower accumulation rates than PFCAs (Scher et al., 2018).

Uptake mechanism for PFAS were studied by Wen et al. (2013) for PFOS and PFOA in corn. They suggested that PFOA and PFOS may have different uptake mechanisms in maize. For PFOA a potential active uptake and entry by anion channels was suggested, while for PFOS a passive entry by aquaporins (water channels) or anion channels (different than the ones used by PFOA) was suggested. Blaine et al. (2013) concluded that passive transport may be the primary mechanism for uptake and translocation due to the linearity of the plant uptake response to soil concentration of PFAS.

Ghisi et al. (2019) reviewed the accumulation of PFASs in agricultural plants. They emphasised two important sources to PFAS contamination of agricultural land: i) field irrigation with contaminated surface or ground waters, and ii) the use of contaminated sewage sludge as a soil conditioner. They pointed out that from agricultural plants PFASs can transfer to humans through the food chain. The authors concluded that PFASs are absorbed by plants to different extents according to their concentrations, chain lengths, functional groups, plant species, variety and organs, growth media (hydroponics vs. soil), and soil and biosolid characteristics. In particular, the abundance and characteristics of soil organic matter are considered among the most important factors.

The C4–C6 compounds appear to accumulate preferably in leaves and fruits, whereas the compounds with longer chain lengths tend to be more concentrated in roots. The authors reported studies with PFBS concentrations up to 1843 µg/kg dw in maize straw, grown on soil with spiking concentration of 1 mg/kg PFAA mixture, and 205 µg/kg dw in lettuce leaves, grown on soil with industrially impacted biosolids.

It was pointed out by Ghisi et al. (2019) that agricultural soils close to airports, fire-fighting training locations, industrial sites and landfills are particularly at risk of PFAS pollution. However, the use of biosolids on agricultural soil must also be considered a potential source of agricultural plant contamination. Growing vegetables in hydroponics should also be discouraged in polluted areas, as it allows pollutants to interact directly with plants in the absence of soil that has the ability to adsorb pollutants.

In a study of whether PFASs in water used for yard and garden irrigation resulted in elevated concentrations of PFASs in soil and home-grown produce at homes with a history

of PFAS-contaminated drinking water, it was found that short-chain PFASs have the highest potential to translocate to and bioaccumulate in edible plants (Scher et al., 2018). The findings were assumed to be globally relevant, as short-chain PFASs serve as commercial substitutes for longer-chain compounds and are increasingly detected in water due to their relatively high solubility and mobility. However, PFBS was not found in the groundwater in this particular area.

Blaine et al. (2013) investigated the accumulation of PFBS in lettuce and tomato grown on biosolid-amended soils and reported bioaccumulation factors (BAF) in lettuce up to 14.5 and PFBS concentrations in lettuce up to 205 µg/kg dw. Furthermore, a BAF of 0.42 was reported in the leaves and fruits from greenhouse tomatoes. PFBS enrichment was also demonstrated in other typical edible crop products, including radish, celery, tomato and peas with shoot concentration factors (SCF) of 3.4, 2.2, 3.7, 4.1, respectively (Blaine et al., 2013; 2014; 2014a) and SCF of up to 7.2 for thale cress (Müller et al., 2016). Felizeter et al. (2012; 2014) examined the uptake of PFASs into hydroponically grown lettuce, tomato, cabbage and zucchini. In the leaves the highest concentration factors were found, with LCF of 11-42, compared to other above-ground parts of the plants. In contrast, concentration factors for PFOS are much lower with 1.1 (Felizeter et al., 2012). A calculated mass distribution of different PFAAs showed that PFBS accumulates mainly in leaves (65%) and roots (21%), whereas PFOS accumulates mainly in roots (68-71%), Felizeter et al. (2014). The high potential for translocation in plants can be attributed to PFBS having a high solubility in water.

Krippner et al. (2014) demonstrated that shorter-chain PFASs are transferred predominantly and at higher concentrations to the shoot in maize plants with shoot/root ratio of 5.5 for PFBS. In contrast, long-chain PFAS accumulated at higher concentrations in the roots of maize plants with a shoot/root ratio of < 1 for PFOS. Krippner et al. (2015) calculated soil to plant transfer factors (TF) for PFBS in maize straw of 1.8-3.8, demonstrating uptake and storage of PFBS preferably in the leaves. However, Navarro et al. (2017) reported TF roots of 5.00 and TF leaves 4.00 for maize.

In wheat grown on biosolid amended soils, PFBS was not detected in grains but in roots and in shoots (Wen et al., 2014). Furthermore, Lan et al. (2018) reported that PFBS accumulates in wheat with lower BAF in root (2-5.6) than in shoot (9.1-10.5). The grass plant *Bromus diandrus* was exposed to 0.5 and 1 µg/mL of PFAA mixture in nutrient solution for 20 days, and a mean TF in shoot of 3.2 for PFBS was determined (García-Valcárcel et al., 2014). In a mesocosm study with two aquatic macrophytes whole plant BCFs of 29.5 and 18.8 were reported for PFBS for one submerged and one free-floating aquatic macrophyte, respectively (Pi et al., 2017).

The leaching of PFAAs from field contaminated soil and their uptake into biota was investigated by Bräunig et al. (2019). Soil from firefighting training sites at two airports with historical usage of aqueous film-forming foam (AFFF) was sampeled and a greenhouse study was carried out to investigate the uptake of PFAAs from soils into earthworms and wheat grass. The uptake of 12 different PFAAs was investigated. PFBS was found to have the third highest accumulation factor in wheat grass, after PFBA and PFPeA (but higher than PFHxS and PFOS). The authors concluded that the high accumulation of shorter chain PFAAs in grass has implications for their movement into the terrestrial food chain.

Navarro et al. (2017) further compared the uptake and distribution of PFASs in plant tissues with polybrominated diphenyl ethers (PBDEs). TF values higher for PFASs than for PBDEs were found in all crop plants: from 2 to 9-fold in spinach, 2 to 34-fold in tomato and 11 to 309-fold in corn. For PBDEs TFs for root and leaf were found in the intervals 0.32-0.50 and 0.01-0.03, respectively, as compared to 5.00 and 4.00 for PFBS. In another study Hurtado et al. (2016) determined the concentration of eight chemicals in leaf of lettuce and in the artificial soil (perlite and sand), in which they were grown in a greenhouse experiment. The tested substances were mostly neutral, except for ibuprofen

and propranolol. Bisphenol-A, caffeine, propranolol and tonalide had calculated BAF values that ranged between 2.3 and 9.1 g_{dwt}/g_{dwt}. Ibuprofen and triclosan had a calculated maximum BAF value of 1.3 g_{dwt}/g_{dwt}, while sulfamethazine was not detected in plants. Carbamazepine had high BAF values ranging from 17 to 247 g_{dwt}/g_{dwt}, PFBS has higher or at least comparable BAFs as the substances bisphenol-A, caffeine, propranolol and tonalide, and higher BAFs than ibuprofen, triclosan and sulfamethazine.

Data on the uptake and concentrations of PFBS in plants is compiled in Table 23.

Table 23: Uptake and concentration of PFBS in plants

Method	Plant	Results	PFBS concentration (µg/kg dw)	Comments	Reference
Field and greenhouse study with soil and industrial impacted biosolids and municipal impacted biosolids PFAA mixture	Lettuce (*Lactuca sativa*) Tomato (*Lycopersicon lycopersicum*)	Greenhouse, Municipal soil L. sativa: BAF: 14.5 Field trial L. sativa BAF: 2.02 L. lycopersicum industrially impacted soil BAF: 0.42 µg/kg dw	Lettuce leaves 205 Tomato fruits 19.4	Greenhouse and field	Blaine et al., 2013
Greenhouse, Plants grown in soils amended with industrial biosolids, loading 49 ng PFBS/g	Radish *(Raphanus sativus),* celery (*Apium graveolens var. dulce*), tomato *(Lycopersicon lycopersicum),* and sugar snap pea *(Pisum sativum var. macrocarpon)*	Shoot concentration factor (SCF) Radish= 3.4 Celery=2.2 Tomato= 3.7 Pea= 4.1	Celery 107 Pea fruits 16 Radish root 62	Greenhouse	Blaine et al., 2014
Greenhous hydroponic system (PFAA-spiked nutrient solution with nominal concentration of 10 ng/L to 10 µg/L	Lettuce *(Lactuca satva)*	Root Conc. Factors (RCF)= 0.002-0.008 Foliage Conc. Factors (RCF)= 0.002-0.008		Greenhouse	Felizeter et al., 2012
Greenhouse – hydroponic system (PFAA-spiked nutrient solution with nominal concentration of 10 ng/L to 10 µg/L	Tomato (*Solanum Lycopersicum* var. moneymaker), Cabbage (*Brassica oleracea* convar.	Root concentration factor (RCF): *cabbage:* PFBS > 10 *zucchini:* PFBS > 10 *tomato:* PFBS >10 - Stem		Greenhouse	Felizeter et al., 2014

Method	Plant	Results	PFBS concentration (µg/kg dw)	Comments	Reference
	capitata var. alba) Zucchini (*Cucurbita pepo* var. Black Beauty	concentration factor (SCF): *cabbage:* PFBS≈ 0.3 *zucchini:* PFBS≈ 1.5 *tomato:* PFBS≈ 2.1 -Leaf concentration factor (LCF) *cabbage*: PFBS≈ 11 *zucchini*: PFBS≈ 18 *tomato*: PFBS≈ 42 Edible part concentration factor (ECF) *cabbage*: PFBS≈ 0.8 *zucchini*: PFBS≈ 0.6 *tomato*: PFBS≈ 0.4 - Edible part/leaf transfer factor: All factors were < 1, which indicates that leafy crops with open leaves (spinach or some lettuce) accumulate higher amounts in the edible part than fruit-bearing crops. Leafy crops pose a higher risk for human exposure			
Hydroponically grown grass plants. With 0.5 and 1 µg/mL PFAA mixture	Grass *(Bromus diandrus*)	TF shoot= 3.3 (0.5 µg/mL) TF shoot= 3.1 (1 µg/mL)		Lab	García-Valcárcel et al., 2014
Corn grown in pots with	Corn *(Zea mays)*	Shoot/root ratio= 5.5		Lab	Krippner et al., 2014

Method	Plant	Results	PFBS concentration (µg/kg dw)	Comments	Reference
nutrient containing 0.1 mg/L PFBS at pH 5,6,7. Plants harvested after 5 d, shoots and roots analysed					
Pot experiment (soil was spiked with an aqueous solution of 0.25 mg individual PFAA/kg soil and 1 mg individual PFAA/kg soil) After 128 days straw and kernels were harvested PFAA mixture, ten compounds	Corn *(Zea mays)*	Transfer factor soil/ straw (TF straw): PFBS= 3.85 (0.25 mg/kg treatment) PFBS=1.84 (1.0 mg/kg treatment)	Treatment 1 mg/kg Maize straw:1843 kernels:5.4	Lab test All concen-trations are measured and based on dry weight.	Krippner et al., 2015
Pot experiment soil spiked with solution of 0.2 and 2 mg/kg PFAA. Harvested after 4 weeks.	Wheat *(Triticum aestivum L.)*	BAF root: PFBS= 2.0 (0.2 mg/kg) PFBS= 5.6 (2 mg/kg) BAF shoot PFBS= 9.1 (0.2 mg/kg) PFBS= 10.5 (2 mg/kg)		Greenhouse	Lan et al., 2018
Hydroponic system, plants exposed to 2 µg/L in growth media for 10 or 14 days.	Thale cress (*Arabidopsis thaliana*)	Root Conc. Factors (RCF)12.6 Shoot Conc. Factors (SCF)= 7.2		Lab test	Müller et al., 2016
Field soil amended with biosolids	Corn *(Zea mays)*	Transfer factor (TF root): PFBS= 5.00 (0.2 mg/kg) (TF leaves): PFBS= 4.00 (0.2 mg/kg)	Maize: roots 150 leaves 120	Field test	Navarro et al., 2017
Semi-static mesocosm study. Uptake phase 15	Submerged and free-floating aquatic macrophytes	Leaf BCF_{ss}: 33.0 / 21.2 Root BCF_{ss}: 12.9 / 14.9			Pi et al., 2017

Method	Plant	Results	PFBS concentration (µg/kg dw)	Comments	Reference
days. Water concentration (nominal and measured): 20 µg/L	*(E. horemanii / E. crassipes)*	Whole-plant BCF_{ss}: 29.5 / 18.8			
Field soil amended with biosolid	Wheat *(Triticum aestivum)*		Wheat roots 22- 60 Wheat shoots 22 **Grains,** husks < MDL	Field test	Wen et al., 2014

MDL: method- detection limit

Data on the uptake of PFBS in terrestrial plants near hotspot pollution sites are presented in Table 24.

Table 24: Uptake factors and concentrations of PFBS in terrestrial plants near hot spots, monitoring data.

Method	Plant	Results	PFBS concentration (µg/kg dw)	Location	Reference
Grass silage and hay cultivated on PFAA contaminated farmland.	Grass		Mean: Grass silage: 68.4 ± 23.1 Hay: 933± 224	Europe, Germany	Kowalczyk et al., 2013
Grass and leaves from 5 locations 3 km within PFAS plant, reference location 85 km away	Grass Leaves from hawthorn, raspberry, silver birch, ash and plane reference location 85 km		**µg/kg fw** <0.1-> 0.3 <0.2- 0.5 1.1	Europe, Netherlands	Brandsma et al., 2019

Method	**Plant**	**Results**	**PFBS concentration (µg/kg dw)**	**Location**	**Reference**
Leaves and bark, soil, water etc collected in 2012 from an area surrounding a large fluorochemical industry park (Jiangsu-Hi) in China. Leaves not washed before analysis.	Tree camphor	Leaf/ accumulation factors LSAF leaves= 2.0 LSAF bark= 0.33 Mean values used to calculate LSAF factors		China	Shan et al., 2014
Leaf and soil samples obtained from a campus of Dalian University, China.	Pine needels (*Pinus massoniana)* Cypress *(Platycladus orentalis)* Ginkgo (*Ginkgo biloba)* Popular, willow, sophora, plane-tree	Leaf/ accumulation factors LSAF leaves=17 pine LSAF leaves=0.59 cypress LSAF leaves=0.12 ginkgo LSAF leaves=0.38 popular, willow, sophora and plane tree.		China	Zhang et al., 2015
Vegetables from five gardens (0.2-1.5 km away from fluorochemical industrial park	Tomato *(Solanum lycopersicum),* Cucumber *(Cucumis sativus),* Eggplant *(Solanum melongena),* Pepper *(Capsicum annuum),* Chinese cabbage (*Brassica rapa ssp. Pekinensis*),		(µg/kg **fw**) 0.2 km 11 0.4 km 11 0.6 km 5.7 1 km <0.2 1.5 km <0.2	China, Fuxin	Bao et al., 2019

Method	Plant	Results	PFBS concentration (µg/kg dw)	Location	Reference
36 samples collected from 13 sampling sites along Qing river, 2013-2014	Submerged plants (*stuckenia pectinate, hydrilla, hornwort*) and three emergent plants (*reed, calamus and scirpus t.*)	BAF mean Stuckenia= 43 Hydrilla= 2 Hornwort= 62		China, Qing River,	Zhou et al 2017

3.7.1. **Measured levels of PFBS in plants**

PFBS has shown a high propensity to transfer and enrich in plants, due to its high mobility in plant xylem (Arp and Slinde, 2018).

In Europe, Kowalczyk et al. (2013) investigated PFBS, PFHxS, PFOS, and PFOA in contaminated feed and the absorption, distribution, and milk secretion of the substances in dairy cows, see Table 24. Hay and grass silage were obtained from an area with a pollution incident in Germany in 2006. The mean content of PFBS in grass silage was 68.4 ± 23.1 µg/kg dw and in hay 993.6 ± 224.4 µg/kg dm, showing that PFBS is taken up in plants used in agriculture. In the Netherlands Brandsma et al. (2019) reported PFBS concentrations in/on grass and leaves of hawthorn, raspberry, silver birch, ash and plane within 3 km of a PFAS plant in the range of >0.1-0.5 µg/kg fw. At the reference location 85 km away from the PFAS plant PFBS concentration of 1.1 µg/kg fw in/on leaves of hawthorn was found.

In Asia, Shan et al. (2014) collected i.a. leaves and bark of camphor trees and soil from the area surrounding a large fluorochemical industry park (Jiangsu-Hi) in China. The authors compared with previous studies on PFOA and hypothesised that PFASs predominantly were transported from the industry sites via atmospheric transportation on particulate matter to the nearby environment and deposited on trees and plants. Hence, tree samples were used to monitor airborne PFASs in the environment. In leaves and bark PFBS was detected with a mean of 0.42 µg/kg dw and 0.07 µg/kg dw, respectively. Compared with other studies in this dossier, it may seem that direct uptake in plants and trees from soil via water may have been somewhat underestimated in this study. However, airborne transport of PFASs on particulate matter may be a significant means of local transportation of PFASs from industrial emission sites to the nearby environment. In any case, the sum of PFASs in the tree leaves decreased significantly as the distance from the facilities increased. For comparison, the leaf/soil and bark/soil accumulation factors were calculated from the mean concentration of PFBS in soil of 0.21 µg/kg dw, 2 and 0.3, respectively, see Table 24.

In another study several PFAAs were monitored in the leaves of coniferous and deciduous forests grown in the urban areas of Dalian, China (Zhang et al., 2015). The results showed that coniferous tree leaves take up more PFAAs than broad-leaved tree leaves. For PFBS calculated leaf/soil accumulation factors (LSAF) were highest for the pine needles 17,

followed by 0.59 for cypress, 0.12 for ginkgo and 0.38 for popular, willow, sophora and plane tree, see Table 24.

A recently published study investigated whether PFAS-contaminated groundwater around a fluorochemical industrial park (FIP) in Fuxin, China, could be introduced into home-produced vegetables in local residences via the application of groundwater for the irrigation or feeding purposes (Bao et al., 2019), see Table 24. Samples of garden soil and vegetables (tomato, cucumber, eggplant, pepper and chinese cabbage) were obtained from five home gardens in the distance of 0.2-1.5 km from the FIP. After PFBA, PFBS was the most dominant contaminant discovered in vegetables with a concentration range between <0.2 and 11 µg/kg fw. This case is described in detail in Section 3.9. The authors conclude that contamination of vegetables with PFBS might be attributed to irrigation with contaminated groundwater, contaminated soil or atmospheric depositions.

Zhou et al. (2017) investigated bioaccumulation of PFAS in aquatic plants in the Qing River (China), an urban river of Beijing receiving i.a water from WWTPs. 36 samples from sixs species, whereof three submerged plants (*stuckenia pectinate, hydrilla, hornwort*) and three emergent plants (*reed, calamus and scirpus t.*) were collected from 13 samplings sites along the river. BAFs in submerged plants increased with increasing chain length, with a calculated mean BAF 11252 for PFOS in *stuckenia pectinate* compared to a mean BAF 43 for PFBS. The authors suggested that aquatic plants preferably absorped long-chain PFAS than short-chain PFAS.

Li et al. (2019) analysed the most prevalent animal protein supplement feeds (APF) for 16 PFAAs, for more details see Section 3.6 In plant derived supplement feeds PFBS contributed with 30% in distillers dried grains with solubles and 13% in soybean meal of the total PFAAs.

In summary, several studies and field data have shown that PFBS can be taken up in plants, especially in its edible parts like leaves, vegetables and fruits. Uptake factors in shoot ranged from 2 to 4 for several crops like radish, celery, tomato and pea (Blaine et al., 2014) until up to 14 in lettuce leaves (Blaine et al., 2013). Leaf concentration factors of up to 42 in tomato were determined (Felizeter et al., 2014). The highest plant concentrations of PFBS were reported in maize straw 1843 µg/kg dw and in vegetables like lettuce with 205 µg/kg dw (Ghisi et al., 2019). Furthermore, monitoring data from crops grown 0.4 km from a PFAS point source showed concentrations of up to 11 µg/kg fw PFBS (Bao et al. 2019), which might be attributed to contamination via groundwater or soil. This case study is an illustrative example of high level of contaminations in the perspective that it might happen in the EU if no action is taken for this substance. Occurrence of PFBS in plant derived supplemental feeds has been reported by Li et al. (2019). Kowalczyk et al. (2013) demonstrated transfer of PFBS from contaminated feed into meat and milk of dairy cows, see Section 3.5.1.4.

3.8. Comparison with other PFASs

In the present section the data from the field studies above are put into context and compared with other PFASs.

PFBS and precursor substances were largely introduced into the market as a replacement chemical for PFOS-substances (Olsen et al., 2009b). Hence, it could be expected that as PFOS use has declined, the PFBS emissions have increased. Below are monitoring data for PFBS put into context and compared with other PFASs in different matrices.

Water. In a global survey of surface water from 2016, both PFBS and PFOS were detected in all samples. However, PFBS was significantly higher in concentration, with the median

PFBS/PFOS ratio being 3.9 (Pan et al., 2018). A recent survey of surface water in Northern Europe, with sampling in 2013, found PFBS to be the dominating PFAS, contributing 21% of the sum PFAS (Nguyen et al., 2017). Similarly, a survey of European and Chinese rivers from 2013-2014 found PFBS to be the dominating PFAS (average 15.6 ng/L), followed by PFOA (4.8 ng/L) and PFPeA (4.7 ng/L) (Heydebreck et al., 2015). In the South China Sea, PFBS was one of the major PFASs analysed, contributing 21% of the total PFAS concentrations, together with PFOA (~26%) and PFOS (~20%). In the Bohai Sea, PFBS was the third most abundant PFAS, after PFOA and PFHxA (Chen et al., 2016).

Drinking water. A study of drinking water from Brazil, France and Spain (Schwanz et al., 2016) reported that PFBS was one of the most frequently detected PFAS, found in 27.2% of the samples, along with PFOS (100%), PFHpA (51.3%) and PFOA (23.0%). A global survey found similar results, with PFBS being the third most frequently detected PFAS in bottled water (47%) and tap water (88%) (Kaboré et al., 2018).

Source zones. Near a PFAS production facility in the Netherlands, PFBS was the dominating PFAS in both river samples (12-27 ng/L) and drinking water (0.5-19 ng/L) (Gebbink et al., 2017). In Finland up to 4.0 µg/L were measured in ditches and streams near airport areas with firefighting training sites. Near the PFAS production facility at Tangxun Lake, China, the two most dominating PFAS in water were PFBA and PFBS, at mean concentrations of 4770 and 3660 ng/L, respectively (Zhou et al., 2013). A remarkable 24-fold increase in PFBS concentration in groundwater was recorded in the vicinity of a PFAS production plant from 872 ng/L to 21 200 ng/L from 2009 to 2017 (Bao et al., 2019).

WWTP and Landfills. In a recent survey of WWTP in Sweden, PFBS, PFOS, and PFHxS had similar concentrations (1.9 ng/L, 1.9 ng/L, and 1.5 ng/L, respectively) (Eriksson et al., 2017). In San Francisco in 2014, PFBS was not the most dominating, but was emitted on average at 2.7 ng/L, which is a considerable level (Houtz et al., 2016). In Ireland high concentrations of PFBS and other PFASs in samples of leachate from landfills were reported by Harrad et al. (2019), with PFBS (arithmetic mean=1100 ng/L) as the predominant compond among the measured PFASs.

Human blood. Glynn et al. (2012) reported that in the period 1996-2010, PFOS in blood decreased, while replacement substances like PFBS and PFHxS increased by 11% and 8.3% per year, respectively. The source of the PFBS exposure was documented to be a local source, see Section 4.3.

Biota. Land et al. (2018) made a literature review and identified six data sets for PFBS trend analysis, out of which one showed significantly declining trends (grey seal), and the other five showed insignificant trends (northern fulmar, thickbilled murre, herring, loggerhead sea turtle and harbor seal). However, indication that PFBS may be rising in biota while PFOS levels are sinking, is found in a time series observed in dolphin livers in the South China Sea, Lam et al., 2016 (reference not included in Land et al. 2018). The ratio of PFBS/PFOS concentrations in the liver was found to have increased from 2002 to 2014. However, trend data are scarce and according to Land et al. "Additional time trend studies are urgently needed for selected PFASs, especially the wide range of alternatives to long-chain PFCAs and PFSAs and their precursors."

In summary, PFBS is in many cases one of the dominating PFASs in the water compartment. Studies show that PFBS is one of the most frequently detected PFASs in drinking water. Close to local PFBS sources, PFBS may be found at increasing levels in human blood. Also in biota rising PFBS concentrations have been reported, while PFOS was said to be declining.

3.9. Case study: Environmental distribution of PFBS – Fuxin, China

In this section a case study for the environmental distribution of PFBS in the vicinity of a point source in China is presented. The case study is based on a publication by Bao et al. (2019). In the surroundings of the Fuxin FIP, northwestern China, the extent of PFAS contamination in several home gardens, groundwater from private wells and garden soils was measured. PFAS contaminations were also characterised in both home-produced vegetables and eggs from the same residences. The study is included to illustrate the environmental fate for PFBS when released from a point source. Although the study is from outside EU and represents high PFBS concentrations, it is included as an illustrative example of high levels of contaminations in the perspective that it might happen in the EU if no action is taken for this substance.

The Fuxin FIP was built in 2004 and contains several fluorochemical production plants, mainly for the manufacture of PFBS and polytetrafluoroethylene (PTFE). High levels of PFAS contaminations have been detected in surface water and groundwater around the FIP in Fuxin over the past few years (Bao et al., 2011). In a previous study, PFBS contamination was determined in river and groundwater samples from the environment surrounding the FIP, with maximum measured concentrations at 0.445 µg/L and 0.872 µg/L, respectively.

In the study by Bao et al. from 2019, ten PFASs were measured to investigate the extent of PFAS contamination in the groundwater, soil and home-produced vegetable and egg samples near Fuxin. Sampling was performed in the home gardens of five residences close to the local fluorochemical plant, Table 25. The distances from the FIP site were 0.2 (H1), 0.4 (H2), 0.6 (H3), 1.0 (H4) and 1.5 (H5) km. Duplicate groundwater samples from shallow aquifers at depths of 5-7 m from five private wells in all home gardens were collected. Duplicate samples of surface soil at a depth of 2 cm from the gardens were also collected. Additionally, five common home-produced vegetable samples (tomato, cucumber, eggplant, pepper and Chinese cabbage) were obtained from all the five gardens. Home produced eggs were acquired from three residences only. Samples were collected of public groundwater normally used for both irrigation of home-produced vegetables and feeding of domestic poultry by the residents around the FIP.

PFBS and PFOA were the predominant PFAS contaminants in the groundwater beneath the FIP with maximum concentrations of 21.2 and 2.51 µg/l, respectively. A 24-fold elevation in PFBS contamination from 0.872 to 21.2 µg/L was detected in the local groundwater from 2009 to 2017. In the soil samples from the three sites closest to the FIP, PFBS was the main contaminant, contributing between 62% and 72% of all the target analytes. The concentrations of PFBS in soil showed a declining trend with increasing distance from the FIP site, ranging from 42.1 to < 0.20 ng/g dw.

Table 25: PFBS concentrations in environmental and dietary samples

Sampling site (distance from FIP)	Ground water (µg/L)	Soil (ng/g dw)	Vegetables (ng/g fw)	Whole egg (ng/g fw)	Egg white (ng/g fw)	Egg yolk (ng/g fw)
H1 (0.2 km)	21.2	42	11	32	22	43
H2 (0.4 km)	20.6	11	11	n.a.	n.a.	n.a.
H3 (0.6 km)	13.1	5.6	5.7	24	19	24

Sampling site (distance from FIP)	Ground water (µg/L)	Soil (ng/g dw)	Vegetables (ng/g fw)	Whole egg (ng/g fw)	Egg white (ng/g fw)	Egg yolk (ng/g fw)
H4 (1.0 km)	0.117	0.75	< 0.20	20	16	39
H5 (1.5 km)	0.064	< 0.20	< 0.20	n.a.	n.a.	n.a.

The contamination of vegetables and soils in residential gardens is likely due to irrigation with groundwater from the local public water system. However, the authors could not rule out a possible contribution from atmospheric depositions of PFAS contaminations in the vicinity of the release points.

Table 26: Estimated daily intakes of PFBS (ng/kg bw/day)[11]

Location	Eggs	Vegetables	Groundwater*
H1	24	34	707
H2	n.a	33	687
H3	19	17	436
H4	15	0.42	3.9
H5	n.a	0.42	2.1

*Possible drinking of groundwater from private wells

Residents around the Fuxin FIP prefer to consume drinking water from the municipal public water system (PWSs), rather than groundwater from their own private wells (PWs), Bao et al. (2011). The estimated daily intake of PFBS through ingestion of groundwater, home-produced vegetables and eggs was 765 ng/kg bw per day for residents living in closest proximity to the FIP (H1), Table 26. The levels detected in groundwater near the FIP (H1, H2, and H3) were exceeding the health advisories for PFBS in drinking water, 7 µg/L, as issued by the Minnesota Department of Health (2011), Table 25.

Contaminations from point sources to the groundwater could be a threat to drinking water quality in many areas around the world. Previous studies have shown that groundwater in shallow aquifers could suffer from migration of PFAS contaminations through the release of AFFF (Moody et al., 2003), discharge from fluorochemical facilities (Hoffman et al., 2011), or the application of biosolids (Lindstrom et al., 2011). In addition to the Fuxin case, there was a large-scale contamination of drinking water with short-chain PFASs in Uppsala, Sweden, and of arable land in Rastatt, Germany. The contamination in Sweden was mainly caused by the use of AFFF at a firefighting training site at a military airport. The Rastatt case, however, concerned contamination of former arable land with short-chain PFASs and precursors due to the use of compost mixed with sludge from paper production. This resulted in severe contamination of drinking water and crops. Considering the dramatic 24-fold increase in PFBS concentration in water between 2009 and 2017 at Fuxin FIP, the levels are likely to increase further.

[11] Adapted from Bao et al. (2019).

3.10. Summary and discussion of environmental fate properties

PFASs are among the most stable organic compounds due to the high bond energy of the C-F bond, which makes the substances resistant to acids, bases, oxidation and reduction, even at high temperatures. PFBS has been found to be stable towards hydrolysis, oxidation and photodegradation in the atmosphere, as well as resistant to degradation in conventional biodegradation tests. In a hydrolysis test no hydrolysis was observed.

PFBS has been found to be a weakly sorbing substance with a high mobility in the environment. PFBS has a preference for distribution to the aqueous phase and is relatively readily transported when water flows through soil. PFBS is a strong acid which is fully deprotonated at environmentally relevant conditions. The corresponding sulfonate anion in solution, as well as dissolved PFBS salts, do not volatilise.

Preference for the water compartment has been confirmed by distribution modelling and by monitoring data, showing that PFBS is frequently found in surface water, groundwater, drinking water, marine water, effluents from waste water treatment plants and in landfill leachate. The concentrations vary from low to very high in proximity to known point sources.

Due to the high aqueous solubility and the low sorption potential, resulting in a preferred distribution to the aqueous phase, PFBS is not readily removed with conventional purification techniques. Both wastewater purification, drinking water production and removal of industrial emissions may suffer from the low purification efficiency for PFBS.

PFBS has a high global contamination potential due to its high environmental stability and high mobility. Long-range transport of PFBS is mainly via the water and sea currents, while sea spray aerosols may also contribute. Long-range transport of PFBS-related substances and degradation to PFBS may take place, often via different routes of transportations than PFBS, like atmospheric transport.

Long-range transport has been demonstrated when PFBS has been found in samples of marine water, surface water, snow, ice, air and biota from remote areas. However, the measured concentrations of PFBS are generally low.

The detection of PFBS in remote areas, like the Arctic, is taken as evidence of the very high persistence and high mobility of PFBS.

As a short-chain, ionic perfluorinated substance, PFBS is a highly mobile substance, and there are no local or intermittent sinks for the pollution stock. Oceanwater is an important compartment for storage and transport of the compound. In costal water PFBS is bioavailable and can accumulate in the marine food chain, and therefore there is a high potential for further increase in the exposure of wildlife to the substance.

PFBS is bioavailable and has been detected in blood and different organs of animals and humans. Bioaccumulation and protein binding is discussed in detail in Sections 3.5 and 3.6, respectively, while the levels of PFBS in humans are presented in Section 4.3. PFBS has been found to bind to serum albumin, and thereby the blood can distribute PFBS within the body resulting in a potential to enrich particularly in blood rich tissues. PFBS has also been shown to bind to different other proteins (peroxisome proliferator-activated receptors (PPARα, PPARγ), human thyroid hormone transport protein transthyretin (TTR), and organic anion transporting polypeptides (OATPs)), although the binding potential is generally lower compared to the longer chain PFAAs. However, tissue distribution and accumulation of PFBS may be affected by its binding to proteins and may be of toxicological significance.

PFBS has been detected in human blood, breast milk, lung, bone, kidney, urine and hair. It has also been detected in human cord blood. A serum elimination half-life of around one month (up to 46 days) has been measured in humans, showing that PFBS has a moderate bioaccumulation potential. The half-life in humans is considerably longer than the half-lives measured for rodents. However, PFBS has a relatively short half-life in the human body compared to the longer chain counterparts. The half-life of PFBS in pigs was reported to be similar to that in humans, and BMF values > 1 were reported for pigs. PFBS has been detected in different species of wild life including green turtles and polar bears, both are threatened species. PFBS has also been detected in marine mammals like dolphins and whales. In the whale it was shown that PFBS transfers to the fetus. Additionally, PFBS was shown to be transfered to the eggs of birds. The findings of PFBS in sensitive life stages and in threatened species are of special concern.

Substances with a low bioaccumulation potential have been estimated to potentially reach similar levels in biota to substances that are known to bioaccumulate, provided that they are sufficiently persistent and mobile in the environment. Calculations even show that with an assumed half-life in water of 10 years and a reported BAF/BCF in crab of 110, the concentrations of PFBS in aquatic biota may be expected to exceed the biota concentrations for a persistent and bioaccumulative substance over time.

The occurence of PFAAs in animal protein supplement feeds (APFs) has been reported. In animal derived supplement feeds PFBS contributed with 17%, 13.3%, 9.2% to the total amount of PFAAs in blood meal, feather meal, meat meal respectively. In plant derived supplement feeds PFBS contributed with 30% in distillers dried grains with solubles and 13% in soybean meal to total PFAAs.

Due to its high water solubility, PFBS is bioavailable to plants. Enrichment in plants has been demonstrated by studies and field data. PFBS can be found in high concentrations in the edible parts of plants like leaves, vegetables and fruits, and long-term exposure of humans and animals can not be excluded.

4. Human health hazard assessment

The studies referred to in this chapter are according to standard tests, guidelines or GLP if stated so. Otherwise they are studies published in scientific peer-reviewed journals or public reports.

4.1. Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1. Non-human information

One study investigated the toxicokinetics of PFBS and perfluorohexanoic acid (PFHxA) in Sprague–Dawley rats and cynomolgus monkeys. A single intavenous dose of 10 mg/kg bw was given to both species followed by measuring the serum concentration of PFBS and PFHxA. The serum clearance (see Table 27) was more rapid in female than male rats for both substances, while no apparent gender difference was seen for monkeys (Chengelis et al., 2009).

In a pharmacokinetic study by Olsen et al. (2009b) in rats and monkeys, PFBS was administered orally or by a single intravenous (i.v.) dose in rats (30 mg KPFBS/kg body weight and monkeys of 10 mg KPFBS/kg body weight), a half-life of 4 hours in female rats, 4.8 hours in male rats and 3.5 days in female monkeys and 4 days in male monkeys were measured. The volume of distribution (Vdss) was estimated and shown to be similar between sexes, and the Vdss suggested that distribution was primarily extracellular regardless of sex according to the authors. Urine appeared to be a major route of elimination (Olsen et al., 2009b).

In a toxicokinetic evaluation, male Sprague-Dawley rats received a single 30 mg/kg bw dose of a PFBS-precursor, Me-FBSA (ECHA dissemination website, CAS 34454-97-2, https://echa.europa.eu/registration-dossier/-/registered-dossier/5299/7/2/2) in propylene glycol by oral gavage at a volume of 5 mL/kg body weight. The vehicle control group rats received a single dose of ethylene glycol at a volume of 5 mL/kg. Necropsies were performed at 4 hours, 28 hours and 100 hours post dose. Animals that received the test article at 30 mg/kg bw had average total fluorine concentrations in serum of 10.8 ppm (4 hours post dose), 23.1 ppm (28 hours post dose) and 1.0 ppm (100 hours after dose). The half-life elimination times for the test compound in the serum were 17 hours. The average liver total fluorine concentrations were 14.1, 18.3 and 2.2 ppm after 4 hours, 28 hours or 100 hours post dose, respectively. The apparent total fluorine elimination rates were similar in both serum and liver for animals treated with the test compound.

Toxicokinetic parameters were reported in a recent study in parallel to the recent NTP-study (NTP, 2019) on three fluorinated compounds (PFBS, PFHxS and PFOS) (Huang et al., 2019a). A single intravenous dose (4 mg/kg bw as 4mL/kg) or gavage administration (three dose levels (4, 20 and 100 mg/kg bw as 5 mL/kg) of PFBS were given to male and female Hsd:Sprague-Dawley rats. Concentrations of these PFAS were measured in the plasma, liver, kidney, and brain. A two-compartment model was the best fit for male rats (intravenous and gavage) and female rats (intravenous) while a one-compartment model was a better fit for the female gavage exposure data. Plasma half-life increased with longer chain length after gavage administration: PFBS-males averaged 3.3 h, females 1.3 h; PFHxS-males averaged 16.3 days, females 2.1 days; PFOS-males and females averaged ~20 days. There were dose-dependent changes in clearance and systemic exposure for all administered chemicals. Overall, concentrations of PFBS were higher in the liver than in the kidney in both sexes. PFBS concentrations in all tissues decreased slightly over time, with females having a faster decrease than males. In males, liver:plasma ratios were generally above 1, dropping below 1.0 at 12 h, while kidney:plasma ratios remained around 0.29 to 0.38. In female rats, the liver:plasma ratios were lower and kidney:plasma

ratios were higher than those in males. Brain:plasma ratios ranged from 0.01 to 0.02 in males and was 0.02 in females at 0.5 h. Sex differences in plasma half-life and tissue distribution were observed for PFBS and PFHxS, but not PFOS.

The tissue distribution of PFBS in mice was characterised (Bogdanska et al., 2014) in the same manner as for the eight-carbon homologue perfluorooctane sulfonate (PFOS) to allow direct comparisons. Following dietary exposure of adult male C57/BL6 mice for 1, 3 or 5 days to 16 mg (35)S-PFBS/kg bw/day, both scintillation counting and whole-body autoradiography (WBA) revealed the presence of PFBS in all of the 20 different tissues examined, demonstrating its ability to leave the bloodstream and enter tissues. After 5 days of treatment, the highest levels were detected in liver, gastrointestinal tract, blood, kidney, cartilage, whole bone, lungs and thyroid gland. WBA revealed relatively high levels of PFBS in male genital organs as well, except for the testis. The tissue levels increased from 1 to 3 days of exposure but appeared thereafter to level-off in most cases. The estimated major body compartments were whole bone, liver, blood, skin and muscle. This exposure to PFBS resulted in 5-40-fold lower tissue levels compared to PFOS. PFBS also showed different pattern of tissue distribution compared to PFOS, including lower levels in liver and lungs relative to blood. In, general, the level in tissues relative to blood were lower for PFBS than for PFOS, with the difference being most pronounced for liver and lungs (Bogdanska et al., 2014).

In another study male and female CD-1 mice were given a single dose with 0, 30, or 300 mg/kg bw PFBS via gavage. Trunk blood was collected at 0.5, 1, 2, 4, 8, 16, 24, and 48 hours and urine at 24 hours after dosing. The half-life of PFBS was estimated to be 4.6 hours in the male mice and 2.5 hours in the females. Within 24 hours, more than 95% of the serum PFBS was excreted into urine (Rumpler et al., 2016). Thus, the half-life of PFBS in mice is short, similar to that of rats (see Table 27 below).

Another study examined the transfer of a mixture of PFAAs from contaminated feed into the edible tissues of 24 fattening pigs and this study showed a tissue distribution of PFBS to blood plasma, liver, kidney, muscle tissue and fat. PFBS was eliminated through feces and urine (Numata et al., 2014). The geometric average half-life was estimated to be 43 days, the individual variability was large and the higher half-life values for PFBS were overlapping with the lower values for PFOA. PFBS have also been detected in milk from dairy cows, although at very low concentrations compared to other PFASs (Kowalczyk et al., 2013).

The enterohepatic circulation of PFASs likely contributes to their extended elimination half-lives in humans. It was demonstrated that PFBS, PFHxS, and PFOS were transported into hepatocytes both in a sodium-dependent and a sodium-independent manner by Na+/taurocholate cotransporting polypeptide (NTCP). PFBS, PFHxS, PFOS and PFCAs with 7-10 carbons are substrates of organic anion transporting polypeptides (OATPs). Chinese Hamster Ovary (CHO) and Human Embryonic Kidney 293 (HEK293) cells were used to demonstrate that human OATP1B1, OATP1B3, and OATP2B1 can transport PFBS, PFHxS, PFOS and the 2 PFCAs (C8 and C9). In addition, it was shown that rat OATP1A1, OATP1A5, OATP1B2, and OATP2B1 transport all 3 sulfonates. This study suggests that besides NTCP and the human apical sodium-dependent bile salt transporter (ASBT), OATPs are also capable of contributing to the enterohepatic circulation and extended human serum elimination half-lives of PFBS and other PFASs (Zhao et al., 2017).

Gomis et al. (2018) predicted the internal PFBS-concentration in the liver in several studies based on modelling. They noted that no robust conclusions could be drawn on the relative internal potencies of PFOS and PFBS. However, they stated that "for PFBS, the administered dose should be up to 2000 times higher to achieve the same magnitude in serum and liver concentrations as PFOS, which is due to the higher bioaccumulation potential of PFOS in serum and in liver". When similar internal dose was reached the health endpoint evaluated (liver) was similar indicating that PFBS have similar potency of effect

as PFOS in µM level. In the NTP (2019) 28-day rat study, internal plasma concentrations were measured. A comparison of the findings based on external dose (mmol/kg/day) and plasma levels on day 29 (µM) in rats showed that PFBS had to be administered at ~1300 higher concentration than PFOS in order to reach similar internal plasma dose in µM. Furthemore, if plasma concentrations at day 29 in the NTP-study are used for comparison, PFBS is markedly more potent. In fact PFBS and PFOS resulted in a similar magnitude of effect on thyroid hormones (decribed in 4.12) although the internal level of PFOS in µM was much higher than for PFBS.

4.1.2. **Human information**

Olsen and co-workers (Olsen et al., 2009b) also performed an elimination assessment in humans where 6 employees (1 woman and 5 men) donated a total of 10 blood samples to obtain serum samples over a 6-month period when the employees were not working in the production line. Spot urine samples were also collected at three time points. Determination of the serum elimination half-lives for the six subjects included all serum PFBS concentrations measured above the LOQ of 5 ng/mL (0.005 µg/mL). The mean serum elimination half-life for PFBS was 27.7 days (95% confidence interval 16.1–39.3). The geometric mean serum elimination half-life was 25.8 days (95% CI 16.6–40.2). The female employee had the longest serum elimination half-life of 45.7 days (see Table 27), while the mean serum half-life of elimination in men was 24.1 days (95% CI 13.1–32.5). Urine seems to be the major route of elimination. Due to the low number of subjects (only one female) and variations in the half-life obtained for one male subject who was tested twice, the elimination half-life obtained should be used with caution. However, a relatively large individual half-life variability for different other PFAAs was also observed using data from up to 66 individuals in a study by Zhang et al. (2013b).

4.1.3. **Summary**

PFBS is transported through the body and is found in many different tissues as it binds to the serum protein, albumin, and several other proteins. The major route of elimination is through urine, although PFBS is also found in faeces and has been detected in milk of dairy cows. The half-life of PFBS in mice and rats are similar with a shorter half-life in female than male rodents. PFBS has a longer half-life in monkeys compared to rodents, while the half-life in pigs seem to be similar to humans. PFBS may also bind to several different transporter polypeptides (NTCP, ASBT and OATPs) which are all capable of contributing to the enterohepatic circulation. Thus, enterohepatic circulation of PFBS may contribute to an extended human serum elimination half-lives of PFBS and other PFAAs. A serum elimination half-life of around one month (up to 46 days) has been measured in humans. Comparing the measured half-lives (Table 27) shows that the half-life of PFBS in humans is considerably longer than the measured half-lives in rodents. Based on the half-life reported for PFBS in human blood compared to the half-lives of PFOA, 2 – 4 years, (B) and PFHxS, 7 - 8 years, (vB), it is concluded that PFBS is less bioaccumulative. No cut-off values for human elimination half-lives for fulfilling the B or vB criteria have been defined. However, the available information indicates at least a moderate bioaccumulation potential.

Table 27: Serum t1/2 of PFBS in different species

Serum t1/2 of PFBS in different species		**Female**	**Male**	**Reference**
Sprague-Dawley rat *PFBS*	half-life for the terminal elimination phase serum T1/2	0.64 hours	2.1 hours	Chengelis et al., 2009

Serum t1/2 of PFBS in different species		Female	Male	Reference
Sprague-Dawley rat *Potassium PFBS*	Terminal mean half-life of serum elimination Two-compartmental model and first-order elimination	4.0 hours	4.8 hours	Olsen et al., 2009b
Sprague-Dawley rat *Potassium PFBS*	Mean half-life was measured in plasma.	1.3 hours	3.3 hours	Huang et al., 2019a
D-1 mice *Potassium PFBS*	Mean serum elimination half-life	2.5 hours	4.6 hours	Rumpler et al., 2016 (abstract)
Cynomolgus monkeys *PFBS*	Half-life for the terminal elimination phase	0.34 days	0.63 days	Chengelis et al., 2009
Cynomolgus monkeys *Potassium PFBS*	Mean serum elimination half-life	3.5 days	4 days	Olsen et al., 2009b
Pigs *PFBS*	Geometric average values reflect both plasma and edible tissue elimination half-lives	43 days	43 days	Numata et al., 2014
Humans *Potassium PFBS*	Mean serum elimination half-life one-compartment model	45.7 days (1 female)	24.1 days (5 male)	Olsen et al., 2009b

4.2. Human exposure routes

Exposure via the environment

The presence of PFBS in drinking water is presented in Section 3.2.5.3, while enrichment of PFBS in plants and exposure through the consumption of vegetables is discussed in Section 3.7. Furthermore, humans are also potentially exposed through consumption of fish or other food such as eggs, see e.g. Section 3.5.3 and Section 3.9 for examples.

Consumer exposure - household dust

Consumer exposure includes exposure from house dust, indoor air as well as dermal contact with consumer products or intake of food contaminated via food packaging.

The presence of PFBS in house dust and exposure via the indoor environment has been documented in the scientific literature. Several studies report findings of PFBS in dust

collected in European homes (D'Hollander et al., 2010; Haug et al., 2011; Jogsten et al., 2012; Bohlin-Nizzetto et al., 2015; Eriksson and Kärrman, 2015; Karásková et al., 2016; Torre et al., 2019). The European PFBS-concentrations in house dust range between 0.01 ng/g (Bohlin-Nizetto et al., 2015) and 72.8 ng/g (Eriksson and Kärrman, 2015) with a detection frequency ranging between 21% (derived from Haug et al., 2011) and 60% (Jogsten et al., 2012).

A study of world-wide indoor exposure to polyfluoroalkyl phosphate esters (PAPs) and other PFASs in household dust by Ericsson and Kärrman (2015) reports that PFBS was detected more frequently (approximately 50%) than in previous studies of dust sampled in 2008/2009 from Norway (Haug et al., 2011) and the U.S. (Fraser et al., 2013; Knobeloch et al., 2012), but at a similar level.

In a recent study by Torre et al. (2019) the concentrations of PFASs were quantified in 65 house dust samples obtained from Belgium, Italy and Spain. The authors report an increase in perfluorobutane sulfonate (PFBS) concentrations in Belgian house dust in comparison to previously published data. The levels of PFBS seem to have increased in the last ten years (0 to 0.40 ng/g; D'Hollander et al., 2010; Torre et al., 2019). No correlations between PFOS and PFHxS with PFBS ($p > 0.05$) were detected. The authors conclude that this suggests different application areas for PFBS. The potassium salt of PFBS is marketed as flame retardants for polycarbonate resins used in electronics (OECD, 2013). The study did not find any significant correlations between PFBS content in dust and information on the presence and use of electric and electronic devices in the homes.

4.3. Levels of PFBS in humans

Human blood has been the most frequently used matrix for determining the internal dose of PFBS in humans as described in detail below. However, PFBS has also been detected in other human tissues, see Table 28. In a study on autopsy tissues from 20 subjects who had been living in Tarragona, Catalonia, Spain, PFBS was detected in bone, lung and kidney, but for bone and kidney only in one sample each (Perez et al., 2013). The concentrations varied between <LOD (14.4 ng/g) and 17.8 ng/g for bone, <LOD (3.4 ng/g) and 80.4 ng/g for kidney and <LOD (2.10 ng/g) to 9.7 ng/g for lung. PFBS was not detected in liver. In a study from Belgium on 30 human hair samples, PFBS was detected in all samples, and the concentrations ranged from 34 to 120 pg/g (Alves et al., 2015). PFBS was also determined in urine (n=24, LOD 0.74 ng/mL) and hair samples (n=30 LOD 0.09 ng/g) from Barcelona, Spain, collected in 2010-2011 and 2009, respectively (Perez et al., 2012). Only one urine sample and two hair samples had detectable concentrations of PFBS, and the concentrations were 1.3 ng/mL in urine, and 0.8 and 10.7 ng/g in hair, respectively.

In humans, blood concentrations of PFBS have been measured (Table 28) in both the general population and populations which have experienced known elevated PFBS exposure, including workers.

In a study comprising human blood plasma samples from the German environmental specimen bank collected between 1982 and 2010 (n=258, age 20-29 years, both genders), PFBS was not found in concentrations above the LOQ of 0.5 ng/mL in any samples (Schröter-Kermani et al., 2013).

In another study from Germany, PFBS was determined in samples (n=396) collected between 2009 and 2016. However, only 1% of the samples had detectable concentrations of PFBS (LOQ 0.4 ng/mL), and the concentrations in these samples were below the LOQ of 0.4 ng/mL (Fromme et al., 2017).

A Norwegian study comprising pooled serum samples of men (n= 24 pools, each comprising >20 samples, age 40-50 years) collected between 1977 and 2006, reported PFBS concentrations above LOQ (0.05 ng/mL) in 16 of 24 samples. The concentrations varied between 0.065 and 0.18 ng/mL. No significant time trend was observed, but all samples from 2001 and onwards were below LOQ (Haug et al., 2009).

In a more recent study from Norway, paired serum, plasma and whole blood samples were collected from 61 adults (men and women) in 2013-2014 (Poothong et al., 2017). PFBS was detected in 51, 81 and 100% of the serum, whole blood and plasma samples, respectively, and the concentrations ranged from below LOQ to 0.22 ng/mL. The LOQs for serum, whole blood and plasma were 0.009 ng/mL, 0.009 ng/mL and 0.018 ng/mL, respectively.

Serum samples of nulliparous women from the Aarhus Birth Cohort Biobank, Denmark (n=1533, median age 29 years) were collected in the period 2008-2013 (Bjerregaard-Olesen et al., 2016). PFBS was detected in 1.2% of the samples (LOD 0.02 ng/mL).

PFBS was determined in 31 pooled samples of human milk from Sweden, collected between 1972 and 2016 in Gothenburg and Stockholm, to assess time trends (Nyberg et al., 2018). The pools comprised 5-116 individual samples per pool. In addition, 36 individual samples collected in 2012 and 10 individual samples collected in 2016 were analysed to evaluate the between subject variability. The concentrations in all 11 pooled samples from Gothenburg collected between 2007 and 2015 were below LOQ (1pg/mL), while in the 20 pools from Stockholm, collected between 1972 and 2014, concentrations above LOQ were reported in 50% of the samples. The concentrations ranged from 1.5 to 5.7 pg/mL, and no clear trend was observed. In the individual samples from Gothenburg, collected in 2012, PFBS was detected in 7 of 16 samples in concentrations between 1.1 and 3.2 pg/mL. PFBS was detected in only one of the 20 individual samples collected in Stockholm in 2012, with a concentration of 1.1 pg/mL. In the samples from Stockholm collected in 2016, PFBS was detected in 7 of 10 samples in concentrations ranging from 4.5 to 21 pg/mL.

During 2001 to 2004 men and women turning 70 years (from Uppsala, Sweden) were invited to donate blood samples (n=1006), to study their PFBS concentrations (Salihovic et al., 2015). Detectable levels of PFBS were found in 14% of the samples from women and in 11 % of the samples from men. The levels ranged from 0.035 to 0.086 ng/mL (LOD 0.01-0.17 ng/mL).

46 blood samples from adults (age 19-53 years, males and females) living in Barcelona, Spain, were investigated, and PFBS was found in 16 of 46 samples, in concentrations up to 0.43 ng/mL (LOD 0.04 ng/mL) (Gómez-Canela et al., 2015). The samples were collected in 2009-2010.

In the US, PFBS was determined in two cross sectional studies comprising blood donors that gave samples in 2006 or 2010 (Olsen et al., 2012). In both sample sets the 75 percentile was below the LOQ (0.025 ng/mL), and the 95 percentile was 0.3 ng/mL. In a follow up study on samples collected from blood donors in 2015 (n=616) 8.4% of the samples had quantifiable concentrations of PFBS, in the range from below LOQ to 4.2 ng/mL (Olsen et al., 2017).

In a recent study from the US National Health and Nutrition Examination Study (NHANES), PFAS concentrations were reported in paired samples of spot urine and serum from 2273 participants aged 6 and older (Calafat et al., 2019). PFBS was not detected in any of the urine samples, but in 9.1% of the serum samples from children aged 6 to 11 years (n=148) and in 0.6% of the serum samples from participants 12 years and older (n=2125). The LOQ was 0.1 ng/mL.

In a French study on women and their newborns (n=100 pairs, age 20-46 years, samples collected in 2010-2013), PFBS was detected (LOD 0.05-0.2 ng/mL) in less than 1% of the paired maternal and cord sera samples (Cariou et al., 2015). The quantified levels were always lower than 0.40 ng/mL. Samples of breast milk from the same mothers were collected and analysed, but no samples had detectable concentrations of PFBS (LODs: 0.01 to 0.04 ng/mL). In a Spanish cohort of mother and children, the concentration of PFBS was assessed in paired samples of maternal and cord blood (N=66 pairs, mean maternal age 32 years) collected in 2003–2006, but no samples had levels above LOQ (0.10 ng/mL) (Manzano-Salgado et al., 2015).

Concentrations of PFBS were assessed in 687 samples of cord blood collected in Shanghai, China between 2011 and 2012 (Wang et al., 2016a). The mean concentration of 0.053 ng/mL, while the highest concentration was 0.46 ng/mL. Concentrations above LOQ (0.009 ng/mL) were found in 97% of the samples. Hence it is demonstrated that foetuses are exposed to PFBS through trans-placental transfer.

In a study of primiparous women from Uppsala, Sweden (n=36 pools, age 19−41 years) the serum concentrations of PFBS increased with 11% per year in the period from 1996 to 2010 (Glynn et al., 2012). The highest concentration was found in a pool from 2010 with a concentration of 0.1 ng/mL (LOD 0.013 ng/mL). The increasing trend of PFBS was later confirmed and linked to contamination of the drinking water in the city of Uppsala (Gyllenhammar et al., 2015). Concentrations of PFBS in serum of first-time mothers (n=297, age 20-41 years) sampled in the Uppsala county in two different time periods were determined (Gyllenhammar et al., 2015). Statistically significantly higher concentrations of PFBS (LOD 0,01 ng/g) were found in the samples collected between 2008–2011 (<LOD-0.80 ng/mL) compared to those collected between 1996–1999 (<LOD-0.21 ng/mL), with median concentrations of 0.019 and 0.027 ng/mL in 1996-99 and 2008-2011, respectively. The highest levels were observed among women living in areas with the most elevated drinking water levels, in both time periods. This support the suggestion that drinking water intake has been the major pathway of exposure for PFBS in Uppsala over the studied period. In another study (Gebbink et al., 2015) on primiparous women living in Uppsala County, Sweden (n= 30 pools, each comprising 9-10 individuals, age 19 to 42 years) PFBS was observed above LOQ (0.009 ng/mL) in around 60% of the samples, in concentrations in the range 0.011 to 0.058 ng/mL. In this study no significant change in concentration was seen in the serum samples for PFBS over the time period 1997 to 2012. In the Gebbink report it is explained that the different results in these studies could be attributed to one or several of the following factors: for the Gebbink et al. (2015) study, serum pools were freshly made and contained fewer individual serum samples in pools from the early part of the study period compared to the Glynn et al. (2012) study. Different sample preparation and analytical techniques (including different types of MS detectors) were used and the study design was different. Moreover, Glynn et al. (2012) investigated a slightly different time period (1996-2010) and included samples collected in all years (except 2003 and 2005) resulting in higher statistical power compared to the Gebbink et al. (2015) study with samples from alternating years collected between 1997 and 2012.

In a study from the Veneto region in Italy where the drinking water had been contaminated with PFASs, including PFBS, elevated serum concentrations were observed among people living in the exposed area (<LOQ – 4.26 ng/mL) compared to the people living in the non-exposed area (<LOQ-0.36 ng/mL) (Ingelido et al., 2018).

The level of PFBS was explored in a study on serum samples collected in 2011 from the general population of Henan, China (n=133, age 0-88 years, male and females) (Fu et al., 2014). In around 30% of the samples PFBS was observed, and the concentrations ranged from 0.03 to 2.2 ng/mL (LOD 0.05 ng/mL).

In a study on 103 males from Guangzhou city, Guangdong province, China, collected between 2012 and 2013, PFBS was detected (LOD 0.013 ng/mL) in all samples with a mean blood concentration of 0.18 ng /mL (95 percentile; 0.43 ng/mL), (Song et al., 2018).

PFBS was determined in a cohort of women (n=335) from Zhejiang, China, included in a case control study on endometriosis-related infertility (Wang et al., 2017). Samples were collected in 2014-2015. As many as 98.5% of the plasma samples had detectable (LOD 0.009 ng/mL) concentrations of PFBS, and the median and interquartile range (IQR) concentrations were 0.091 ng/mL (0.088-0.097 ng/mL) and 0.089 ng/mL (IQR 0.085-0.095 ng/mL) for cases and controls, respectively.

In 2004, a fluorochemical industrial park was built in Fuxin, China, for the production of PFBS and other PFAAs. Serum samples from non-occupationally exposed residents from Fuxin, China (n=120, age 0.1 to 87 years, males and females) were collected in 2009 (Bao et al., 2011). PFBS was found above LOQ (0.07 ng/mL) in 93% samples, with a median concentration of 0.12 ng/mL while the max concentration was 1.3 ng/mL. In another study on non-occupationally exposed healthy residents from Fuxin, China (n=100, 50 males and 50 females, age 1-87 years), with samples collected in 2015, PFBS was detected in all serum samples (LOQ: 0.05 ng/mL), Bao et al. (2017). The median (0.14 ng/mL) and max concentrations (1.5 ng/mL), were very similar to those observed in the samples collected in 2009. In a later study from the same area it was demonstated that the area is considerably contaminated with PFBS, see Section 3.9, (Bao et al., 2019).

In a Chinese study, serum samples from fishery employees and their families, living close to the Tangxun Lake with elevated PFBS concentrations were compared to serum samples from people in a reference area in the Hubei Province (Zhou et al., 2014). PFBS was detected (LOQ 0.10 ng/mL) in 72% and 29% of the serum samples of the fishery employees and fishery families, respectively, whereas it was detected only in one serum sample from the reference area. The concentrations in the fishery employees (detected in 28 of 39 samples) were highest with a median concentration of 11.3 ng/mL, followed by the fishery families (detected in 2 of 7 samples) with a median concentration of 2.34 ng/mL. The PFBS concentration measured in the sample from the reference area (detected in 1 of 9 samples) was 0.12 ng/mL.

Concentrations of PFBS in the range < LOD (0.02 ng/mL) to 0.09 ng/mL have been reported in blood serum from Australian fire fighters (n=149, age 17-66 years), (Rotander et al., 2015). In a study on blood levels in employees of the 3M Company (n=18, including six researchers, six production workers, six corporate employees, age 29-79 years), PFBS was measured (Ehresman et al., 2007). Most samples were below LOQ, but this was rather high, 5 ng/mL. However, in the samples from some of production workers PFBS concentrations in the range 7-32 ng/mL were observed.

For comparison, the European Food Safety Authority (EFSA, 2018) reported a median of the values reported as median concentrations of PFOA in a number of studies in European general populations of 1.9 and 3.3 ng/mL for adults and children, respectively. Thus, the higher concentrations of PFBS reported so far are in the range of PFOA concentrations, even though the PFBS levels in the general populations are considerably lower than those of PFOA.

Table 28: Concentrations of PFBS in human tissues (ng/g ww) and blood (ng/mL)

Location	Year	Mean +/- SD ng/mL	Min-Maks (Median) ng/mL	n (det. Freq)	Remark	Reference
Germany, Münster	1982 - 2010	nd		258 (0%)	Plasma	Schröter-Kermani et al., 2013
Germany	2009 - 2016	<0.4		396 (1%)	Plasma	Fromme et al., 2017
Norway	1997 - 2006		0.065-0.18	24 pools (67%)	Serum (men 40 – 50 years)	Haug et al., 2009
Norway	2013 - 2014	0,05; 0,13; 0,04 in serum, whole blood, plasma respectively	<LOQ-0.22	61 (51, 81, 100% of serum, whole blood, plasma respectively)	Paired serum, plasma and whole blood (adult men and women)	Poothong et al., 2017
Denmark	2008 - 2013			1533 (1.2%)	Serum (nulliparous women, median age 29)	Bjerregaard-Olesen et al., 2016
Italy, Veneto	2018	<LOQ	<LOQ - 4.26	257 (31 %)	Blood (exposed areas)	Ingelido et al., 2018
Italy, Veneto	2018	<LOQ	<LOQ - 0.36	250 (18 %)	Blood (non-exposed areas)	Ingelido et al., 2018
France	2010 - 2013		LOD (0.05-0.2) - 0.4 (max)	< 1% of paired samples	Paired maternal and cord sera (women and their newborns)	Cariou et al., 2015
Spain, Tarragona	2008	3.2*	17.8 (max)		Bone	Pérez et al., 2013
Spain, Tarragona	2008	<LOD			Brain	Pérez et al., 2013
Spain, Tarragona	2008	17.8*	9.7 (max)		Lung	Pérez et al., 2013
Spain, Tarragona	2008	8*	80.4 (max)		Kidney	Pérez et al., 2013
Spain, Barcelona	2009 - 2010	0.20 (0.11)	< LOQ - 0.43	46 (35%)	Blood adults (age 19-53, males and females)	Gómez-Canela et al., 2015

Location	Year	Mean +/- SD ng/mL	Min-Maks (Median) ng/mL	n (det. Freq)	Remark	Reference
Spain	2003 - 2006		< LOQ	66	Paired samples maternal and cord blood (mean maternal age 32 years)	Manzano-Salgado et al., 2015
Sweden, Gothenburg and Stockholm	1972 - 2016		<1-21 pg/mL	n= 31 pools and 46 individual samples individual samples	Human milk, collected between 2 weeks and 3 months after delivery	Nyberg et al., 2018
Sweden, Uppsala	1996 - 1999	0.03 ± 0.00	<MDL - 0.21 (0.02)	132 (77 %)	Serum pregnant mothers (1996-1999)	Gyllenhammar et al., 2015
Sweden, Uppsala	2008 - 2011	0.06 ± 0.01	<MDL - 0.80 (0.03)	134 (86 %)	Serum pregnant mothers (2008-2011)	Gyllenhammar et al., 2015
Sweden, Uppsala	1996 - 2010		<LOD - 0.10 (0.09)	36 pools (72 %)	Serum primiparous mothers	Glynn et al., 2012
Sweden, Uppsala	1997 - 2012		<LOD (0,009) – 0,058	30 pools (60%)	Serum primiparous mothers	Gebbink et al., 2015
Sweden, Uppsala	2001 - 2004	0,068(0.05) for women and 0.055 (0,03) for men	0.035 - 0.086	1006 (14% women and 11 % of men)	Blood (Men and women age 70)	Salihovic et al., 2015
USA	2006 and 2010				Plasma, blood donors	Olsen et al., 2012
USA	2015		<LOQ-4.2	616 (8,4%)	Plasma, blood donors	Olsen et al., 2017
USA			< LOQ (5)- 32		Serum, plasma and blood (Fluorochemical workers)	Ehresman et al., 2007
USA	2013 - 2014			9.1% (6-11 years) 0.6% (12 years and older	Serum Serum	Calafat et al., 2019

Location	Year	Mean +/- SD ng/mL	Min-Maks (Median) ng/mL	n (det. Freq)	Remark	Reference
				0% (6-11 years) 0% (12 years and older)	Urine Urine	
Australia			<LOD (0.02)-0.09	149	Serum (Fire fighters)	Rotander et al., 2015
China, Henan	2011		0.03 - 2.2	133 (30%)	Serum (0-88 years, male and females)	Fu et al., 2014
China, Guangzhou city	2012 - 2013	0.18	(95 percentile 0.43)	103 (100%)	Blood	Song et al., 2018
China, Zhejiang	2014 - 2015		Case: median 0.091 (IQR: 0.088-0.097) Controls: median 0.089 (IQR: 0.085-0.095)	335 (98.5%)	Plasma, women (case study) endometriosis-related infertility	Wang et al., 2017
China, Shanghai	2011 - 2012	0.053	LOD - 0.46 (0.05)	687 (97 %)	Cord blood (ng/mL)	Wang et al., 2016a
China, Tangxun Lake			Employee: 11.3 (median) fishery family: 2.34 (median) ref. area: 0.12 (median)	39 employees (72%) 7 family members (29%) 9 background exposed (11%)	Serum, fishery employees, fishery family, reference area population	Zhou et al., 2014
China, Fuxin	2009	0.19 ± 0.22	0.01 - 1.30 (0.12)	120 (93 %)	Serum	Bao et al., 2011
China, Fuxin	2015	0.23 ± 0.27	0,05-1.5 (0,14)	100 (100%)	Serum	Bao et al. 2017

* ng/g

4.3.1. **Summary**

PFBS has been detected in human blood, breast milk, lung, bone, kidney, urine and hair. Concentrations of PFBS in human blood have been analysed in a number of studies from Europe, US and Asia. The PFBS level is often below the quantification limit, with a concentration frequently below 0.5 ng/mL. However, in a few studies concentrations up to 5 ng/mL were observed. In persons with known elevated exposure to PFBS, e.g. from drinking water or in occupational settings, also higher levels have been measured. Further, PFBS has been detected in human cord blood with a mean concentration of 0.053 ng/mL, while the highest concentration was 0.46 ng/mL.

Studies from Uppsala, Sweden, as well as a study from the Veneto region in Italy, in both cases with known elevated PFBS exposure from drinking water, have shown that persons experiencing elevated exposure to PFBS have elevated PFBS concentrations in their blood compared to people from the same region experiencing only the general background exposure. Furthermore, elevated PFBS blood concentrations were observed in fishery employees and their families working and living nearby a lake in China with elevated PFBS concentrations. High blood PFBS concentrations were also observed in employees from a company manufacturing PFBS. Hence, it has been demonstrated that elevated exposure to PFBS can lead to elevated blood concentrations.

4.4. Repeated dose toxicity

4.4.1. **Non-human information**

4.4.1.1. In vitro data

An in vitro study showed that PFBS treatment for 6 days extensively promoted the differentition of 3T3-L1 preadipocytes to adipocytes, resulting in significantly increased triglyceride levels in cells (Qi et al., 2018). In particular, the treatments with PFBS (10, 50, 100 and 200μM) at the early adipogenic differentiation period (day 0–2) were positively correlated with increased triglyceride accumulation on day 6. Protein and messenger-RNA (mRNA) levels of the transcription factors in adipocyte differentiation; CCAAT/enhancer-binding protein α (C/EBPα) and peroxisome proliferator-activated receptor gamma (PPARγ), along with acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), the key proteins in lipogenesis, increased after PFBS treatment. PFBS significantly activated the phosphorylation of extracellular signal-regulated kinase1/2 (ERK1/2) after 4-h treatment, and the effect of PFBS on triglyceride was abolished by U0126, a specific MAPK/ERK kinase (MEK) inhibitor. In conclusion, PFBS increased the adipogenesis of 3T3-L1 adipocytes, in part, via MEK/ERK-dependent pathway. In another study, human mesenchymal stem cells (hMSC) were exposed to different concentrations (50, 100, 150, 200 and 250 µmol/L) of several short chain PFASs, PFOA and PFOS during a 21 day differentiation process (Liu et al., 2020). PFBS exposure caused a decrease in CD90 of the hMSC, which could stimulate adipogenesis. Furthermore, the authors reported that short-chain PFASs, including PFBS, stimulated to adipogenic differentiation, evidenced by upregulation of several common adipogenesis marker genes. The effects of PFBS were however not as pronounced as for PFOS and PFOA and no cytoplasmic lipidaccumulation was seen for PFBS. Liu et al. (2020) also report that four short-chain PFASs, including PFBS, did not affect human mesenchymal stem cell osteogenic differentiation, contrary to PFOA, which is able to do so.

4.4.1.2. In vivo data: oral

Data available on the toxicity of PFBS in animals have identified the liver, kidneys, stomach and hematological systems as targets of toxicity. However, the data are limited. A statistically significant dose dependent decrease in hemoglobin and hematocrit levels were observed in male rats administered orally 200 mg/kg bw/day and 600 mg/kg bw/day PFBS for 90 days (Lieder et al., 2009a). A significant decrease in erythrocyte levels were observed at 600 mg/kg bw/day. Furthermore, the absolute and relative (to body and brain) spleen weight was decreased at all dose levels in males compared to their controls. The latter effect was however not considered of toxicological significance according to the study authors. The NOAEL was determined to be 60 mg/kg bw/day in male rats (based on haematological parameters) and 600 mg/kg bw/day in females (based on kidney effects described below) (Lieder et al., 2009a). Administration of 600 mg/kg bw/day for 90 days resulted in tubular and ductal papillary epithelial hyperplasia in the kidneys and necrosis and hyperplasia/hyperkerosis in the forestomach of rats (Lieder et al., 2009a). Increases in absolute and relative liver weight were reported in male rats administered 900 mg/kg bw/day for 28 days (3M, 2001). At 300 mg/kg bw/day treatment-related microscopic changes were observed in the kidney and liver for males and kidneys for females, and at 1,000 mg/kg bw/day hepatocellular hypertrophy was observed in a 2-generation study (Lieder et al., 2009b). →Klimisch reliability 1 - well documented, guideline study. No histological changes or abnormalities in bone marrow of male rats were observed at 600 mg/kg bw /day (Lieder et al., 2009a). *→Klimisch reliability 1 - well documented, guideline study.*

A study by 3M reported no significant hematological alterations in rats dosed with up to 900 mg/kg bw /day PFBS by gavage for 28 days (3M, 2001). A significant decrease in serum phosphorus and potassium levels in male rats that received 300 and 900 mg/kg bw/day was observed, giving a NOAEL value of 100 mg/kg bw/day for males. The decrease for potassium was approximately 20% of the control values at a level of significance of $p<0.05$ in both treatment groups. The decrease for phosphorus was approximately 16% of control values at a level of $p < 0.05$ in both treatment groups. Treatment of male rats with 900 mg/kg bw/day PFBS by gavage for 28 days induced a significant increase in absolute and relative liver weight (25–30%) relative to controls, which was no longer detected following a 14-day recovery period (3M, 2001). Clinical chemistry tests of liver function were unremarkable, and there were no chemical-related microscopic alterations. The NOAEL for liver weight effects was 300 mg/kg bw/day. Treatment of female rats with 900 mg/kg bw/day PFBS by gavage for 28 days caused a significant increase (9–11%) in absolute and relative kidney weight, but no significant alterations in the microscopic appearance of the kidneys was observed (3M, 2001). The weight of the kidneys returned to control levels following a recovery period of approximately 14 days. The NOAEL for kidney weight effects was 300 mg/kg bw/day PFBS (3M 2001, NICNAS 2005) *→Klimisch reliability 1 - guideline study.*

In the recent 28-day NTP study (NTP 2019) all dose groups consisted of 10 male and 10 female rats. All test compounds were administered in deionised water with 2% Tween 80 by gavage, 7 days per week for 28 days; control animals received the vehicle only. Doses were selected on the basis of a maximum tolerated daily dose and kinetic information obtained from toxicokinetic studies (Huang et al., 2019). PFBS was administered twice daily at one-half the dose for total daily doses of 0, 62.6, 125, 250, 500, or 1,000 mg/kg body weight. Compared to vehicle controls, there were dose-related and significant increases in the relative liver weight in 62.6 mg/kg/day males and absolute and relative liver weights in the 125, 250, and 500 mg/kg/day males. In females, there were dose-related and significant increases in the relative liver weight in the 125 mg/kg/day group and absolute and relative liver weights in the 250, 500, and 1,000 mg/kg/day groups. The organ weight changes in liver appeared to correlate with histopathologic changes observed in the liver. Male kidney weights (absolute and relative to body weight) were increased in the 500 mg/kg/day group. In females, dose-related and significant increases in the relative

right kidney weights occurred in all the dose groups. Absolute spleen, heart, and thymus weights in the 500 mg/kg/day females were significantly lower than those of the vehicle controls. The biological significance of these changes is not clear. In addition, a significant prolongation of diestrus was observed at the 250 mg/kg bw/day dose and the animals were reported to be not cycling at 500 mg/kg bw/day (NTP 2019). Vaginal samples were not collected from 1000 mg/kg bw/day group.

It is unclear if the lack of hepatocyte hypertrophy in the PFBS 90-day study versus the observation in the current study is due to once-daily dosing versus the twice-daily dosing (e.g., Cmax differences). There was no clinical pathology interpretation for the 1,000 mg/kg/day male and female rats due to the high mortality in these groups. Reduced growth of both male and female rats was also seen at the highest dose (NTP, 2019) *→Klimisch reliability 1 - guideline study.*

A humanised transgenic mouse model (APOE*3-Leiden.E3L CETP) which expresses a human-like lipoprotein profile was used to study the effect of PFBS on lipid metabolism. PFBS was administered daily to the transgenic male mice at 30 mg/kg bw/day for 4-6 weeks and resulted in a modestly reduced plasma triglycerides and cholesterol, and increased clearance of triolein (a triglyceride) (Bijland et al., 2011). Importantly, these mice had wild type PPARα which may still contribute to the higher expression levels and sensitivity of mouse PPARα to PFASs as compared to the human PPARα to lipid and lipoprotein metabolism.

4.4.1.3. In vivo data: inhalation

In a subchronic study, Crl:CD SD rats were exposed (whole body) to vapours of perfluorobutane sulfonyl fluoride (PBSF) for 6 hr/day, 5 days/week for 4 weeks (ECHA dissemination website). The mean analysed exposure concentrations of the chemical were 47, 162 and 459 ppm. Clinical signs observed immediately post exposure included vocalising and agitation when handled, walking on toes (abnormal gait) and hyperactivity, consistent with a neurological effect. These signs were transient and generally resolved the following day with no evidence of sustained neurotoxicity. There were no effects on haematological or blood chemistry parameters. Histopathological examination of the respiratory tract revealed no treatment-related findings (ECHA dissemination website[12]).

4.4.2. **Human information**

One low-confidence study (Zeng et al., 2015) used the controls from the case-control study of asthma described below (Dong et al., 2013) and examined the association between PFBS exposure and serum lipids. The models were adjusted for age, gender, bodymass index (BMI), parental education level, exercise and ETS (environmental tobacco smoking) exposure. There was a statistically significant increase in total cholesterol (β = 19.3 mg/dL increase per 1 µg/l increase in PFBS, 95% CI = 0.6−38.0, $p<0.05$) but when PFBS exposure was analysed in quartiles, no exposure-response gradient was observed.

10 different PFASs were analysed in cord plasma samples (N=687) collected in Shanghai between 2011 and 2012, a region widely polluted with PFASs in China. The PFAS levels were compared with different maternal factors, including BMI, and it was found an inverse association with PFUnDA and PFDoDA but a positive association with PFHxS and PFBS (Wang et al., 2016a).

[12] https://echa.europa.eu/registration-dossier/-/registered-dossier/10698/7/6/3

4.4.3. **Summary and discussion of repeated dose toxicity**

The 28-day and the 90-day oral rat study show effect on liver, kidneys, stomach and hematological systems as PFBS-targets of toxicity. A dose dependent decrease in hemoglobin and hematocrit levels were observed in male rats with NOAEL determined to be 60 mg/kg bw/day (based on haematological parameters). In the 90-day study epithelial hyperplasia was observed in the kidney, and necrosis and hyperplasia/hyperkerosis was observed in the forestomach, both in male and female rats at 600 mg/kg bw/day. The NOAEL is 200 mg/kg bw/day in this study based on these microscopic effects seen in kidney and forestomach. 3M reported a NOAEL of 300 mg/kg bw/day for increased kidney weight. Furthermore, an investigation on mice indicated modest changes in lipid metabolism after 4-6 weeks daily exposure to PFBS (30 mg/ kg bw/day). As supporting evidence for lipid metabolism distortion, an in vitro study showed that PFBS increased the adipogenesis of 3T3-L1 adipocytes resulting in significantly increased cellular triglyceride levels, in part, via MEK/ERK-dependent pathway. In addition, PFBS exposed hMSC led to elevated markers that may promote adipogenic differentiation. A zebra fish study also showed that PFBS can disrupt pancreatic organogenesis and perturb expression of genes involved in lipid metabolism (described in Chapter 5). These data together with human data linking PFAS to an increased BMI or changes in blood cholesterol or triglycerides, points towards a possible metabolic effect related to PFBS-exposure. However, other data in the literature may be less clear. Thus, the currently available data is so far insufficient for conclusion. Furthermore, a sub-chronic rat inhalation study with PFBS reported a transient effect on neurological parameters.

4.5. Mutagenicity

4.5.1. **Non-human information**

4.5.1.1. In vitro data

According to NICNAS (2005) the potassium salt of PFBS, KPFBS, was not found to be mutagenic to bacteria or clastogenic to Chinese Hamster Ovary-W-B1 cells under relevant test conditions. This is supported by the recent NTP study (NTP, 2019).

A study by Eriksen et al. (2010) examined possible PFAS-induced reactive oxygen species (ROS) generation and oxidative DNA damage in HepG2 cells (a liver cell line). PFBS did not induce any ROS or oxidative DNA-damage which is seen for PFOS and PFOA. Hence, the available information does not indicate that PFBS has in vitro mutagenic potential under testing conditions.

4.6. Carcinogenicity

4.6.1. **Non-human information**

4.6.1.1. In vivo data- Carcinogenicity: oral

There are no two-year carcinogenicity studies available for PFBS. In a repeated dose toxicity 90-day study, kidney hyperplasia was reported at 600 mg/kg bw/day. However, histopathological evaluation of the kidney by an independent expert reported that no consistent changes were seen in the kidneys (NICNAS, 2005; Lieder et al., 2009a). Increased incidence of necrosis in epithelial cells in the forestomach of male and female rats at 600 mg/kg bw/day was observed, including a thickening of mucosa due to hyperplasia and hyperkeratosis. In the two-generation study of Lieder et al. (2009b) treatment related microscopic changes were observed in the kidney of first generation

(F1) female rats in the 300- and 1000 mg/kg bw/day dosage groups. The changes were minimal to moderate severity of hyperplasia in the epithelium of the inner medulla at the dose groups mentioned, respectively. Taken together, a few repeated dose toxicity studies have reported treatment related hyperplastic changes in the kidney, increased hypertrophy and cytoplasmic alteration in the liver and necrosis in forestomach at high doses which may indicate a precursor to cancer development.

4.7. Toxicity for reproduction

4.7.1. **Effects on fertility**

4.7.1.1. Non-human information

From the 2-generation study conducted by Lieder et al. (2009b) parental-generation (P) rats were dosed orally by gavage with 0, 30, 100, 300 and 1000 mg KPFBS/kg bw/day for 10 weeks prior to and through mating (males and females), as well as during gestation and lactation (females only). F1 pups were dosed similarly, beginning at weaning. Second generation (F2) pups were not directly dosed but potentially exposed to PFBS through placental transfer and nursing. In this study, no effect on mating, fertility, sexual maturation of female pups (vaginal patency) or histological alterations in female reproductive tissues were observed. In P-generation females estrus cycling was unaffected by treatment. In F1 females, the number of animals with prolonged (≥6 days/21 days) diestrus was statistically significantly increased in the 100 mg/kg/day dose group ($p \leq 0.01$) and statistically significantly ($p \leq 0.05$) decreased in the 1000 mg/kg/day group when compared to control group (7/30, 10/30, 15/30, 7/30, 0/29, at 0, 30, 100, 300 and 1000 mg/kg bw/day, respectively). However, there were no statistically significant differences in the average numbers of estrus stages per 21 days among the F1 dose groups. The authors did not consider the statistically significant changes in the number of animals with prolonged diestrus treatment-related since they were not dose dependent and they had no effect on fertility and mating. However, a prolonged diestrus have been reported also in the mouse developmental study (Feng et al., 2017) described in 4.8.1 and in the rat 28 day NTP study (NTP 2019). Furthermore, modulation of estrus cyclicity in the mice developmental study may be considered relevant in the light of the thyroid hormone effects discussed below. There were no treatment-related microscopic changes in sex organs of males although a statistically significant decrease in the number of spermatids per gram testes and increased incidence of abnormal sperm were noted at the highest dose. However, this may be considered incidential since this was not seen in the F1-generation and was within historical variations. Mating and fertility parameters were seemingly unaffected in P and F1 generation (Lieder et al., 2009b). Furthermore, administration of up to 900 mg/kg bw/day PFBS to rats by gavage for 28 days did not cause any significant gross or microscopic alterations in primary or secondary sex organs from males or females (3M, 2001).

Caenorhabditis elegans (C. elegans) has been proposed as an alternative model to screen for reproductive toxicity in mammals (Bressers et al., 2018). It is however not officially accepted by EU regulatory bodies or validated by ECVAM. A recent study by Chen et al. (2018) shows that egg production and brood number of *C. elegans* decreased markedly following exposure to PFBS, although at a much higher exposure concentration than what was seen for PFOS. However, the internal concentrations of PFBS and PFOS were similar. The results indicate that PFBS exerts reproductive toxicity in *C. elegans* via germ cell apoptosis resulting from elevated levels of reactive oxygen species. However, since the relevance to mammalian toxicity has not been evaluated properly the data must be interpreted with care.

4.7.1.2. Human information

In China and Taiwan, two studies examined different reproductive outcomes in women and men (Song et al., 2018; Zhou et al., 2016). Biomonitoring of PFAAs in blood and semen samples from 103 male participants from Guangdong province in China was performed cross- sectionally to investigate potential link between PFAAs exposure and semen mobility in China. Negative correlations were significantly observed between sperm motility and PFBS concentrations in semen ($p<0.01$), as well as for other PFASs in semen. (Song et al., 2018). Another study, (Zhou et al., 2016) in 225 Taiwanese adolescents, looked at associations between PFBS levels and reproductive hormones and they reported no clear correlations in males or females. However, both of these studies are considered of low-confidence and low sensitivity due to low exposure levels of PFBS and low participant numbers, and thus there is a lack of clear associations.

4.8. Developmental toxicity

4.8.1. **Non-human information**

In a two-generation reproductive toxicity study in rats (OECD 416) administered 0, 30, 100, 300 and 1000 mg/kg bw/day of potassium PFBS (as reported above in more detail), there was no clear evidence of adverse effects on reproduction, fertility and lactation at the highest dose tested of 1000 mg/kg bw/day (Lieder et al., 2009b). The NOAEL for reproductive toxicity in males and female P (parent) generation is 1000 mg/kg bw/day. The P generation male and female rats LOAEL toxicity was 300 mg/kg bw/day based on treatment-related microscopic changes in the kidney and liver for P-generation males and kidneys for P-generation females, respectively. The mean number of liveborn F1 pups was statistically significantly decreased in the 30 mg/kg bw/day group, but this change was not dose-dependent. The viability index in F1 pups and the lactation index in F1 and F2 pups showed statistically significant changes at various doses but were not dose-dependent (Lieder et al., 2009b). Similarly, no clear effects were observed in delivery and litter parameters (e.g., implantations, litter sizes, live foetuses, corpora lutea, and early resorptions) following prenatal exposure from gestational day (GD) 6 to 20 (Lieder et al., 2009b). There were no significant effect on sexual maturation in female offspring (vaginal opening) or development of sex organs in either sex. In F1 males preputial separation was statistically significantly delayed at 1000 mg/kg, but this delay was attributet to body weight reduction in this dose group. F1 litter developmental NOAEL was 300 mg/kg bw/day based on body weight effects for males in the high dose group. The F1 generation male and female rats LOAEL was 300 mg/kg bw/day based on treatment-related microscopic changes in the kidney and liver for males and kidneys for females. No adverse effects were observed in F2 pups in doses as high as 1000 mg/kg bw/day. → *Klimisch reliability 1- well documented, guideline study*.

A recent publication, reporting a non-guideline study performed by Feng et al. (2017), describes effects on dams and offspring mice. Dams were exposed to potassium PFBS (50, 200 and 500 mg/kg bw/day) administrated orally from GD1-GD20. Only effects on female offspring were reported. The study was conducted on twelve week old ICR Mice (China), 30 dams in each dose group were randomly signed to one of 3 experimental groups examening; 1.survival, growth, pubertal onset and overian and uterine development (50 offspring/10 dams), 2.Thyroid- and gonadal hormone measurements in offspring: Postnatal day (PND) 1 (n=30), PND30 (n=10), PND60 (n=10) and group 3. Levels of serum PFBS were measured (n=10 dams). Pregnant mice were housed individually in nesting boxes and administered PFBS orally in a volume of 150 µl from GD1 to GD20. Dosages were adjusted daily for BW changes. Dams did not exhibit fetal loss or abnormal behavior during the administration of PFBS. The pups were born by natural delivery and housed in the same stainless steel cages with their dams under the same laboratory conditions. On PND 21, all offspring were weaned. Female offspring were transferred to other cages (2–4 per cage). Their female offspring (PFBS-offspring) were born alive and

were pink in color. The numbers of neonatal PFBS-offspring in the 50 mg/kg (9.8 ± 0.51, n=10 dams), 200 mg/kg (11.5 ± 0.54, n=10 dams), and 500 mg/kg (12.1 ± 0.57, n=10 dams) dosage groups were not significantly different from that in the control group (10.8 ± 0.63; P>0.05;n=10 dams). Compared with control offspring, the PFBS-offspring (in the 200 and 500 mg/kg bw/day groups) remained underweight throughout the weaning (P<.01, n=40/10;) and pubertal periods, PND36 (P<.01, n=40/10). Male offspring effects were not reported. A statistically significant delay in eyes opening, vaginal opening, and first estrous was observed in the two highest dose (200 and 500 mg/kg bw/day).

Ovarian and uterine size (% of BW), as well as follicle and corpus luteum numbers were significantly reduced in the 200 and 500 mg/kg bw/day dose group (P<.05, n=10/10) in adult PFBS exposed offspring (PND 60) compared to controls. They exhibited fewer primordial follicles, primary follicles, secondary follicles, early antral follicles, antral follicles, and preovulatory follicles, as well as fewer corpora lutea at both 200 and 500 mg/kg bw/day compared to control offspring (P<.05, n=10/10) at diestrus (Feng et al., 2017). Importantly, no effect on body weight gain of dams exposed to PFBS was observed indicating that PFBS did not cause marked general toxicity in dams at any dose (Feng et al., 2017). Notably, PND40–60 PFBS-offspring (200/500 mg/kg bw/day) exhibited a prolongation of diestrus compared with control (P<.05, n=30/10) Furthermore, pubertal and adult PFBS-offspring exhibited statistically significant (P<.05) decreases in serum estrogen (E2) at 200/500 mg/kg bw/day at both PND 30 and 60 and progesterone (P4) levels at the same doses but only at PND60, with the elevation of luteinising hormone levels at PND30. These results indicate that prenatal PFBS exposure (≥200 mg/kg bw/day) causes delay in pubertal onset and reproductive organ development in female offspring mice. In addition, total T4 and total triiodothyronine (T3) was reduced significantly at 200 and 500 mg/kg bw/day (P<.05) in offspring at PND1, 30 and 60 compared to controls, with an increase in thyroid stimulating hormone (TSH) at the same exposure doses at PND30. In addition, PFBS-dams exhibited statistically significant (P<.05 and 0.01) decreases in total T4 and T3 levels and free T4 levels and increases in TSH levels, but no changes in E2 and P4 levels (described in Section 4.11.2). Serum-PFBS level were measured to be 74, 332 and 720 ng/mL at GD20 in dams exposed to 50, 200 and 500 mg/kg bw/day respectively. Taking into account that serum thyroid hormone levels were reduced also in dams in the absence of marked general toxicity, this indicate that prenatal PFBS exposure (≥200mg/kg/day) causes permanent hypothyroxinemia accompanied by deficits in perinatal growth, pubertal onset, and reproductive organ development in female mice. → *Klimisch reliability 2- well documented, not a guideline study*.

4.8.2. **Human information**

A recent study reported that prenatal exposure to PFBS was positively associated with the risk of preeclampsia and overall hypertensive disorders of pregnancy (HDP) (Huang et al., 2019). PFASs were measured by liquid chromatography system coupled with tandem mass spectrometry in 687 umbilical cord plasma samples collected between 2011 and 2012 in Shanghai, China. Information on HDP including gestational hypertension and preeclampsia was collected from medical records. In this fully adjusted statistical model, PFBS from cord blood plasma had an increased odds of preeclampsia [adjusted odds ratio (AOR): 1.81, 95% CI: 1.03–3.17], and overall HDP (AOR: 1.64, 95% CI: 1.09–2.47). However, this study found that preeclampsia was not associated with PFOA and PFOS, which were different from that found in the US-based C8 Health Project which reported a probable link between PFOA and pregnancy-induced hypertension. In addition, PFBS was measured in cord plasma and not maternal plasma which may indicate a reverse causation since preeclampsia might affect liver and kidney functions leading to less secretion of PFBS and more accumulation in the body. Due to the short carbon-chain of PFBS high transmission through the placenta might be expected and cord plasma level may well reflect maternal level. In a recent human mother-child cohort study (Gao et al., 2019) similar

concentrations of PFBS were detected in both cord blood and maternal blood, and in 70% of the cases PFBS was detected in both the cord and respective maternal sample.

Chen et al. (2019b) investigated the association between prenatal exposure to PFASs, including PFBS, and childhood adiposity at 5 years of age in a birth cohort study in Shanghai. The study involved 1140 pregnant women from 2012 to 2017. The median concentration of PFAS in the cord plasma ranged from 0.05 (PFBS) to 6.74 ng/mL (PFOA). The findings indicated that in girls, PFBS had a significant positive association with waist circumference and waist to height ratio in a multivariable linear regression model. Girls in the highest tertile of PFBS concentrations had more fat mass, as well as higher body fat percentage, waist circumference, and waist to height ratio compared to those in the lowest tertile. The authors concluded that high prenatal exposure to PFBS may be of concern for childhood adiposity.

4.8.3. **Summary and discussion of reproductive toxicity**

The two-generation reproduction study in rats, performed in accordance with OECD TG 416 (2001), indicated that PFBS exposure has a low potential to produce effects on fertility, reproductive organs or development in rats under the testing conditions used. Thyroid-hormones were not measured in this rat study. No clear adverse effects on functional aspects of reproduction were observed in either generation. In both the P- and F1-generation males, minimal to mild hepatocellular hypertrophy in males and histomorphological changes in the kidney in both males and females was observed microscopically in the 300 and 1000 mg/kg bw/day KPFBS dose groups. This study has a Klimisch reliability of 1. However, prenatal exposure to PFBS in a mouse developmental study examining effect in dams and female offspring indicated reproductive toxic effects on several parameters such as delay in perinatal growth and pubertal onset in addition to changes in reproductive organ development from 200 mg/kg bw/day, assessed until PND60. This study has a Klimisch reliability of 2. The marked effects on F1 ovary weight and histopathology in the mouse study was not observed in the rat 2-generation study. The rat study did not observe any delay in pubertal onset either, as seen in the mice study. The reason for these differences is unclear. There were some differences in the study set up and the endpoints examined and reported perhaps explaining the deviations (i.e. pituitary gonadal or thyroidal hormones, uterus weight, corpus luteum numbers was reported in Feng et al., 2017 but not by Lieder et al., 2009b). Notably, in both studies, number of offspring with prolonged diestrus was increased, although in the 2-generation study this was only observed at the 100 mg/kg bw/day dose and there was a significant decrease in the number of offspring with prolonged diestrus at high dose group compared to control offspring. A possible PFBS-mediated prolonged diestrus is supported by the 28 day rat NTP-study, were a significant prolongation of diestrus was observed at the 250 mg/kg bw/day dose and the animals were reported to be not cycling at 500 mg/kg bw day (NTP 2019). This study has a Klimisch reliability of 1. Sprague Dawley rats were used in both the 2-generation study and the 28 day study. Clear developmental effects was observed in the mice study while no clear developmental effects was seen in the 2-generational OECD guideline rat study.

4.9. Other effects: Immunotoxicity

4.9.1. **Non-human information**

4.9.1.1. In vitro

The release of the pro-inflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) was evaluated in liposaccharide stimulated primary human lymphocytes and in the THP-1 cell line, while the release of IL-10 and IFN-γ was evaluated in PAH stimulated human peripheral blood lymphocytes (Corsini et al., 2012). After exposure to

several PFAS, TNF-α production was suppressed, and PFBS showed to inhibit IL-10 release. Mechanistic investigations demonstrated that inhibition of TNF-α release in THP-1 cells occurred at the transcriptional level. All PFAS, including PFBS, decreased LPS-induced NF-κB activation. None of the PFAS tested, except PFOA, were able to activate PPARα driven transcription in transiently transfected THP-1 cells, excluding a role for PPARα in the immunomodulation as observed. PFBS and PFDA prevented LPS-induced I-κB degradation. Overall, this study suggests that PFAS, including PFBS, affect NF-κB activation, which directly suppresses cytokine secretion by immune cells.

The US EPA ToxCast program has tested KPFBS. A positive activation of the G-protein coupled receptor (GPCR) LTB4r (Leukotriene receptor B4) was noted for KPFBS. This GPCR NovaScreen assay reported in ToxCast, assess binding to the guinea pig LTB4r. Several other PFASs (i.e. PFOS, PFHxS, PFOA, PFHxA) show binding to this receptor in ToxCast as well. The functional relevance of this finding is still unclear, however it is known that this receptor is involved in pro-inflammatory responses and inhibition of this receptor is used in asthma prescribed drugs.

4.9.1.2. Respiratory effects in rats

Administration of PFBS at gavage doses of up to 900 mg/kg bw/day for 28 days or 600 mg/kg bw/day for 90 days had no significant effect on the gross or microscopic morphology of the lungs and trachea in rats (3M, 2001; Lieder et al., 2009a); no increases in nasal lesions were observed in the 90 day study (Lieder et al., 2009a).

4.9.2. **Human information**

Associations between different PFAS and various infectious diseases (i.e. common cold, lower respiratory tract infections, otitis media, pneumonia, respiratory syncytial virus infection and/or varicella) in children have been reported (Granum et al., 2013; Pennings et al., 2016; Dalsager et al., 2016; Goudarzi et al., 2017; Impinen et al., 2018). However, PFBS was not among the PFAS in these studies.

The relationship between PFAS and asthma- and allergy related outcomes has been investigated in several studies. The findings were not consistent between the different studies and PFBS was not a part of most of the investigations (Granum et al., 2013; Impinen et al., 2018; Smit et al., 2015). However, a case-control study on 231 asthmatic children and 225 non-asthmatic control children (aged 10–15 years) from Northern Taiwan showed that asthmatics had significantly higher serum PFAAs concentrations compared to the healthy controls (Dong et al., 2013). The adjusted odds ratios (OR) for asthma among those with the highest versus lowest quartile of PFAS exposure ranged from 1.81 (95% CI: 1.02, 3.23) for PFDoA, 1.90 (95% CI 1.08–3.37) for PFBS to 4.05 (95% CI: 2.21, 7.42) for PFOA. When stratified by gender, a greater number of significant associations between PFAAs and asthma outcomes were found in males than in females. Among males, adjusted odds ratios for asthma among those with the highest versus lowest quartile of PFAAs exposure ranged from 2.59 (95% CI: 1.14, 5.87) for the PFBS to 4.38 (95% CI: 2.02, 9.50) for PFOS.

To evaluate the association between serum PFAA concentrations and T-lymphocyte-related immunological markers of asthma in children, and to assess whether gender modified this association, serum concentrations of ten PFAAs and serum levels of TH1 [interferon (IFN)-γ, interleukin (IL)-2] and TH2 (IL-4 and IL-5) cytokines were measured (Dong et al., 2013; Zhu et al., 2016). The results showed that serum PFAAs, including PFBS, were associated positively with TH2 cytokines and inversely with TH1 cytokines among male asthmatics. In addition, a 16-kDa club cell secretory protein (Clara) (CC16), which plays a key role in anti-inflammatory and antioxidant functions in epithelial cells and a prominent biomarker for asthma, was measured in a later study (Zhou et al., 2017). A negative association between several PFAAs, except PFBS, and CC16 was observed. When effects of

multiplicative interactions between asthma and individual PFAAs on the CC16 outcome were estimated among children, a marginally significant interaction effect ($p < 0.15$) on CC16 levels was found between asthma and PFOS, PFOA, PFBS and PFBS in all participants.

4.9.3 Summary and discussion of immunotoxicity

Taken together, *in vitro* studies as well as epidemiological studies indicate an association between PFAS and different immunological outcomes, with lower potency for the shorter chain PFBS. Only very few studies have analysed PFBS among the PFAAs and results show a weak relationship between PFBS and asthma-related outcomes. As immunotoxicity is an effect of increasing concern across several members of the larger PFAS family, the lack of studies evaluating this outcome following PFBS exposure is a limitation in the database.

4.10. Other effects: Neurotoxicity

4.10.1. Non-human information

4.10.1.1. In vitro data

In vitro studies exposing transformed neuronal PC12 cells (a standard in vitro model for neuronal development) with different fluorinated compounds showed that all compounds tested had an adverse effect on neuronal development of these cells, seemingly through different mechanisms (Slotkin et al., 2008). The rank order of adverse effects on PC12 cells was PFOSA>PFOS>PFBS≈PFOA. In the case of PFBS, PC12 cell differentiation was supressed for two different neurotransmitter phenotypes. PFBS, unlike other fluorinated chemicals, induced a concentration dependent reduction of ChAT (choline acetyl transferase) and TH (tyrosine hydroxylase) activity needed for differentiation into two distinct neurotransmitter phenotypes, i.e. acetylcholine (Ach) and dopamine (DA), respectively.

A study by Liao et al. (2009) shows that PFASs exhibit adverse effects on cultured primary rat hippocampal neurons to various extents, which was generally dependent on the carbon chain length and functional group attached to the fully fluorinated alkyl chain. PFBS had a significant effect on calcium homeostasis by increasing voltage dependent calcium influx through calcium channels after exposure to high concentration (100 µM). In addition, exposure to 50 µM PFBS disturbed neuronal developmental processes by significantly reducing the total length of neurites and changing the pattern of the number of bifurcated neurites.

4.10.1.2. In vivo data

The 90-day oral rat study (test guideline OECD 408) of potassium PFBS included a FOB (Functional Observational Battery) and motor activity assessment but not a peripheral neuropathy assessment per se. The authors did not find any significant alterations in motor activity or performance on functional observation tests at doses up to 600 mg/kg bw/day (Lieder et al., 2009a). However, neurological alterations were reported in an oral 28-day study in rats (NTP, 2019), where treated males differed from control males in a decrease in tail flick, rotorod latency and foot splay, but did not exhibit a dose-response at the doses tested. In contrast, treated females exhibited an increase in rotorod latency.

A recent study examined long-term potentiation (LTP) in rats after exposure to PFOS and its alternatives, such as PFBS, aiming to provide some evidence about the potential to affect cognitive ability. Different dosages of PFOS, PFHxS, PFBS and chlorinated polyfluorinated ether sulfonate (Cl-PFAES), were dosed to rats via acute intracerebroventricular injection. The field excitatory postsynaptic potential (fEPSP) amplitude of the input/output functions, paired-pulse facilitations, and LTP in vivo were

recorded. PFOS and its alternatives including PFBS inhibited LTP in varying degrees without significant effects on the normal synaptic transmission. There was a higher potency of PFHxS and PFOS than PFBS to inhibit LTP and points to the possibly higher neurotoxicity potential of the long carbon chain perfluoroalkyl compounds. The results suggest that acute exposure to PFOS and its alternatives, including PFBS, impaired the synaptic plasticity by a postsynaptic rather than a presynaptic mechanism (Zhang et al., 2016).

4.10.2. Summary and discussion of neurotoxicity

In summary, there are only very few studies providing some evidence that the nervous system is a sensitive target for PFBS. Clinical observations and morphological examinations of the nervous system in the 28-day study reported a decrease in tail flick latency test in males after PFBS exposure and acute PFBS exposure of rats had an inhibiting effect on LTP (although at a lower potency than PFOS) which indicate a potential to affect cognitive ability. In addition, PFBS has shown to disturb neuronal development in vitro in two separate studies. However, more studies are needed to verify the significance of these results.

4.11. Other effects: Endocrine disruption

4.11.1. *In vitro*

An *in vitro* PFAS-exposure study on human cell lines such as MCF-7, H295R, LNCaP and MDA-kb2 was set up in order to study the underlying mechanisms of the reproductive toxic effects and in particular study the endocrine properties of different PFASs including PFBS (Behr et al., 2018). The obtained in vitro data suggested that the PFASs examined in this study displayed either a weak or no (anti)-estrogenic and no (anti)-androgenic activity in human cells and had only weak impact on steroid hormone secretion as well as on steroidogenic gene expression. The steroidogenesis assay in H295R-cells was conducted following OECD TG 456. Effects were predominantly observed for PFOA and PFOS and not for their substitutes. Moreover, these effects were only observed at high concentrations, at least three to four orders of magnitude above human blood serum concentrations which are in the range of about 10 nM. PFBS was not found to have (anti)-estrogenic or (anti)-androgenic activity or effects on steroid hormone secretion or steroidogenic gene expression at concentrations up to 10 µM. The authors concluded that PFASs do not affect estrogen or androgen receptor signalling or steroid hormone biosynthesis at concentrations relevant to human exposure.

PFASs have been shown in vitro to disturb steroidogenesis in human placental choriocarcinoma cell line (JEG-3) by inhibiting CYP19 aromatase gene expression, increasing several membrane lipids (Gorrochategui et al., 2014). The study highlights the ability of the PFAS mixture to alter cellular lipid pattern at concentrations well below those that generate toxicity, and the potential for PFBS to significantly inhibit aromatase activity in placental cells (IC50 of 68 µM).

The US EPA ToxCast program has tested potassium PFBS (KPFBS) for its ability to disrupt androgen, estrogen, thyroid or stereoidogenesis pathways using a screening battery of endocrine bioactivity assays. ToxCast summary showed no significant activity in the tests conducted on these parameters.

In vitro studies are useful as supporting information and may suggest that the tested chemical acts via a particular mode of action. However, in vitro assays do not reflect the true complex hormonal axis as present in a living organisms and results must be interpreted with care. In addition, several important endocrine mechanisms, e.g. additional mechanisms for disruption of thyroid hormone signaling, are currently not covered in the ToxCast assays.

4.11.2. ***In vivo***

In the 28-day rat study by 3M in 2001 (3M, 2001) the thyroid weight and histology was reported to be normal after PFBS exposure. However, no thyroid hormones were measured. In the 90-day and two-generation reproductive rat study with KPFBS by Lieder et al. (2009a and b) (reported in Sections 4.4 and 4.8, respectively), thyroid hormones were not measured either. They reported however that no effects of PFBS were seen on thyroid histology in the 90-day rat study. Thyroid weight was not reported in the 90-day study nor in the 2-generation study, although it is recommended in the OECD 416 guideline. Additional requirements on thyroid measurements have been added to the updated OECD guideline for the 90-day study from 2018. One statistical significant finding in the 2-generation study that may relate to endocrine disruption (ED) was an increase in the number of pups showing ≥6 days of diestrus cycle at the 100 mg/kg bw/day, although this was not dose response related. → *Klimisch reliability 1- well documented, guideline studies according to the guidelines used.*

In the 28-day PFBS exposure study by NTP (2019), statistically significant dose-dependent decreases in total T3, total T4, and free T4 levels were reported in both male and female rats at all doses tested ≥62.6 mg/kg bw/day (PFBS doses tested: 0, 62.6, 125, 250, 500, 1,000 (mg/kg bw/day). The reported reductions in total T3 were up to −57% and −43% in male and female rats, −86% and −77% in free T4, and −97% and −71% in total T4, respectively. Similar effects were evident for the other sulfonates tested (PFOS and PFHxS), as total thyroxine (T4), free T4, and total T3 largely decreased in a dose-response manner. In general, the magnitude of the effect was stronger in PFBS and PFOS rats compared to the PFHxS exposed rats. Of note, the T4:T3 ratio appeared to be reduced in all groups of PFBS-exposed rats based on the data presented in the summary Table 7 in NTP (2019) suggesting an increased rate of T4 to T3 conversion.

Although the thyroid hormone levels decreased in a dose-response manner in the 28 day study (NTP, 2019), TSH concentration was not consistently increased (although a small trend was seen) across the chemicals or sexes. Thyroid gland weight and thyroid histopathology were not changed after 28 days of PFBS exposure in male or female rats at up to 1,000 mg/kg bw/day (NTP, 2019). However, in this timeframe of exposure (28 days), the lack of significant abnormalities related to thyroid weight and histopathology is not unexpected. However, one would have expected a greater elevation of serum TSH than what was observed for this degree of hypothyroidism within this timeframe. In rodent studies, a similar "enigmatic" pattern of reduced total and free serum T4, without corresponding increases in TSH, are observed also for other PFASs as well as for some polybrominated diphenyl ethers and polychlorinated biphenyls (European Commission, 2017). These observations underline the complexity of regulation of thyroid hormone homeostasis. In the absence of endpoints sensitive to thyroid hormone disruption, as neurodevelopmental testing or thyroid hormone effects at the level of the target organs, it is difficult to evaluate the functional consequences of the thyroid hormone reductions. However, the marked reduction in thyroid hormones represents a clear concern, in particular for developmental neurotoxicity.

It has been shown that PFAS can bind to serum proteins including albumin and TTR, TTR being the main transport protein for thyroxin in adult rats. A study evaluating the competitive binding of various PFASs to TTR was done by Weiss et al. (2009) and confirmed the ability of these sulfonates to bind to TTR, with PFBS having a lower binding capacity than the longer carbon-chain counterparts being more than 100 times less potent than T4. It has been argued that the measured reduction in free T4 could be related to the assay used. It has been shown for PFOS that displacement of T4 from carrier proteins may affect the measurement of free T4 when using the RIA assay and not the preferred equilibrium dialysis-analog radioimmunoassay, ED-RIA (Chang, 2007). That the observed marked reduction in serum T4 is mainly caused by its displacement from TTR is not

obvious. This argument is supported by the relatively low PFBS concentrations (154 ng/ml) in the low dose female group in which the total T4 concentration was reduced by 50% (from 3.1 to 1.48 µg/dl) in the NTP study (2019). Nevertheless, total T4 and T3 were also reduced and the serum T4/T3-binding proteins serve as an extrathyroidal storage of thyroid hormones and this contributes to the control of thyroid hormone homeostasis.

However, alternatively, the decreases in total T4 and T3 may be related to activation of the constitutive androstane receptor (CAR) that regulates the expression of thyroxine-UDP-glucuronosyltransferase and may thus accelerate degradation of thyroxine by the liver. An increased rate of thyroid hormone excretion by PFBS is suggested by the marked upregulation of the two CAR-activity marker genes (*Cyp2b1* and *Cyp2b2*) included in the NTP 2019 study. It is noteworthy that PFHxS-K had a lower response in CAR activity with a lower effect in thyroid hormones (NTP, 2019) → *Klimisch reliability 1- well documented, guideline study*.

The concern for thyroid disruption is strengthened by the study of Feng et al. (2017). This study, described in more detail in Section 4.8.1, showed that when KPFBS (200 and 500 mg/kg bw/day) was orally administered to pregnant mice (10 dams in each group) on gestational days 1–20, their female offspring exhibited a statistically significant decrease in perinatal body weight gain, delay in eye opening, significant delay of vaginal opening and prolonged first estrus and diestrus. The ovarian and uterine size, as well as follicle and corpus luteum numbers were reduced in adult offspring. Furthermore, pubertal and adult offspring exhibited decreases in serum estrogen (E2) and progesterone (P4) levels with the elevation of luteinising hormone levels. Notably, decreases in serum total thyroxine (T4) and T3 levels were observed in neonatal, pubertal, and adult offspring in conjunction with slight increases in thyroid-stimulating hormone (TSH) and thyrotropin-releasing hormone levels. In addition, dams exhibited a significant decrease in total T4 and T3 levels and free T4 levels and increases in TSH levels, but no changes in E2 and P4 levels. The body weights of GD20 PFBS-dams were not different from those of control dams, irrespective of dosage. These results indicate that prenatal PFBS exposure (≥200 mg/kg bw/day) may cause hypothyroxinemia accompanied by deficits in perinatal growth, pubertal onset, and reproductive organ development in female mice. → *Klimisch reliability 2- well documented, non-guideline study.*

In support for this, both PFHxS (Ramhøj et al., 2016) and PFOS (Yu et al., 2009; Lau et al., 2003) have been found to reduce total T3 and/or total T4 level(s) in rodents leading to hypothyroxinemia in pups. PFHxS dose-dependently decreased dam T4 level on GD15 and pup T4 and T3 levels on PND 16/17 from 5 mg PFHxS/kg/day while gestational exposure to PFOS (3.2 mg PFOS/kg) alone significantly decreased T4 levels in pups on PNDs 21 and 35. Postnatal exposure to PFOS alone also induced T4 depression on PNDs 21 and 35. Thus, there seems to be a decrease at least in thyroxine (T4) levels in both offspring and dams exposed to either PFOS (Yu et al., 2009), PFHxS (Ramshøj et al., 2016) or PFBS (Feng et al., 2017) with decreasing potency from PFOS to PFBS. The thyroid hormones are apparently common targets for PFBS, PFHxS-K, and PFOS as also seen in the NTP-study, and similar effects were observed with the PFAS carboxylates. Thus, given the importance of normal thyroid hormone levels in multiple systems, the significant reduction of thyroid hormone availability can justifiably be considered a critical effect of PFBS exposure.

The regulation of maternal thyroid hormone levels is known to be a crucial element during developmental life stages as foetal neurodevelopment is known to be dependent on maternal production of thyroid hormones. Low maternal thyroid hormone levels may lead to neurodevelopment and growth deficiencies in pups. The PFBS database is limited by the lack of developmental neurotoxicity test data that could have strengthened the thyroid deficiency adversity evaluation. It should thus be recognised that disturbances of thyroid hormones raise specific concerns as a number of important physiological functions (growth, metabolism, brain deveopment) are dependent on a normal thyroid hormone

axis. The offspring of mice exposed during gestation to PFBS (dams that experienced decreased thyroid hormon levels), exhibit decreases in serum estrogen and progesterone levels with the elevation of luteinising hormone levels and deficits in perinatal growth, pubertal onset, and reproductive organ development in females (Feng, 2017). Thyroid hormones are essential for normal development and the regulation of basal metabolism (Jomaa, 2015). In addition, the review of Choksi et al. (2003) concludes that developmental hypothyroidism alters female reproductive tract development in rats. Low thyroid hormone levels may thus delay perinatal growth, pubertal onset, and disturb reproductive organ development in female mice which in fact was observed in Feng et al., 2017. The observed PFBS-mediated reduction in TT4 in several studies may perhaps be more challenging and problematic for the foetus compared to adults. As the foetus is dependent on T4 and T3 from the maternal production it is important that the circulating protein-bound T4 depot is adequate.

The effects of PFBS and its salts via thyroid hormone disturbances are not fully characterised, due to their possible large scope as well as to the insufficient capacity of regulatory tests to explore these functions in detail. It is also noticed that the ECHA and EFSA (2018) guidance states that "Using the current understanding of thyroid physiology and toxicology (European Commission, 2017), it is proposed that the following be applied when interpreting data from experimental animals: […]2). Substances that alter the circulating levels of T3 and/or T4 without histopathological findings would still present a potential concern for neurodevelopment". ECHA and EFSA (2018) guidance, developed in the context of pesticides/biocides (i.e. not REACH specific), states also that in the absence of substance-specific data which provide proof of the contrary, humans and rodents are considered to be equally sensitive to thyroid-disruption (including cases where liver enzyme induction is responsible for increased thyroid hormone clearance. There is therefore a concern that a safe level cannot be established with sufficient certainty regarding PFBS and its salts. In particular this relates to effects on the developing foetus and with regard to life-long exposure.

4.11.3. **Summary**

The available in *vitro* studies as well as ToxCast screening show little or no support that the KPFBS substance acts via the endocrine pathway. However, *in vivo* studies give supporting evidence for possible endocrine disturbing properties of PFBS.

In mice, a decrease in both T3 and T4 accompanied by moderate increases in TSH was seen after prenatal PFBS-exposure, together with deficits in perinatal growth, pubertal onset and reproductive organ development at doses ≥200 mg/kg bw/day (Feng et al., 2017). These decreases were of a concerning magnitude in both dams and offspring and they were shown to persist at least 60 days after gestation. Unfortunately, levels of thyroid hormones were not measured in the two-generation reproductive rat study by Lieder et al. (2009b). However, in a recent 28-day study, marked decreases in total T3, total T4, as well as in free T4 were observed in female and male adult rats at doses ≥62.6 mg/kg bw/day (NTP, 2019). These thyroid hormone reductions occurred in the absence of changes in TSH or thyroid pathology, a pattern that is also reported for other PFASs as well as for PBDE and some PCBs (European Commission, 2017).

Taken together, there is sufficient evidence from different *in vivo* studies in both mice and rats that the substance has the potential to disturb thyroid hormone homeostasis in intact organisms as the findings seem to be consistent. However, there is a lack in the available database of studies specifically addressing thyroid hormone sensitive endpoints that could give more information on the adversity of the hormone reductions. In vitro studies are useful as supporting information and may suggest mode of action but do not reflect the true complex hormonal axis as present in a living organism. In addition, several important

endocrine mechanisms, e.g. for thyroid disruption, are currently not covered in the in vitro ToxCast assays. No information concerning these endpoints in humans is available, however, animal models are considered informative for evaluating the potential for thyroid effects of chemicals in humans (Zoeller et al., 2007). The thyroid hormonal axis seems therefore to be a potential target for PFBS toxicity in animals as well as in humans.

4.12. Summary and discussion of human exposure and health hazard assessment

In blood PFBS binds to albumin, is transported through the body and is found in many different tissues. PFBS has also been detected in human cord blood. PFBS binds to a number of other proteins, including receptors. A chain-length of C4 is generally reported to have a low binding affinity to albumin and other proteins, however, PFAAs containing the sulfonic acid group may bind stronger to proteins than their carboxylic acid counterpart. PFBS may also bind to several different transporter polypeptides (NTCP, ASBT and OATPs) which are all capable of contributing to the enterohepatic circulation and thus extended human serum elimination half-lives of PFBS and other PFAAs.

Since PFBS has a relatively high aqueous solubility, it can be found in different crops and in drinking water, and it can be taken up in the food chain, which may result in increased exposure. Household dust has also a relevant source of exposure for humans to PFBS.

Concentrations of PFBS in human blood have been determined in a number of studies. In the studies many samples were below the quantification limit, and in most studies the maximum concentration was below 0.5 ng/mL. However, in some studies concentrations up to around 5 ng/mL were observed. Studies of people with known elevated exposure to PFBS, for example via drinking water or in an occupational setting, have demonstrated that increased exposure leads to higher PFBS concentrations in the blood, even though the half-life of PFBS is relatively short (around 1 month) compared to for instance PFOA (2 – 4 years). Table 29 shows an overview of health effects caused by PFBS.

Table 29: Overview of PFBS-induced effects in rodents

Target organ	Type of effect	NOAEL/LOAEL	Reference	Klimisch score
Liver	Weight increase/ hepatocyte hypertrophy, cytoplasmic alteration. Lesions were minimal to mild severity, increased liver enzymes (ALT, ALP, AST)	LOAEL: 62,6 mg/kg bw/day for female NOAEL: 62,6 mg/kg bw/day for male	NTP, 2019	1
	Weight increase	LOAEL: 600 mg/kg bw/day NOAEL: 300 mg/kg bw/day	3M, 2001 Lieder et al., 2009a	1
	Modest changes in lipid metabolism	LOAEL: 30 mg/kg bw/day	Bijland et al., 2011	3
Kidney	Absolute and relative kidney weight increase in the highest dose group for male and relative kidney weight was increased in all dose groups for female, papilla necrosis at the highest dose.	NOAEL: 250 mg/kg bw/day for male LOAEL 62,5 mg/kg bw/day for female	NTP, 2019	1
	Absolute and relative kidney weight, hyperplasia was observed in the kidney, and necrosis and hyperplasia/ hyperkerosis was observed in the forestomach, both in male and female rats	LOAEL: 600 mg/kg bw/day NOAEL: 200 mg/kg bw/day	Lieder et al., 2009a	1
Haematologic system	Decrease in hemoglobin and hematocrit levels	LOAEL: 200 mg/kg bw/day NOAEL (male): 60 mg/kg bw/day	Lieder et al., 2009a	1
Repro/Developmental Tox	Delay in perinatal growth, pubertal onset vaginal opening and first estrus cycle, and diestrus was prolonged in F1	LOAEL: 200 mg/kg bw/day NOAEL: 50 mg/kg bw/day	Feng et al., 2017	2
	Rat F1 litter developmental NOAEL was based on body weight effects for males in the high dose group. The F1 generation male and female rats LOAEL was 300 mg/kg bw/day based on treatment-related microscopic	NOAEL: 300 mg/kg bw/day	Lieder et al., 2009b	1

Target organ	Type of effect	NOAEL/LOAEL	Reference	Klimisch score
	changes in the kidney and liver for males and kidneys for females Females displayed abnormal cyclicity in the 250 mg/kg bw/day dose group with respect to diestrus and females in the 500 mg/kg bw/day dose group were not cycling	NOAEL 125 mg/kg bw/day LOAEL 250 mg/kg bw/day	NTP 2019	1
Endocrine effects	Decrease in thyroid hormones: freeT4, total T4 and T3 Decrease in total T4 and T3 and free T4 levels and an increases in TSH levels dams and a decrease in total T4 and T3 at PND 0, 30 and 60 and an increase in TSH at PND30 in pups (Feng et al., 2017)	LOAEL: 62.6 mg/kg bw/day in rats LOAEL: 200 mg/kg bw/day NOAEL: 50 mg/kg bw/day (74 ng/mL in plasma) in mice	NTP 2019 Feng et al., 2017	1 2

Different studies in rodents, supported by in vitro studies, provide evidence for potential adverse health effects. Repeated dose toxicity studies in rat have shown PFBS-mediated effects on liver, kidneys, and hematological systems, although at relatively high doses. A dose dependent decrease in hemoglobin and hematocrit levels was observed in male rats with a NOAEL determined to be 60 mg/kg bw/ day (90 days).

A study on mice indicated modest changes in lipid metabolism after 4-6 weeks daily exposure to PFBS (30 mg/ kg bw/day). Only a few studies have, as of yet, seen similar effects on lipid metabolism. Together with human data indicating that PFBS, as well as other PFAAs, have been associated to increased BMI or changes in blood cholesterol and triglycerides, these data may point towards an effect of PFBS on metabolic disorder or disturbance of the lipid metabolism. However, the current data is insufficient to allow a conclusion to be drawn.

Evidence for genotoxic or mutagenic effects of PFBS has not been found. The current knowledge of carcinogenic effects of PFBS is insufficient. However, some indications for carcinogenicity were found in a study showing minimal to moderate effects on the kidneys. Indications for reproductive toxic effects of PFBS were found when prenatal exposure to PFBS was linked to a delay in pubertal onset and reproductive organ development in female offspring mice.

Hormonal disturbances, such as decrease in free T4, total T3 and T4 levels, have been shown after prenatal PFBS-exposure, both in mother and their offspring, together with deficits in perinatal growth, pubertal onset and reproductive organ development in mice at doses ≥200 mg/kg bw/day. Also statistically significant dose-dependent decreases in total T3, total T4, and free T4 levels were reported in both male and female rats in a 28

day study at all doses tested (≥62.6 mg/kg bw/day) . Hence, PFBS exposure seems to disturb the levels of thyroid hormones in intact organisms. Other effects of PFBS, such as neurotoxicity and immunotoxicity, have also been reported, but evidence that these endpoints are sensitive targets is as yet lacking. However, the regulation of maternal thyroid hormone levels is known to be a crucial element during developmental life stages as the foetuses are dependent on maternal production of thyroid hormones. Low maternal thyroid hormone levels may lead to neurodevelopment deficiencies in offspring, and the database is limited by the lack of developmental neurotoxicity studies in particular.

Taken together, effects on thyroid hormone disturbances were observed in both rats and mice. These effects are serious and of particular concern since the foetus is dependent on maternal production of thyroid hormones. Cycling disturbances were also observed in rats and mice after adult and/or developmental exposure to PFBS, along with developmental effect in mice. In addition, effects on liver, kidney and haematological system were observed in rats. Toxicological data obtained for PFBS point towards similar health effects as seen for other PFAAs, although the potency seems to be lower for PFBS than for the C6 or C8 counterparts for several of the endpoints. This was seen in a report on relative toxic potency to liver for 20 different PFASs, investigated by Zeilmaker et al. (2018). PFBS was found to have lower liver toxicity compared to C6 and C8 PFSAs and C4 to C18 PFCAs. However, in general the differences in potencies between short and longer chained PFASs may be lower or even comparable when looking at internal liver organ concentrations and not external doses (Gomis et al., 2018). In the NTP (2019) 28-day rat study, internal plasma concentrations were measured. A comparison of the findings based on external dose (mmol/kg/day) and plasma levels (µM) on day 29 showed that PFBS had to be administered at ~1300 higher concentration than PFOS in order to reach similar internal plasma dose in µM. Liver pathology data and thyroid hormone measurements in males and females were presented for all perfluorinated sulphonates included in the NTP-study. A toxicity comparison based on dose administered showed PFOS to be the most potent and PFBS to be the least potent with PFHxSK closer to PFOS in potency than to PFBS. However, if plasma concentrations at day 29 are used for comparison, PFBS is markedly more potent. In fact PFBS and PFOS resulted in a similar magnitude of effect on thyroid hormones although the internal level of PFOS in µM was much higher than for PFBS. It should be noted that due to the higher protein binding of PFOS than PFBS, the levels of free PFBS may be higher than the free PFOS at similar serum levels.

It should also be recognised that the half-life of PFBS in humans is considerably longer than the half-lives measured for rodents.

Due to the very high persistence of PFBS in the environment and documented uptake and distribution in the human body, continuous exposure through drinking water, food as well as dust may cause higher levels in the blood, leading to unforeseen and unwanted health challenges. In addition, the combinatorial effect when exposed to a mixture of PFASs is of concern. The co-exposure of PFBS and other very persistent fluorochemicals present in the environment may lead to a combination of effects on human health.

5. Environmental hazard assessment

5.1. Aquatic compartment (including sediment)

5.1.1. **Fish**

5.1.1.1. Short-term toxicity to fish

Summaries of two standard acute toxicity tests are available on the dissemination website for KPFBS. Both tests followed the OECD TG 203 guideline. Fathead minnows were exposed to nominal concentrations (0, 204 (actual 220), g/L, 408 (actual 437) mg/L, 816 (actual

888) mg/L, 1632 (actual 1655) mg/L and 3263 (actual 3341) mg/L) of PFBS for 96 hours in a static system. Two negative control groups were kept in blank test medium. No mortality was observed at concentrations up to 888 mg/L, however 30% cumulative mortality was observed at 1655 mg/L and 100 % cumulative mortality was observed at 3341 mg/L. The 96-hour LC_{50} of KPFBS to fathead minnow was 1938 mg/L. → *Klimisch reliability 1- well documented, guideline study*.

Furthermore, bluegill sunfish were exposed to nominal concentrations between 612 (actual 629) mg/L to 9790 (actual 9433) mg/L of KPFBS in a static test. Control fish were kept in blank test medium. The NOEC for bluegill sunfish was 2715 mg/L. At 5252 mg/L 15% cumulative mortality was observed, and 100% mortality was observed at the highest exposure concentration. Reported LC_{50} value for bluegill sunfish was 6452 mg/L. → *Klimisch reliability 1- well documented, guideline study*.

Hagenaars et al. (2011): A zebrafish study assessed the structure activity relationship of PFBS and three other PFASs following a prolonged zebrafish early life stage test according to OECD 236 guideline Fish embryo toxicity test (FET) The test procedure is previously described by Nagel (2002). Normal fertilised zebrafish eggs were exposed to nominal concentrations (50, 100, 250, 500, 1000 and 3000 mg/L) of PFBS potassium salt. Groups of twenty normally shaped fertilised eggs per exposure concentration were divided over a 24-well plate and each egg was placed individually in 2 mL of the test solution. Remaining four wells were filled with clean water and used for control eggs. 3,4-dichloroaniline (3.7 mg/L) was used as a positive control and resulted in mortality between 72% and 92%. Two replicate plates were used for each exposure concentration (i.e. 40 embryos per exposure concentration). The eggs were exposed until 120 hours post fertilisation (hpf). The embryos were checked for mortality at 8 hpf, and for mortality and hatching every 12 h until 120 hpf to include each developmental stage. Heart rate was recorded at 48 hpf (non-hatched) and 72 hpf (hatched). Malformations of the head were apparent in exposed larvae starting from 96 hpf. Another effect was uninflated swim bladder which resulted in abnormal swimming. The results also demonstrated significant altered heart rates in embryos exposed to PFBS. At 3000 mg/L PFBS significantly increased heart rates were detected after 48 hpf, however the heart rates significantly decreased after 72 hpf. The EC_{50} value of PFBS was 1529.32 mg/L and was a regression estimate of the concentrations at which 50 % of the embryos showed an effect (mortality or malformation). There was a low mortality rate of PFBS, reported LC_{50} was >3000 mg/L. NOEC at 96 hpf and 120 hpf were 500 mg/L and 250 mg/L respectively (Table 30). *Klimisch reliability 2- modified guideline study*.

Ulhaq et al. (2013a): Zebrafish embryos were exposed to nominal (actual) concentrations (blank, 10 mg/L, 30 mg/L, 100 mg/L, 1000 mg/L and 3000 mg/L) of PFBS and six other PFASs following OECD TG 236 with an extended exposure period and additional sublethal endpoint observations. Normally developed fertilised eggs were distributed on a 48 well plate along with 750 µL of the exposure medium (PFASs and reconstituted water). A total of 24 embryos were tested per PFAS concentration, as well as 24 embryos in the water control group. The embryos were exposed until 144 hpf. Observations of lethal and sublethal endpoints were made after 24, 48, 120 and 144 hpf. Heart rate was recorded at 48 hpf. Effects observed in the literature following PFBS exposure were affected heart rate and pericardial oedema, which is a sign of compromised cardiac output. A combined EC_{50} (450 mg/L) was determined for lethal and non-lethal effects due to inadequate statistical power. NOEC (heart rate) for PFBS was 300 mg/L. Similar sublethal effects in zebrafish embryos following exposure to other PFASs, such as PFOA and PFOS, have been observed in the studies by Zheng et al. (2012) and Hagenaars et al. (2011). *Klimisch reliability 2- modified guideline study*.

Ulhaq et al. (2013b): Locomotor behavior of zebrafish larvae was also assessed on the same exposed individuals described in the previous section. Sublethal behavioural endpoints were recorded at 144 hpf using an automated video tracking system. The effect

on larvae activity was determined both in light and darkness. The reported EC_{50} (450 mg/L) in this study was based on the combined sublethal and lethal embryotoxicity effect data from the study described in the previous paragraph (Ulhaq et al., 2013a; Ulhaq et al., 2013b). Overall activity was reduced at 1000 mg/L PFBS, but active swimming speed was increased to that of the control group. Compared to the control group, there was a reduction in overall activity in the group exposed to the highest concentration of PFBS (3000 mg/L). This effect is considered as a disturbance in the behavioural response pattern in zebrafish larvae. In this study, PFBS was the compound that exhibited the highest impact on the tested behavioural endpoints. Exposure to PFBS was positively correlated with active swimming speed and negatively correlated to all the other endpoints. *Klimisch reliability 2- modified guideline stud*

Sant et al., 2019: Zebra fish embryos were exposed to PFBS (0, 4.8 mg/L and 9.6 mg/L) daily, beginning at 1-day post fertilisation (dpf) until 4 and 7 dpf.The test fish were two transgenic lines bred for homozygosity. Embryos were examined for viability following the OECD Fish Embryo Acute Toxicity Test Guidelines No. 236 (OECD, 2013), and examined under microscope for hatching at 3 dpf and swim bladder inflation at 4 dpf. Incidences of fish with significantly stunted growth and truncated exocrine pancreas length was significantly increased after PFBS exposure, although these two effects occurred independently. Pancreatic islet morphology revealed an increased incidence of hypomorphic islets (areas lower than the 1st percentile of controls) and an elevated occurrence of fragmented islets. RNA Seq data (4 dpf) also identified disruptions in regulation of lipid homeostasis. The findings demonstrate that PFBS can disrupt pancreatic organogenesis and perturb expression of genes involved in lipid metabolism. However, since the test fish were from transgenic lines the ecological relevance of these results are less than that of the studies by Hagenaars et al. and Ulhaq et al. The overall impact of PFBS described by Sant et al. is not as severe when compared to findings with embryonic PFOS exposures, as reported by Sant et al. (2017). *Klimisch reliability 2- modified guideline study.*

Table 30: Overview of short-term effects on fish

Species	Duration	Endpoint	Effect level	Concentration	Comment	Reference
Fathead minnows	96 h	Mortality	LC_{50}	1938 mg/L	OECD 203	ECHA disseminatio n website; NICNAS, 2005
Bluegill sunfish	96 h	Mortality	LC_{50}	6452 mg/L	OECD 203	ECHA disseminatio n website; NICNAS, 2005
Zebrafis h larvae	96h/120 hpf	Lethal, sublethal and developme ntal	LC_{50} EC_{50} NOEC	>3000 mg/L 1900.78/ 1529.32 mg/L 500/ 250 mg/L	OECD 236	Hagenaars et al., 2011

Species	Duration	Endpoint	Effect level	Concentration	Comment	Reference
Zebrafish larvae	144 hpf	Developmental Heart rate	EC_{50} LC_{50} NOEC	450 mg/L 1500 mg/L 300 mg/L	OECD 236	Ulhaq et al., 2013a
Zebrafish larvae	144 hpf	Locomotor behaviour	EC_{50}	450 mg/L		Ulhaq et al., 2013b
Zebra fish	1dpf, 4dpf and 7dpf (day post fertilisation)	Viability and organ development	LC_{50} (estimated)	393.13 mg/L (1310 µM)	OECD 236	Sant et al., 2019

In summary, exposure to high concentrations of PFBS resulted in disturbances in heart rate, morphological malformation and altered swimming behavior in zebra fish. In the presented acute and long-term studies, an overall low mortality rate was observed in exposed fish. In the currently available literature, there is a large range in observed effect concentrations for zebra fish. However, due to the high lethal concentrations reported in the acute toxicity studies, it cannot be excluded that toxicity is caused by the counterion, e.g. potassium (Mount et al., 1997).

5.1.1.2. Long-term toxicity to fish

Five papers based on the same series of studies on PFBS expoure using the marine medaka (*Oryzias melastigma*) have been published by Chen et al. (2018a; 2018b; 2018c; 2019a; 2019c). The use of standardised test guidelines (i.e. OECD/ISO test guideline) under GLP conditions was not documented. However as stated in the Guidance on information requirements and chemical safety assessment Chapter R.4: Evaluation of available information, by ECHA (2011), the use of a guideline method or GLP does not necessarily reflect the reliability of a study, as long as the study is well documented and scientifically acceptable. All of the available studies follow the same method of fish rearing. *O. melastigma* is not a widely used OECD model fish but has been used in some ecotoxicity studies as a model teleost in marine toxicology (Dong et al., 2014a), probably because it is closely related to Japanese medaka (*Oryzias latipes*) which is a well described test model.

The chosen experimental concentrations presented herein were identified after performing a concentration range test where hatching, mortality, and malformation of marine medaka larvae were not changed significantly at 15 days postfertilization (dpf). Following this, a life-cycle experiment starting with exposure of F0 marine medaka to solvent control, 1.0, 2.9 and 9.5 µg/L PFBS from embryo stage (newly spawned) until reached sexual maturity (6 months). Exposure concentrations used in the studies are environmentally realistic considering the high occurence of PFBS in leachate of landfill sites in Singapore and Ireland, as well as near point sources in China (Bao et al., 2019; Harrad et al., 2019; Yin et al., 2017). F1 eggs were collected by pairing adult F0, and eggs were then cultured in

clean artificial seawater until 6 months. F2 eggs were collected by pairing F1 adults and eggs were cultured in artificial seawater until 15 days post fertilisation (dpf). Following reproduction, F0 and F1 fish were terminated and dissected. The tissue samples were snap frozen in liquid nitrogen and stored at -80°C until further analysis.

The exposure to PFBS was performed in a semi-static system containing fully aerated, charcoal-filtered artificial seawater (25‰) under constant ambient temperature (24±0.5 °C). The medaka juvenile and adults were fed twice daily with flake food and newly hatched nauplii of *Artemia*. Three replicates, each containing approximately 150 embryos in 100 ml test solution were used per treatment. At day 30 post fertilisation the larvae were transferred to 4 L media and after 2 months the fish were transferred to 20 L media. The test solutions were renewed daily. Water concentrations of PFBS were monitored regularly after the renewal of seawater by collecting 1 L aliquots of seawater from each exposure tank (n=3). The water concentrations of PFBS were monitored regularly. Nominal (1; 3; 10 µg/L) and measured (1±0.1; 2.9±0.1; 9.5±0.3 µg/L) concentrations did not deviate more than 10% during the full duration of exposure.

Comments regarding the methods used: .

Use of dimethylsulphoxide (DMSO) (0.001% v/v) in this study should have been justified as the physicochemical properties of PFBS suggests it should be easily dissolved directly in water (water solubility of KPFBS salt is 52.6 g/L (Table 5) whereas PFBS is fully miscible at 20 °C). However a study on zebrafish showed that with respect to the fish embryo test, results indicate that DMSO may be used without complications as a solvent (Kais et al., 2013), but only at a maximum concentration of 0.01% (0.1 mL/L) as already indicated in the OECD difficult substances paper (OECD, 2000). In the studies described below the DMSO concentration was 0.001%. It is noted that there was no negative (untreated/seawater) control included in current exposure.

It may seem that the size of the vessels is small and that there may be an issue with overcrowding. However from the data in the publication by Chen et al. (2019), the egg weight was about 0.8 mg/egg. Thus the total biomass loading in the initial part (until 15 dpf) of the study, was around 1.2 g/L which is slightly above the recommendations in OECD guidelines for fish toxicity tests (e.g. TG 203, 215 and 234).

Upon request, additional information regarding the study design was received by the corresponding author of the studies which provided details needed to decide on the reliability of the studies. On these grounds, the series of four Chen studies described below is given a *Klimisch score* 2 – reliable with restrictions. The studies are generally well designed and performed, but there are some minor flaws in the documentation and setup.

Conclusions made on the effects of PFBS on body length and weight are not well documented in the publications. However it appears that the data concerning weight of F0 can be derived from Figure 1 in the Chen et al. (2018b) study. F0 weight is affected in a dose-dependent manner for both sexes. No data on the body lengths of the fish are available. In addition there are some uncertainties regarding the changing trends in the sex ratio following exposure to PFBS. Given these weaknesses in the study design by Chen et al., some of the authors' conclusions are somewhat uncertain.

Chen et al. (2018a): The effects on thyroidal axis, thyroid hormone (TH) levels, thyroxine-binding globuline (TBG) contents, expression of genes along the HPT axis, body weight and length were investigated to elucidate multigenerational thyroid disrupting potency of PFBS across three generations (F0, F1 and F2), Table 31. The data presented on both functional levels of organization (hormone and gene transcription) are not clearly displaying a coherence as most of the data lack statistical significance. In the female F0 there were indications of hormonal disruption of T4 and the down-stream gene Dio 2, however since the gene transcription is not significantly down-regulated and there is no

effect on the TBG, the presented results are not strong enough by themselves to predict whether PFBS is a thyroid disruptor. The authors suggest that there is a clear relationship between the hormonal increase of T3 and TBG in F1 larvae from parents exposed to 1.0 µg/L, which further is supported by gene transcription of pivotal genes (Dio1 and 3, hhex) and delayed hatching.

The increase in T3 and TBG in F1 larvae was not occurring at higher exposure concentrations, but this was not supported by pivotal genes such as Dio2 or Dio3 transcription. However, a more general parameter which displays thyroidal disruption on a morphological organismal level is retarded growth. The authors report that there was a reduction in growth of both adult F1 males and females. F0 adult female and male fish are reported as having significantly decreased body weight (male and female) and body length (male) when exposed to 2.9 and 9.5 µg/L PFBS. Data concerning weight of F0 medaka is not reported specifically, but it can be derived from Figure 1 in the Chen et al. (2018b) study. No data on the body lengths of the fish are available.

The combined data of the decreased T3 levels in adult female F1 and the decreased gene transcription of Dio1, 2 and 3 is not consistent and does not follow the previous argumentation by the authors: "In accordance with previous reports the current results provide additional evidence that the increased T3 content, as a characteristic of hyperthyroidism, suppresses the transcription and activity of Dio2." The overall gene transcription in adult F1 females is significantly affected in F0 parents exposed to the two highest concentrations 2.9 and 9.5 µg/L. This is however not clearly reflected in the hormonal levels measured.

The authors are suggesting that the significant increase in the gene transcription of TBG is supporting the significant increase of T4 in parents exposed to 9.5 µg/L PFBS, Table 31. Furthermore, there is a slight increase (non-significant) in T3 in the same exposure concentration, however there is an increase of Dio1 expression, which is the opposite of the findings previously described (increase in TH levels will cause a down-regulation of Dio1). The results are therefore hard to interpret. The authors conclude that the results presented herein support a significant disruption of the thyroidal axis in F0 adult medaka exposed to realistic concentrations of PFBS. Transgenerational disturbances in the thyroid endocrine system of F1 and F2 generations were noted.

The authors' claim that the study demonstrates that PFBS disrupts the thyroidal axis at the concentrations tested is quite general as they only assessed a few endpoints on a lower level of organization (hormones) and transcriptional regulation of genes in the HPT axis. As the authors mentioned themselves "differences in translational rates, post-translational modifications, or protein degradation cause discrepancies in gene transcription and protein expression levels" where the protein expression is considered to be the most reliable level of organization due to it being more stable over time, especially during chronic exposure.

Table 31: Overview of transcriptional effect in F1 and F2 offspring (larvae).

Modified from Table S4 in the supplementary material by Chen et al. (2018a). Significant upregulation is shown by upwards arrow and significant downregulation is shown by downwards arrow. A dash indicates non-significant results.

Genes	F1 offspring (15 dpf)			F2 offspring (15 dpf)		
Genes	1.0µg/L	2.9 µg/L	9.5 µg/L	1.0 µg/L	2.9µg/L	9.5µg/L
CRH	↓	-	↓	-	↓	-
CRHR	-	-	-	-	-	-
TSHβ	-	-	-	-	-	-
TSHR	↓	↓	-	-	-	-
NIS	↓	↓	-	-	-	-

Genes	F1 offspring (15 dpf)			F2 offspring (15 dpf)		
Genes	1.0µg/L	2.9 µg/L	9.5 µg/L	1.0 µg/L	2.9µg/L	9.5µg/L
TPO	↓	↓	↓	↓	↓	-
TG	↓	↓	↓	-	↓	-
TBG	↓	↓	↓	-	↓	↑
TRα	-	-	↓	-	-	-
TRβ	↓	-	-	-	↓	-
Dio 1	↓	↓	↓	-	↓	↑
Dio 2	-	-	-	-	-	-
Dio 3	↓	↓	↓	-	↓	-
UGT1	↓	↓	↓	-	-	↑
SULT	↓	↓	↓	-	↓	-
MCT8	-	-	-	-	-	-
SLCO1c1	↓	↑	↓	-	↓	-
pax8	-	-	-	-	↓	-
hhex	↓	↓	↓	-	-	-

*

Chen et al. (2018b): The objective of the second study by Chen et al. (2018b) was to investigate the toxic effects of PFBS on vision in marine medaka.

The measured endpoints were PFBS accumulation, contents of various neurotransmitters, enzyme activity in eyes, AChE activity, MAO activity and protein expression. Eyes from five fish of the same sex were pooled as a replicate. PFBS exposure resulted in concentration dependent accumulation of PFBS in the eye tissue, and impaired both visual development and function in a sex-dependent manner. No clear dose response relationships were reported.

In exposed females, eye weight (wet weight) was significantly decreased, and water content in eyes increased. However this effect was not supported by data on dry weight. Concentrations of PFBS in the eyes increased in a dose dependent manner. Multiple neural signalling processes were perturbed by PFBS life-cycle exposure, including cholinergic, glutameric, GABAergic and monoaminergic signals. Levels of norepinephrine and epinephrine in the eyes were increased, which may adaptively decrease the intraocular hypertension. PFBS exposure also adversely affected expressions of eye proteins that are related to visual functions and motor activity, and thus the muscular control of the eyes of the fish.

These results highlight the eye as a sensitive organ to environmental pollutants. However, the biological relevance of these effects are unclear since no anomalies in visual function were reported.

Chen et al. (2018c): In the third paper, Chen et al. (2018c) reported effects of PFBS exposure on the gut microbiota of marine medaka. It was found that even at environmentally realistic concentrations, PFBS caused dysbiosis of gut microbiota which was durable in parents and persisted in the offspring. Decreased relative abundances of *Cetobacterium* were observed in intestines of F1 males derived from parents exposed to 1.0 µg/L PFBS. Fluctuations in abundances of *Cetobacterium* might affect the supply of vitamin B12 in the intestine, which could result in deficiencies and mental disorders.

Chen et al. (2019a): In the fourth paper, Chen et al.(2019a) investigated the effects on the reproductive system and the underlying mechanisms related to endocrine disruption and transgenerational toxicities.

A wide range of endpoints were included in the study: sex ratio, egg production, PFBS elimination rate, PFBS concentration, histology, measurement of sex steroid hormones (testosterone (T), 11-ketotestosterone (11-KT), 17β-estradiol (E2)) and regulatory hormones of the hypothalamus-pituitary -gonad (HPG) axis in brain tissue or plasma

(Gonadotropin releasing hormone (GnHR) (brain), Luteinizing hormone (LH) (plasma), Follicle stimulating hormone (FSH) (plasma)), global methylation level, plasma proteomics, and gene transcription. The sex ratio of the fish was determined based on the development of secondary sex characteristics. Sexually mature male medaka will have a larger and parallellogram-shaped anal fin. To calculate egg production of the F0 generation, spawn was collected daily during the final 2 weeks of the exposure period.

The authors claim that the sex ratio was statistically significantly affected in the highest test concentration of 9.5 µg/L with an increased proportion of males (128% vs 83.2% in control). However, in our view the difference in sex ratio between the exposure groups and the controls is so small that the calculated statistical significance may represent a false positive effect. The gonadosomatic index (GSI) was significantly affected with ovaries in the 9.5 µg/L exposure group significantly smaller than in the control group. Egg production was significantly lower in the 2.9 µg/L and 9.5 µg/L groups compared to the control. During the exposure of F0 from 4 months to 6 months the PFBS whole tissue concentration increased in a gradual and dose-dependent manner, while only trace amounts were detected after 2 months of depuration. Accumulation of PFBS in exposed ovaries reached 3.0, 3.6 and 10.0 ng/g dry weight (dw) in the 1.0, 2.9 and 9.5 µg/L exposure groups, respectively.

The GSI values were significantly lowered in the F0 females exposed to 9.5 µg/L. This corresponds to the observed shift in F0 oocyte distribution where a significant increase in previtellogenic and significant decrease in vitellogenic and postvitellogenic oocytes were observed. This delay in oogenesis could in turn result in the observed significant decrease in F0 fecundity. The systemic effects on the gonads shown by GSI, delayed oogenesis and reduced fecundity are cohesive and indicate an effect of PFBS on reproduction. No histological abnormalities were observed in liver and testes. Oocyte development was blocked in the ovaries in the highest exposure group. In the 9.5 µg/L treated fish ovaries, the proportion of previtellogenic oocytes was significantly increased, whereas the proportions of vitellogenic and post-vitellogenic oocytes were decreased.

There were no significant changes in brain GnRH levels and plasma LH levels in males or females. Plasma FSH levels were significantly higher in both sexes exposed to 1.0 µg/L and 2.9 µg/L but not in the fish exposed to 9.5 µg/L PFBS. Levels of T were significantly decreased in males exposed to 2.9 µg/L and 9.5 µg/L PFBS. The plasma E2 levels in females were decreased in a concentration-responsive manner with only the concentration in fish exposed to 9.5 µg/L PFBS being statistically different from control. In both sexes, 11-KT was significantly decreased compared to control. Ovary tissues from the 9.5 µg/L exposure group were significantly hypermethylated. There were no significant differences in the ovary tissues in the other concentration groups or on global methylation levels of testicular DNA among groups. Proteomic analysis of control and 9.5 µg/L exposed groups revealed differentially expressed protein (DEPs) between the two groups. DEPs were mainly associated with immune response and blood pressure regulation compared to control group. No significant differences in the abundance of the estrogenic biomarkers vitellogenin (VTG) and choriogenin (ChG) proteins were detected.

The observed significant decrease in hormonal level of E2, 11-KT and E2/T in F0 females exposed to 9.5 µg/L are indicative of an anti-estrogenic effect. However, these changes could also be a result of the stage of the oogenesis as the hormonal levels vary throughout the gonadal recrudescence (Nelson et al., 2013). In case of systemic effects on female gonads leading to delayed oogenesis, changes in hormonal and transcriptional levels would be expected as is observed in this study. In F0 males, significant increased levels of E2/11KT and E2/T was observed. However, the increase in these ratios are probably due to the significant decrease in 11KT and T levels as there were no significant changes in E2 levels in the F0 males. Thus, PFBS appears to affect the androgens in males and might have an anti-androgenic effect. The 11-KT was significantly reduced in female F0 as well.

In Japanese eel, *Anguilla japonica*, it was found that 11-KT appeared to induce the early stage of oocyte growth (Sudo et al., 2012).

Egg spawn of the F1 generation in the 9.5 µg/L exposure group had a significantly lower weight compared to control group. The hardness of the F1 egg envelope in eggs from exposed parents (2.9 and 9.5 µg/L) was significantly increased while the protein and lipid loads were significantly decreased. However, no method is indicated for determining hardness of egg envelope. Comparison of F1 and F2 generation control egg weight suggests that normal variation of egg weight is greater than the range shown across exposure within the F1 generation. Parental exposure to 9.5 µg/L PFBS led to a significant reduction in global methylation levels at 0 dpf in F1 offspring. At later timepoints (2, 5, 10 and 15 dpf) there were no significant differences in global methylation among the groups. F1 larva mortality increased significantly after parental exposure at 2.9 and 9.5 µg/L PFBS. 0.2, 0.9, and 2.9 ng/g dw of PFBS was transferred from exposed parents to F1 eggs in the 1.0, 2.9 and 9.5 µg/L exposure groups, respectively. No residual PFBS was detected in F1 adults or F2 eggs. HPG hormone levels recovered to approximately control levels in F1 adults (not exposed), with only GnRH and LH concentrations in females being significantly higher than the control group. There were no significant changes in F1 sex ratio, GSI values or fecundity. Compared to the control group, the weight, lipid load and protein load of F2 eggs from parents ancestrally exposed to 1.0 µg/L PFBS was significantly increased. No significant effects on egg weight, lipid load and protein load were observed for the other groups. No significant effect on mortality of F2 larva was observed.

In exposed medaka, positive regulation of the feedback loop was initiated in the hypothalamus and pituitary in response to the inhibited synthesis and imbalanced homeostasis of sex hormones. Down-regulation of estrogen receptors in liver would be responsible for the decreased transcription of VTG and ChG. It is difficult to know if the observed effects are mediated by systemic effects on the gonads resulting in hormone imbalance and reduced egg production, or if the effects observed in the gonads are a result of specific effects targeting hormonal receptors as is often associated with endocrine disruption. However, the identification of PFBS as an endocrine disrupter according to the WHO criteria (WHO-IPCS, 2002: WHO-UNEP, 2012) is not in the scope of the present SVHC proposal.

Chen et al. (2019c): In a recent publication, DNA methylation was examined with comparison to swimming behaviour in larvae of exposed parents. By mixing and mating control and exposed medaka (male or female), a crossbreeding strategy was employed to produce offspring eggs from various crossbreds, with the aim of differentiating the maternal and paternal influences. Significant increase in locomotor activity was demonstrated by fish larva with parent crosses of control females x 3 µg/L PFBS exposed males ($p < 0.001$) and control females x 10 µg/L PFBS exposed males ($p < 0.05$). The activities of offspring of exposed females crossed with control males were not significantly different from the controls and did not demonstrate elevated locomotor activity. The authors' found that inheritance of exposed paternal methylome marks in offspring may be partially responsible for abnormal swimming behaviour, although different toxic mechanisms may be involved depending on the exposure concentration.

The current data generated by Chen et al. (2018a; 2018b; 2018c; 2019a; 2019c) are considered as evidence for the hormonal disturbing properties of PFBS, albeit the identification of PFBS as an endocrine disruptor according to the WHO criteria is not in the scope of the present SVHC proposal. The GSI values were significantly lowered in the F0 females exposed to 9.5 µg/L. This corresponds to the observed shift in F0 oocyte distribution where a significant increase in previtellogenic and significant decrease in vitellogenic and postvitellogenic oocytes were observed. This delay in oogenesis could in turn result in the observed significant decrease in F0 fecundity. The systemic effects on the gonads shown by lowered GSI, delayed oogenesis and reduced fecundity are cohesive and show an effect of PFBS on reproduction. Based on these effects on reproduction in

marine medaka it is concluded that PFBS fulfils the T criteria for the environment of Annex XIII of REACH (i.e. NOEC or EC_{10} for marine or freshwater organisms less than 10 µg/L).

5.1.2. **Aquatic invertebrates**

5.1.2.1. Short-term toxicity to aquatic invertebrates

A 96 hour acute toxicity test performed on mysid shrimp (*Americamysis bahia*, former scientific name *Mysidopsis bahia*) is described in the registration dossier for KPFBS and reviewed by NICNAS (2005). The test was conducted following EPA OPPTS 850.1035 (Mysid Acute Toxicity Test) in 2000 mL polyethylene buckets containing 1500 mL of test or control medium and nominal concentrations ranged from 31 to 1000 mg/L KPFBS. Negative controls were kept in blank test medium. The mysids were subjected to a photoperiod of 16 h light and 8 h dark, temperature was held at 25 ± 2 ^{0}C, and measurements of pH ranged from 8.0 to 8.3. Observations were made at 4, 24, 48, 72 and 96 hours after test initiation. The 96 h LC_{50} of mysid shrimp was 372 mg/L. → *Klimisch reliability 1- well documented, guideline study*.

A 48 hour immobilisation test was performed on *Daphnia magna* according to OECD TG 202 (ECHA dissemination website; NICNAS, 2005). Analytical exposure concentrations were < LOQ, 234 mg/l, 470 mg/L, 886 mg/L, 1707 mg/L and 3767 mg/L. Negative controls were kept in blank test medium. The daphnids were subjected to a photoperiod of 16 h light and 8 h dark, the temperature was held at 20 ± 1 ^{0}C, and measurements of pH ranged from 7.9 to 8.5. Observations were made after 4, 24 and 48 hours after initiation to determine the numbers of mortalities and immobile organisms. The EC_{50} (48 h) was 2183 mg/L based on immobilisation of *Daphnia magna*. → *Klimisch reliability 1- well documented, guideline study*.

Table 32: Summary of short-term effects on aquatic invertebrates

Species	Duration	Endpoint	Effect level	Concentration	Comment	Reference
Mysid shrimp	96 h	Lethality	LC_{50}	372 mg/L	EPA OPPTS 80.1035	ECHA dissemination website; NICNAS, 2005
Daphnia magna	48 h	Immobilisation	EC_{50}	2183 mg/L	OECD 202	ECHA dissemination website; NICNAS, 2005

5.1.2.2. Long-term toxicity to aquatic invertebrates

The chronic toxicity of KPFBS was determined in a 21 day *Daphnia magna* life-cycle chronic toxicity test equivalent to OECD guideline TG 211. The study is described in the registration dossier for KPFBS. The daphnids were exposed to six different concentrations of KPFBS (nominal/actual concentrations were 63/60; 125/121; 250/247; 500/502; 1000/995; 2000/1876 mg/L). Negative controls were kept in blank test medium. Temperature was held at 20 ± 1 °C except for two occasions when the temperature was 18 °C. The daphnids were subjected to a photoperiod of 16 h light and 8 h dark, temperature was held at 20 ± 1 ^{0}C, and measurements of pH ranged from 7.9 to 8.5. The daphnid neonates were less than 24 hours at test initiation. The first generation was observed daily for survival, onset of reproduction, number of neonates and signs of toxicity. Length and dry weight of surviving first generation daphnids was determined. The 21 day NOEC was 502 mg/L,

while LOEC was 995 mg/L. The study showed that the chemical was practically non-toxic to daphnids after chronic exposure. → *Klimisch reliability 1- well documented, guideline study*.

Table 33: Summary of long-term effects in aquatic invertebrates

Species	Duration	Endpoint	Effect level	Conc.	Comment	Reference
Daphnia magna	21 days	Several endpoints, see text	NOEC LOEC	502 mg/L 995 mg/L	OECD TG 211	ECHA dissemination website; NICNAS, 2005

5.1.3. **Algae and aquatic plants**

A 96-h EbC_{50} (algal biomass) and ErC_{50} (growth) of 2347 mg/L and 5733 mg/L study, following OECD TG 201, has been reported for *Raphidocelis subcapitata* (formerlyformerly known by the scientific names *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*) on the ECHA dissemination website and is also reviewed by NICNAS (2005). The EbC_{50} and ErC_{50} values are the concentration at which 50% reduction of biomass or growth rate is observed, respectively. The test was carried out by exposing *Selenastrum capricornutum* to nominal concentrations of KPFBS (nominal/ actual concentrations; 313/285; 625/563; 1250/1077; 2500/2216; 5000/4561; 10000/9478 mg/L) for 96 h. Negative controls were kept in blank test medium. Temperature was held at 24 ± 2 ^{o}C and the algae were held under continuous cool-white fluorescent lighting. The effect of PFBS exposure on cell count, cell morphology, cell densities, area under growth curves, growth rates and percent inhibition values were determined (NICNAS, 2005). Based on the observed results in this study, the PFBS potassium salt was practically non-toxic to algae. → *Klimisch reliability 1- well documented, guideline study*.

Rosal et al. (2010): The toxicity of PFBS towards *P. subcapitata* was also examined. Chronic toxicity was determined following an algal growth inhibition test according to OECD TG 201 *P. subcapitata* open system, using 96-well microplate in which the algae were cultured in a total volume of 200 µL. Low toxicity was observed, with 37 % growth inhibition at 20250 mg/L. → *Klimisch reliability 2* - comparable to guideline study with acceptable restrictions

Table 34: Summary of effects on algae

Species	Duration	Endpoint	Effect level	Concentration	Comment	Reference
Raphidocelis subcapitata	96 h	Biomass Growth	EbC_{50} ErC_{50}	2347 mg/L 5733 mg/L	OECD 201	ECHA dissemination website; NICNAS, 2005
Raphidocelis subcapitata	72 h	Growth	EC_{50}	>20 250 mg/L	37 % growth inhibition	Rosal et al., 2010

5.1.4. **Sediment organisms**

Stefani et al. (2014): A multigeneration toxicity test on *Chironomus riparius* aimed to investigate the evolutionary consequences of exposure to PFBS, PFOS and PFOA. The test

was performed according to OECD TG 233, but extended to 10 generations exposed at a low concentration (nominal concentration of 10 µg/L). Native *C. riparius* L3 larvae were collected and bred until emergence and egg deposition. Six-hundred larvae were then bred per treatment and per generation until emergence and egg deposition under a nominal concentration of 10 µg/L of contaminants. New-born larvae were used to start the next generation. Total exposure time was 300 days and the study followed ten generations until emergence of adults in generation ten. The test temperature was 20±1 ºC and measured pH was 7.8-8.2. Exposure to PFBS resulted in an increased mutation rate based on five microsatellite loci, which indicated a stronger genetic variability in the PFBS exposed population compared to that of controls. This may indicate a potential risk of mutational load caused by exposure to PFBS. The authors concluded that the present study provides an indication for long term relevant effects on population genetics on *C. riparius* caused by PFBS and PFOS. A pattern of increased mutation rate emerged as the main transgenerational effect. → *Klimisch reliability 2 - comparable to guideline study with acceptable restrictions. Test extended to 10 generations at low concentration.*

Table 35: Summary of effects on sediment organisms

Species	Duration	Endpoint	Effect level	Exposure concentration	Comment	Reference
Chironomus riparius	300 days	Emergence rate, sex ratio and reproduction	NOEC	10 µg/L (exposure concentration)	Extended OECD 233	Stefani et al., 2014

5.1.5. **Other aquatic organisms**

The toxicity of PFBS was examined by using two bioluminescence inhibition assays on the marine bacterium *Vibrio fischeri* and the cyanobacterial recombinant strain *Anabeana CPB4337* (Rosal et al., 2010). Bioassays with the photo-luminescent bacteria *Vibrio fischeri* were performed according to ISO 11348-3 standard protocol (International Organization for Standardization, 2007). The incubation period for this assay was 15 min. This bioassay measures the decrease in bioluminescence induced in the cell metabolism due to the presence of a toxic substance. A third bioassay was performed using the recombinant bioluminescent cyanobacterium *Anabeana CPB4337* and was based on the inhibition of constitutive luminescence caused by the presence of any toxic substance (Rodea-Palomares et al., 2009). Low toxicity was observed in *V. fischeri* and *Anabeana*, with effect concentrations (EC_{50}) of 17520 mg/L and 8386 mg/L respectively (Rosal et al., 2010). → *Klimisch reliability 2* - comparable to guideline study with acceptable restrictions.

Lou et al. (2013): A study on *Xenopus laevis* aimed to investigate comparatively adverse effects of longterm exposure to PFOS and PFBS on the growth and sexual development of amphibians. *X. Laevis* is the most used model amphibian species in toxicological studies (Cevasco et al., 2008). Adult *X. Laevis* female and male (3 years old) were raised separately in glass tanks and fed with chopped pork liver and commercial amphibian diet three times a week. Breeding was induced in a pair of *X. laevis* by injecting human chorionic gonadotropin. Fertilised eggs were incubated. On the fifth day post-fertilisation, tadpoles were exposed to PFOS (0.1; 1; 100; 1,000 µg/l), PFBS (0.1; 1; 100; 1,000 µg/l), 100 ng/l 17-beta-estradiol (E2), and 100 ng/l 5 alpha-androstan-17-beta-ol-3-one (DHT), respectively until 2 months postmetamorphosis, with water renewal every other day to maintain the appropriate exposure concentrations. Dimethylsulphoxide (DMSO) (0.001% v/v) was used as a carrier solvent. Stock solutions were diluted by charcoal filtered tap water to prepare test water. The tadpoles were fed three times daily with *Artemia*. *X. laevis* exposure was conducted in 18L water tanks. Three replicates of 25 tadpoles were used for each exposure concentration. On termination of the experiment the survival rate

was recorded and frogs were weighed and dissected. Liver tissue was weighed and HIS calculated. Sex or intersex was determined examining the gross gonadal morphology with a stereo microscope. Gonads and liver were histologically examined. Finally, total RNA was extracted from the brain and liver, and RT-PCR conducted for sex-related genes (estrogen and androgen receptor, aromatase, and ribosomal protein L8).

It was found that PFBS did not affect growth or survival (see Table 36), but had the potential to promote expression of estrogen and androgen receptor activity. However, aromatase activity in the brain was not altered by PFBS. The authors indicated that the increase in expression of ER and AR suggests an increase in the responsiveness to the corresponding sex hormone and potential adverse effects on sexual development, see Section 5.6.

Furthermore, exposure to PFOS and PFBS resulted in similar adverse effects on hepatohistology at >100 µg/L (P <0.05). PFBS induced hepatocyte degeneration in both male and female frogs. Liver tissues also exhibited hepatocyte hypertrophy in both sexes following exposure to PFBS at the highest concentration (1000 µg/L, P <0.05). Hepatohistologial impairment caused by PFBS exhibited no sex-difference. In conclusion, these results show that PFBS has adverse effects on hepato-histology and potential to alter the sexual development of *X. laeveis*. *Klimisch score 2 – reliable with restrictions, non-guideline study.*

Table 36: Summary of effects on other aquatic organims

Species	Duration	Endpoint	Effect level	Concentration	Comment	Reference
Vibrio fischeri	15 min	Bioluminescence inhibition	EC_{50}	17520 mg/L	ISO 11348-3	Rosal et al., 2010
Anabeana CPB4337	15 min	Bioluminescence inhibition	EC_{50}	8386 mg/L	ISO 11348-3	Rosal et al., 2010
Xenopus laevis	13 weeks	Weight, mortality, hepatosomatic index, sex ratio, gonadal histology	NOEC	1 mg/L	Non-guideline	Lou et al., 2013

5.2. Terrestrial compartment

5.2.1. Toxicity to soil micro-organisms

Wójcik et al. (2018): The effects of PFBS and other water soluble perfluorinated pollutants on phospholipids in model soil decomposer membranes were investigated. PFASs are toxic to a wide range of soil bacteria and this biocide activity is related with their membrane activity (Chen et al., 2013; Pasquini et al., 2013). Such effects can lead to lowered degradation of dead organic matter by decomposer organisms.

Phospholipid Langmuir monolayers were used as a simplified model of bacterial membranes and their interactions with selected environmentally relevant PFASs, such as PFOS, PFOA and PFBS were studied (Wójcik et al., 2018). The effects of PFASs on the texture of model membranes were studied by Brewster angle microscopy, while the influence on molecular packing in the 2D bacterial crystal lattice was searched by the Grazing Incidence X-ray diffraction technique. PM-IRRAS spectroscopy was used to study the effect of PFASs on the phospholipid polar head group conformation, while penetration tests were used to monitor the effectiveness of PFASs incorporation into the model

membrane. Results showed that Gram negative bacteria are more susceptible to PFASs than Gram positive species.

PFBS caused significant changes in the model membranes. Both PFOS and PFBS changed the orientation of the phospholipids within the 2D crystal lattice inducing the tilt of the hydrocarbon chains from the monolayer normal. PFBS turned out to be more structure breaking than PFOS. The tilt of the hydrophobic chains induced by the PFASs presence in the subphase was greater for PFBS than for other PFASs. Results also showed that PFBS was not built into the hydrophobic regions of the membranes, but rather interacted with the polar headgroups of the monolayer. Tests proved that PFBS and PFOS behave similarily in that they can be incorporated to the monolayer at low surface pressure. However, the practical impact on microbes was not determined. The authors concluded that the switch from eight-carbon atom PFASs to shorther chained homologues does not necessarily lower their environmental toxicity.

5.2.2. **Toxicity to terrestrial plants**

Lan et al. (2018): The adverse effects of six perfluoralkyl acids PFBA, PFHxA, PFOA and PFBS, PFHxS, PFOS on the growth of wheat seedling were studied at two different spiking levels (200 and 2000 µg/kg soil). Biomass and chlorophyll levels were analyzed, and plant uptake and translocation were calculated. Pot experiments with pre-germinated seedlings of wheat (*Triticum aestivum*) in spiked soils were conducted in greenhouse for four weeks, with four replicates of each treatment. Fresh weight of shoot and roots were determined per pot and the chlorophyll contents (chlorophyll a and b) were measured at two different wavelengths (663 nm and 645 nm) with a spectrophotometer.

The exposure to PFBA/PFBS reduced the synthesis of Chlorophyll *a* in wheat by 35% at spiking levels of 2000 µg/kg, indicating their phytotoxicity. In contrast, PFOA/PFOS can promote the wheat chlorophyll content. Shoot biomass was significantly reduced (22.5% for PFBA/PFBS) at spiking levels of 2000 µg/kg PFAAs and the reduction increased with the carbon chain length of PFAAs. However, wheat root biomass showed a promotion at both spiking levels, possibly due to the enhancement in permeability of nutrients.

Qu et al. (2010): In another study PFOS stimulated the growth of wheat seedlings and induced the synthesis of chlorophyll and soluble protein in wheat seedlings at concentrations less than 10 mg/L. However, PFOS concentrations >10 mg/L treatment could exert inhibition to the elongation and biomass of roots and leaves, and lead to damage to chlorophyll accumulation and soluble protein synthesis. When the concentration of PFOS was raised up to 200 mg/L, the activity of superoxide dismutase (SOD) and peroxidase (POD), two enzymes controlling oxidative stress in plants, decreased significantly with 12.6% and 33.7% inhibition for roots respectively, indicating that the antioxidative defensive system in wheat seedlings might be damaged by PFOS. Oxidative stress in *Arabidopsis thaliana* caused by PFOA had also been reported by (Yang et al., 2015).

The exposure of wheat seedlings to PFBS/PFBA led to a reduced chlorophyll a content of 35% and reduced shoot biomass of 22.5%, even though the test concentrations were much higher than concentrations detected in monitoring studies. High concentration of PFCAs and PFSAs can lead to a decrease in biomass in wheat and can cause oxidative stress, indicating their potential for phytotoxicity.

5.3. Microbiological activity in sewage treatment systems

Inhibition of microbial activity by KPFBS was tested on sewage microorganisms according to OECD TG 209 (NICNAS, 2005; Echa dissemination website). The nominal concentrations were 100 mg/L, 1000 mg/L and 10000 mg/L. Two negative controls were included in the

study. 3,5- dichlorophenol was used as positive control. The exposure period was 3 hours. There were two inoculum controls. No clear dose-response was observed, and no toxic effects on respiration were observed in the tested inoculum as compared to controls. The results from this study indicated that KPFBS was not inhibitory to sewage microorganisms (EC_{50} > 1000 mg/L). → *Klimisch reliability 1- well documented, guideline study*.

5.4. Toxicity to birds

Newsted et al. (2008): One acute dietary study on two bird species and one reproduction study on bobwhite quail have been performed by Newsted et al. (2008). The acute dietary study was performed according to OECD 205 and the species tested were bobwhite quail and mallard. Nominal test concentrations of potassium PFBS were 0 (control), 1000, 1780, 3160, 5620 and 10 000 mg PFBS/kg feed. Control birds were fed a plain diet. The bobwhite quails and mallards were exposed through their feed for 5 days at 39 ^{0}C and 31 ^{0}C respectively, with a 16 hours light and 8 hours dark period. Few treatment related effects were observed. There was a statistically significant reduction in body weight gain in quail in the 5620 and 10000 mg/kg group during day 1-8. Statistically significant reductions in body weight gain in the mallards was observed in the 10000 mg/kg group only. Hence, the dietary LC50 values were determined to be >10000 mg/kg for both species. The NOEC was determined to be 3160 mg/kg for bobwhite quail and 5620 mg/kg for mallard.

For the bobwhite quail the NOEC was 3160 mg/kg due to reduction in body weight gain in the 5620 and 10000 mg/kg dose groups. The NOEC for the mallard was 5620 mg/kg due to reduction in body weight gain only in the 10000 mg/kg dose group. Regarding the reproduction study, the estimated NOEC for northern bobwhite quail exposed to PFBS in diet was 900 mg/kg, the highest concentration tested. Chronic dietary exposure of adult quail also led to concentration-dependent increase in PFBS accumulation in offspring eggs and juveniles, indicating maternal transfer to the progeny. → *Klimisch reliability 1- well documented, guideline study*.

The reproduction study was conducted according to OECD 206. Eighteen-week-old northern bobwhite quail were exposed to potassium PFBS. The nominal dietary concentrations were 100, 300 and 900 mg/kg, and the exposure period lasted 21 weeks. Each group consisted of sixteen pairs consisting of one female and one male bird per pen. Sixteen breeding pairs were included as negative controls and were fed an untreated diet. Recorded effects were adult health, body weight gain and feed consumption. Eggs were studied for egg shell thickness, egg shell cracks/abnormalities, infertility or embryo mortality. Cracked and abnormal eggs were discarded. Remaining eggs were incubated and allowed to hatch on day 21 of incubation. Evaluated reproductive endpoints were egg production, embryo viability, hatchability and hatchling health and survival. Hatchlings were housed in brooding pens and fed an untreated diet. Blood samples were collected from one 16-week old chick from each brooding pen. Adult birds and chicks were then euthanised and subjected to gross necropsy. There were no treatment related mortalities in any of the treatment groups. No PFBS related effects were noted on any lethal or nonlethal endpoints measured in the study and the dietary NOEC was determined to be 900 mg/kg ww feed. The authors concluded that PFBS exposure does not pose a significant risk to avian species. → *Klimisch reliability 1- well documented, guideline study*.

Two chronic studies with NOEC values of 200 (pilot study, Klimisch score 2) and 900 mg/kg feed with birds have also been reported on ECHAs dissemination website.

Table 37: Summary of effects on birds

Species	Duration	Endpoint	Effect level	Concentration	Comment	Reference
Northern bobwhite quail (*Colinus virginianus*)	5 days	Acute toxicity	LC_{50} NOEC	>10000 mg/kg 3160 mg/kg	OECD TG 205	Newsted et al., 2008
Northern bobwhite quail	21 weeks	Reproduction	NOEC	900 mg/kg	OECD TG 206	Newsted et al., 2008
Mallard (*Anas platyrhynchus)*	5 days	Acute toxicity	LC_{50} NOEC	>10000 mg/kg 5620 mg/kg	OECD TG 205	Newsted et al., 2008

5.5. Mammalian wildlife

The hazards described in Chapter 4 are also relevant for mammalian wildlife, especially to wildlife species with low reproductive output, because any negative effect on development or reproduction has a high likelihood of leading to serious effects at the population level for such species. Effects of PFBS on hormone levels or/and development/reproduction are of particular concern. In mice (Feng et al., 2017) and rats (NTP, 2019) decreases in total T4 and T3 levels and free T4 levels, and in addition for the mice study, increases in TSH levels. In the mice study (Feng et al., 2017) PFBS exposure caused a delay in perinatal growth, vaginal opening and first estrus cycle, and diestrus was prolonged (see Chapter 4).

PFBS has been shown to enrich in the edible parts of plants (see Section 3.7). For herbivorous mammalian wildlife species eating plants as the sole energy source this may be of particular concern. A quantitative risk characterisation is, however, not part of the SVHC assessment.

5.6. Other effects

Vongphachan et al. (2011): A gene expression assay was performed on avian embryonic neuronal cells of domestic chicken (*Gallus domesticus*) and herring gull (*Larus argentatus*) investigating key genes in the thyroid hormone pathway. Primary cultures of chicken embryonic neuronal (CEN) and herring gull embryonic neruonal (HGEN) cells were prepared for an in vitro screening method to determine the effects of PFAS exposure on the mRNA expression of TH-responsive genes. CEN and HGEN cells were prepared from the cerebral cortices of day 11 and 14 embryos, respectively. The cells were treated with PFBS at different concentrations 0.01, 0.1, 1, 3, 10, and 50 µM, the latter concentration was administered to the CEN cells only. Due to a decrease in cell viability following PFHxA treatment at 30 µM and 50 µM, 10 µM was the highest PFAS concentration used for subsequent mRNA expression in CEN and HGEN cells. The cells were incubated for 24 h. Cell viability was estimated using the Calcein-AM assay. RNA was extracted and complementary DNA (cDNA) was synthesised using reverse transcriptase (RT) prior to PCR assay. To investigate gene expression mRNA levels were quantified using real-time PCR. T3 was used as a positive control for gene expression analyses.

PFBS significantly altered the mRNA of TH-responsive genes in primary cultures of CEN and HGEN cells. The positive control triiodothyronine (T3) caused increased expression of TRalpha, TR-beta, Dio2, and RC3 in CEN, with reduced TTR expression and no changes in expression of Dio3, Oct-1, or MBP. In contrast, PFBS caused increased expression of D3

and RC3. RC3 expression was increased 2-fold at 10 µM (3 mg/L) PFBS. These effects could affect TH-dependent processes, while changes in RC3 expression could have consequences in synaptic plasticity, associative with learning and memory (Iniguez et al., 1993; Iniguez et al., 1996). HGEN cultures were substantially less sensitive to T3 (TR-alpha and TR-beta required ca. 10x greater concentration to show the same increase as in CEN, and RC3 required ca. 1000x greater T3 concentration to show the same increase as in CEN). HGEN showed no response to T3 by D2 or Oct-1. It should be noted that Octamer motif-binding factor 1 (Oct-1) transcription is not mediated by the thyroid system but may be activated by stress response. Lack of activation by T3 is expected. It should be noted that HGEN cell cultures showed significant activation by 3 mg/L PFBS. Again, this should be interpreted, not as a thyroid effect, but a general stress response in cell culture. The observed effects described in this study indicate that PFBS and other PFASs may have the potential to affect TH homeostasis in bird cells.

Lou et al. (2013): A study on *Xenopus laevis* aimed to investigate comparatively adverse effects of longterm exposure to PFOS and PFBS on the growth and sexual development of amphibians using *X. laevis*. Effects on gonads, livers, and sex-related genes were investigated. It was found that PFBS did not affect growth or survival but PFBS did not cause intersex and had no effect on the sex ratio of the frogs. PFBS induced a significant increase in expression of estrogen and androgen receptor activity in female and male brains at all concentrations. Aromatase activity in the brain was not altered by PFBS. A significant increase in mRNA expression of ER in the liver was observed in both male and females. In liver, the AR expression was only increased at 1000 µg/L for both sexes. However, there were no clear dose-response relationship. Positive controls for estrogen receptor and androgen receptor did not show notable effects on transcription in brain and liver tissue extracts. The authors indicated that the increase in expression of ER and AR suggests an increase in the responsiveness to the corresponding sex hormone and potential effects on sexual development. However, these results are not suffcient to conclude on an endocrine mode of action.

5.7. Summary of the environmental hazard assessment

Ecotoxicity data are available for PFBS and the potassium salt of PFBS, KPFBS, which is the form of PFBS most often used in laboratory testing. With regards to ecotoxicity of the potassium salt, several proprietary GLP studies have been included in the registration dossier. Ecotoxicological studies, supported by *in vivo* studies in rodents and in vitro studies, provide evidence for adverse effects.

A full acute base set is available showing low acute ecotoxicity of PFBS. The acute toxicity (LC_{50}) to fish was reported to be 1938 mg/L (fathead minnow). PFBS exposure caused similar effects as PFOS in zebrafish, although at higher concentrations (Hagenaars et al., 2011; Ulhaq et al., 2013a; Ulhaq et al., 2013b). Exposure to PFBS of zebrafish larvae resulted in malformations, altered heart rates, oedemas and distinct changes in behavioral patterns, such as abnormal swimming behavior.

A few long-term toxicity studies on fish are available. The current data on marine medaka fish are considered as evidence regarding the thyroidal hormonal disturbances associated with exposure to PFBS. Similar thyroid disruptive effects have also been reported in rat and mice studies, see Section 4.11.2. PFBS exposure also adversely affected expressions of eye proteins that are related to visual functions and motor activity in marine medakas, and thus the muscular control of the eyes of the fish. However, the biological relevance of these effects are unclear since no anomalies in visual function were reported.

Furthermore, GSI values were significantly lowered in F0 marine medaka females exposed to 9.5 µg/L PFBS. This corresponds to an observed shift in F0 oocyte distribution where a significant increase in previtellogenic and significant decrease in vitellogenic and postvitellogenic oocytes were observed. This delay in oogenesis could in turn result in the

observed significant decrease in F0 fecundity. The systemic effects on the gonads in marine medaka shown by lowered GSI, delayed oogenesis and reduced fecundity are consistent and indicate an effect of PFBS on fish reproduction. Based on these effects on reproduction in marine medaka it is concluded that PFBS fulfils the T criteria for the environment of Annex XIII of REACH (i.e. NOEC or EC_{10} for marine or freshwater organisms less than 10 µg/L).

Acute algal toxicity tests (96 hour duration) with the freshwater algae *Pseudokirchneriella subcapitatata* indicate EC_{50} values > 5000 mg/L. While bioluminescence inhibition assays on bacteria and cyanobacteria indicated similar EC_{50} values, exposure to PFBS caused significant changes in model bacteria membranes. Data from acute studies with the freshwater crustacean *Daphnia magna* were similar with 48 hour EC_{50} value of >2000 mg/L. A 96 hour acute toxicity test with the marine crustacean *Mysidopsis bahia* is reported with an EC_{50} value of 372 mg/L. A chronic 28 day daphnid reproduction test resulted in a NOEC value of 502 mg/L. Long-term relevant population effects were reported in *C. riparius* following exposure to 10 µg/L PFBS. In addition, two acute toxicity studies with birds were reported by Newsted et al. (2008) with LC_{50} values >10000 mg/kg feed.

Exposure of wheat seedlings to PFBS/PFBA led to a reduced chlorophyll a content of 35% and reduced shoot biomass of 22.5%. PFCAs and PFSAs can cause a decrease in biomass in wheat and oxidative stress, indicating their potential for phytotoxicity.

Effects on mRNA expression of hormone receptors, and genes associated with the thyroid pathway have been shown in tadpoles and in avian neuronal cells, respectively (Lou et al., 2013; Vongphachan et al., 2011).

6. Conclusions on the SVHC Properties

6.1. CMR assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57 (f) of the REACH Regulation.

6.2. PBT and vPvB assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57 (f) of the REACH Regulation.

6.3. Assessment under Article 57 (f)

In the present support document it is evaluated whether perfluorobutane sulfonic acid (PFBS) and its salts should be regarded as "substances for which there is scientific evidence of probable serious effects to human health or the environment which give rise to an equivalent level of concern to those of other substances listed in Article 57 points (a) to (e) of the REACH Regulation".

The assessment follows a case-by-case approach and is based on available information in a weight of evidence evaluation. All studies and publications referenced in this dossier are considered relevant for the equivalent level of concern consideration for PFBS and its salts, and are used in this weight of evidence assessment. Despite the fact that some studies are not conducted according to standard guidelines under GLP conditions, or may not be considered highly relevant as standalone studies, there is no reason to discard any study.

As a strong acid, PFBS is readily deprotonated and forms sulfonate salts with bases, e.g. sodium, potassium and ammonium salts. In aqueous solution the salt forms and sulfonate ion will exist in equilibrium with PFBS itself. In the human body and in the environment PFBS will quickly be deprotonated by available bases and will be present dissolved in its anionic form. The concentrations reported in environmental and human monitoring studies will always be a sum of the acid PFBS, its anionic sulfonate form and its salt forms, see Chapter 1.

Several different precursors to PFBS exist and are used in mixtures and in products, including side-chain fluorinated polymers. Such related substances may degrade to PFBS during the use-phase of a product, in the waste stage or in the environment and may be used in higher overall volumes compared to PFBS itself or its salts. However, the concern in this evaluation assessment is first and foremost related to the ultimate degradation product PFBS that the precursor substances eventually will turn into, see Section 1.3.3.

6.3.1. **Summary of the data on the hazardous properties**

6.3.1.1. Persistency/degradation

The perfluoroalkyl chain is persistent due to the high stability of the C-F-bond, while the sulfur atom in the sulfonic acid group is at its highest oxidation state and cannot be oxidised further under environmentally relevant conditions. There are experimental data showing that the shorter chain trifluoromethane sulfonic acid was non-degradable in a hydrolysis study and in a biodegradation screening test (based on oxygen consumption) and could not be degraded by a bacterial strain which was able to defluorinate difluoromethane sulfonate and utilise it as a source of sulfur. The structurally similar longer chain sulfonic acids PFHxS and PFOS have already been identified as (very) persistent fulfilling the persistence criteria of REACH Annex XIII or the Stockholm convention (UNEP, 2006) (see Section 3.1.4).

Based on the available data, abiotic or biotic degradation of PFBS at relevant environmental conditions is expected to be very slow or negliglible, see Section 3.1. This is supported by read-across to trifluoromethane sulfonic acid and PFOS. For PFOS, UNEP reported in 2006 that no biodegradation had been demonstrated and it was found to be hydrolytically stable under environmental conditions with a hydrolytic half-life of 41 years (UNEP, 2006). This has been confirmed in later studies. At optimised lab conditions certain bacterial strains have been shown to degrade PFOS, see Section 3.1. However, at environmentally relevant conditions, such transformations have not been observed.

QSAR predictions and screening tests confirmed that PFBS is meeting the screening criteria for "potentially persistent/very persistent" according to ECHA Guidance on PBT/vPvB assessment (ECHA, 2017a). Very high persistence is also demonstrated by the presence of PFBS in remote areas where there is no indication of a possible direct emission source.

Based on experimental data, QSAR modelling information, read-across with relevant perfluoroalkane sulfonic acids like PFOS and trifluoromethane sulfonic acid and the detection of PFBS in remote areas like the Arctic, it is concluded that PFBS by far fulfils the P- and vP-criteria in REACH Annex XIII.

6.3.1.2. Partitioning in the environment and mobility

PFBS is a highly water-soluble, weakly sorbing substance with a high mobility in the environment. PFBS has a preference for distribution to the aqueous phase which is confirmed by distribution modelling and by field data demonstrating that PFBS is found in marine and surface water, and in ground water and drinking water samples throughout the world. Furthermore, PFBS is readily transported when water flows through soil. Being a strong acid, PFBS will be fully deprotonated at environmentally relevant conditions, and volatilisation of PFBS will be negligible, see Section 3.2.

Near fluorochemical industry facilities high PFBS concentrations are often found in surface water and groundwater. At an industrial site in China a 24-fold increase of the PFBS-concentration in the local groundwater was registered from 2009 to 2017.

6.3.1.3. Decontamination and release reduction for removal of PFBS from the environment and from drinking water

Due to the preference for the aqueous phase in the environment, the most important compartment for PFBS decontamination is water. PFBS is not readily removed with conventional water purification techniques. The same properties that make PFBS a highly mobile substance in the environment are also the reason why removal of PFBS is challenging. Due to the high aqueous solubility and the low sorption potential, PFBS will only to a low degree bind to adsorption materials and will rather remain in the water phase through the purification process. Drinking water production, as well as purification of wastewater and industrial effluents may suffer from the low purification efficiency for PFBS, see Section 3.3.

The presence of PFBS precursors may complicate the water treatment process even further as the precursors may behave differently through the purification steps and may break down to PFBS either during or after purification.

The methods available today to remove PFBS from drinking water and lower the human exposure to PFBS are expensive and not commonplace. In a recent global survey on drinking water, PFBS was the third most frequently detected PFAS in bottled water (47% of the samples) and tap water (88% of the samples).

The Swedish National Food Agency has recommended limits for drinking water based on the presence of 11 PFASs (PFBS, PFHxS, PFOS, 6:2 FTSA, PFBA, PFPeA, PFHxA, PFHpA,

PFOA, PFNA and PFDA)[13]. If the sum of these 11 PFASs occurs at concentrations greater than 0.09 µg/l, the Agency recommends that measures are taken as soon as possible to reduce the pollution. That PFBS is specified as one of the 11 PFASs covered by the limit value, demonstrates that there is specific concern associated with this particular compound.

6.3.1.4. Potential for long-range transport

The long environmental lifetime and the high mobility of PFBS results in a high global contamination potential. A characteristic travel distance of 17616 km and an overall environmental persistence, P_{OV}, of 220 days have been estimated. Long-range transport of PFBS is mainly via water and sea currents. Long-range transport of PFBS-related substances and degradation to PFBS may take place via other routes of transportation than those of PFBS, such as atmospheric transport. This also contributes to the concentrations of PFBS in the environment, see Section 3.4.

Sea spray aerosols contribute to the global transport of PFAAs, including PFBS, through the capacity to circulate significant amounts of PFAAs between the oceans and the atmosphere. A portion of the mass emitted from the oceans will deposit on land, thus re-entering the terrestrial system. Hence, exposure of humans and the environment to PFAAs like PFBS might continue even if emissions of the substances are stopped.

The global contamination potential has been demonstrated by findings of PFBS in samples of surface water, snow, ice, air and marine water in remote areas such as the Arctic, as well as in the Antarctic. PFBS has also been detected in Arctic biota, including polar bears and killer whales.

6.3.1.5. Bioaccumulation

Measured BCF values in fish are in the range 0.36 to 27.5. The highest fish BAF measured (field study) is 1736, however we considered this study unreliable with a Klimisch score 3. In another field study a mean BAF for fish of 69 was reported, while for crab a mean BAF of 110 was measured. Compared to the numeric criteria in REACH Annex XIII it is concluded that PFBS is not bioaccumulative in aquatic organisms (BCF < 2000).

However, PFBS is bioavailable and has been shown to enrich in blood and internal organs of animals and humans. Unlike the enrichment of contaminants in adipose tissue, the accumulation in specific organs has a higher potential to cause adverse effects, see Section 3.6.PFBS has been detected in different species of wildlife, including green turtles and polar bears which are both listed on the IUCN red list of threatened species. PFBS was also detected in birds and in marine mammals like dolphins and killer whales. In the killer whale the substance was shown to transfer from mother to foetus. Likewise, PFBS has been found to transfer from mother to eggs in birds. Hence, birds and animals, including endangered species, are exposed to PFBS at sensitive life stages.

In humans PFBS has been detected in blood, breast milk, lungs, bone, kidneys, urine, hair and cord blood. A serum elimination half-life of around one month (up to 46 days) has been measured in humans. The half-life in pigs was reported to be similar to that in humans (43 days), and BMF values > 1 have been reported for pigs (1.2 (whole pig), 0.8 (meat), 6.4 (liver), 14 (blood plasma), 2.2 (kidney) and 0.9 (fat), indicating that PFBS might be bioaccumulative in pigs. No cut-off values for human elimination half-lives for fulfilling the B or vB criteria have been defined. Based on the lower half-life reported for PFBS in human blood compared to the half-lives of PFOA, 2 – 4 years, (B) and PFHxS, 7 -

[13] https://www.livsmedelsverket.se/en/food-and-content/oonskade-amnen/miljogifter/pfas-in-drinking-water-fish-risk-management?AspxAutoDetectCookieSupport=1

8 years, (vB), it is concluded that PFBS is less bioaccumulative. It should be remembered that the data on human half-life is scarce and that a great individual variability in the data on half-lives of other PFAAs in humans has also been observed. Furthermore, even though blood is used as a proxy for the entire body, the half-life in blood is not necessarily identical with the whole-body elimination half-life in humans. However, based on the available information it is considered that PFBS has a moderate bioaccumulation potential. The presence of PFBS in human cord blood is of particular concern since the foetus is highly vulnerable to exposure to toxic substances.

6.3.1.6. Enrichment in plants

Field irrigation with contaminated surface or ground waters and the use of contaminated sewage sludge as a soil conditioner are sources to PFASs in agricultural plants. From the plants PFASs can transfer to humans through the food chain. Several studies and field data have demonstrated the uptake of PFBS in plants. PFBS and other short-chain PFASs tend to enrich significantly in edible parts like leaves, vegetables and fruits. Uptake factors in shoot are in the range 2 – 4 for several crops like radish, celery, tomato and pea, while uptake factors of up to 14 in lettuce leaves and 42 in tomato leaves have been measured. Considerable enrichment of PFBS has also been demonstrated for cabbage, zucchini and maize.

In plant derived supplement feeds PFBS contributed 30% of the total amount of PFAAs in distillers dried grains with solubles and 13% in soybean meal (Li et al., 2019b). Furthermore, in an area with a pollution incident in Germany in 2006, PFBS was determined in grass silage and hay as feed for dairy cows at 68.4 ± 23.1 µg/kg dm and 993.6 ± 224.4 µg/kg dm, respectively. This shows that PFBS in this particular case was actually taken up in plants used as feed in agriculture, see Section 3.7.

6.3.1.7. Protein binding

PFBS can bind to serum albumin, and thereby the blood can distribute PFBS within the body, resulting in a potential to enrich particularly in blood rich tissues. Furthermore, PFBS acts as a substrate of organic anion transporting polypeptides, which function as an aid for uptake, elimination or reabsorption from urine to blood. It has also been demonstrated that PFBS can be transported into hepatocytes by the bile acid transporters, see Sections 3.6 and 4.1.1.

PFAAs resemble fatty acids in structure and concerns have been raised that PFAAs may disrupt fatty acid binding to transporter proteins or receptors and thus affect lipid metabolism. For example, PFBS was shown to bind to the liver fatty acid binding protein (L-FABP), which partly may explain the concentrations found in liver tissues or in kidneys where L-FABP is expressed. PFBS has also shown a potential to bind to and/or to activate the peroxisome proliferator-activated receptors (PPARα, PPARγ).

PFBS was found to compete with T4 for binding to the human thyroid hormone transport protein transthyretin (TTR), although the binding potency of PFBS was found to be considerably lower compared to T4, as well as compared to PFOS.

In animal derived supplement feeds PFBS contributed 17%, 13.3%, 9.2% of total PFAAs in blood meal, feather meal and meat meal, respectively.

Altogether, PFBS has been shown to bind to different transport proteins and receptor proteins, although the binding affinities and activating potentials in general are lower for PFBS than for the longer chain counterparts.

6.3.1.8. Effects on human health

Repeated dose toxicity studies in rats have documented PFBS-mediated effects on liver, kidneys and hematological system. A dose dependent decrease in hemoglobin and hematocrit levels was observed in male rats with a NOAEL at 60 mg/kg/day.

A developmental study in mice has documented endocrine disturbances, such as decrease in T3 and T4 following prenatal PFBS-exposure, together with deficits in perinatal growth, pubertal onset and reproductive organ development at doses ≥200 mg/kg/day. Decreased thyroid hormone levels were also observed in a 28-day rat study (all doses tested ≥62.6 mg/kg bw/day). These observations are of particular concern since low maternal thyroid hormone levels may lead to e.g. neurodevelopment deficiencies in the foetus.

Evidence for genotoxic or mutagenic effects of PFBS has not been reported, but the current knowledge of possible carcinogenic effects is still limited.Other effects of PFBS, including effects on lipid metabolism, neurotoxicity and immunotoxicity have been reported, but the database is limited.

In general, toxicological data point towards similar health effects for PFBS as documented for some long-chain PFAAs, although the potency seems to be lower. However, the adverse health effects observed for PFBS may contribute to concern for co-exposure, see Section 6.3.2.6.

6.3.1.9. Effects in the environment

Ecotoxicity data are available for PFBS and the potassium salt of PFBS, KPFBS, which is the form of PFBS most often used in laboratory testing. With regards to ecotoxicity of the potassium salt, several proprietary GLP studies have been included in the registration dossiers. A full acute base set is available showing low acute ecotoxicity of PFBS.

Exposure of wheat seedlings to PFBS/PFBA led to a reduced chlorophyll a content of 35% and reduced shoot biomass of 22.5%. PFCAs and PFSAs can cause a decrease in biomass in wheat and oxidative stress, indicating their potential for phytotoxicity, see Section 5.2.2.

Effects on mRNA expression of hormone receptors and genes associated with the thyroid pathway have been shown in tadpoles and in avian neuronal cells, respectively.

The ecotoxicological data showing effects on reproduction in F0 marine medaka fish (lowered GSI, delayed oogenesis and reduced fecundity) at 9.5 µg/L PFBS fulfils the T criteria for the environment of Annex XIII of REACH (i.e. NOEC or EC_{10} for marine or freshwater organisms less than 10 µg/L). Thyroid hormonal disturbances have also been observed in marine medaka fish.

6.3.2. **Concerns arising from the substance properties**

The Guidance on the preparation of an Annex XV dossier for the identification of substances of very high concern, v2. (2018)[14] provides general instructions to support the drafting. On the interpretation of the equivalent level of concern (ELoC), as laid down in Article 57 (f) of REACH, further support has been developed to assist all interested parties in the assessment of substances that may meet this category of concern. In 2012, ECHA, the Commission, and Member States prepared a discussion paper on the identification of substances as SVHC under Article 57 (f) with sensitisers as an example[15]. Some years later the Joint Research Centre (JRC) repeated this exercise for the example of neurotoxic and immunotoxic substances, testing to what extent the same assessment elements as

[14] https://echa.europa.eu/documents/10162/23036412/svhc_en.pdf/8faef33c-b46e-4186-8b7c-8cfbeccd0812
[15] https://echa.europa.eu/documents/10162/13657/svhc_art_57f_sensitisers_en.pdf

for sensitisers could be used on those health concerns[16]. It was concluded by JRC and discussed among Member States, ECHA, and the Commission that this same set of relatively generic assessment elements could be applied to assist the more detailed case-by-case assessment of an equivalent level of concern for these two endpoints. Since then, several substances were included into the Candidate List on the basis of an Equivalent Level of Concern for endocrine disrupting effects in the environment, for endocrine disrupting effects on human health and on effects on specific target organs after repeated exposure, i.e. kidney and bone. Assessment of these different cases suggests several additional elements that could be considered in the context of evaluating an equivalent level of concern for the environment like e.g. the possibility to adversely impact future generations and the possibility to impact regions or environmental organisms that are spatially remote from the point of environmental release of the substance.

In the ELoC assessment for PFBS, hazard-related arguments and arguments related to environmental abundance and fate play an important role in concluding on the weight of evidence . This is in line with a recent judgement of the Court (Case C-323/15 P)[17] where ELoC "*encompasses the possibility of taking into consideration, for the purposes of comparison, material going beyond merely the hazards arising from the intrinsic properties of the substances concerned*" (paragraph 34).

6.3.2.1. Concern for an irreversible and increasing presence in the environment

The available information on the physicochemical properties of PFBS, and its degradation and environmental presence, gives rise to concern that once the substance enters the environment, its presence will be irreversible. PFBS is stable towards both abiotic and biotic environmental degradation and has been shown to by far exceed the limits of being a very persistent (vP) substance, see Section 3.1. PFBS will persist in the environment and expose future generations. PFBS tends to distribute to the aqueous phase and is readily transported when water flows through soil. There are no natural barriers to prevent PFBS from being distributed to surface waters, groundwater and oceans once PFBS has entered the environment.

Hence, PFBS may remain in the environment for such long times that it becomes increasingly difficult to predict possible exposures. As long as PFBS-releases to the environment continue, including the degradation of precursor compounds, its presence and concentrations may continue to increase. Consequently, slowly increasing environmental concentrations may be unavoidable and irreversible.

Cousins et al. (2019) point out that since actions to reduce emissions of PFAAs started in 2000–2002, there has been no evidence of consistent declines of any PFAA in the environment or biota. This illustrates the irreversibility of releases of such persistent chemicals as PFAAs.

6.3.2.2. Intrinsic substance properties result in irreversible and increasing contamination of surface water, marine water and groundwater

Building on the concern laid down in Section 6.3.2.1, the information presented in Section 3.2 gives rise to the additional concern that once PFBS enters the environment, the

[16] Identification of Substances of Very High Concern (SVHC) under the 'equivalent level of concern' route (REACH Article 57(f)) – neurotoxicants and immunotoxicants as examples; http://publications.jrc.ec.europa.eu/repository/bitstream/JRC96572/jrc96572-identification%20svhc%20reach%20article%2057f.pdf

[17] http://curia.europa.eu/juris/liste.jsf?language=en&num=C-323/15

substance is free to move with the water phase and may thus lead to irreversible contamination. The combination of very high persistence and high mobility allows PFBS to distribute on a great spatial scale in the environment even far from the release sources.

The high solubility (52.6 g/L at 22.5-24 °C), low adsorption potential (Log K_{OC} 1.2-2.7) and low volatility (Henry's Law constant: 0.040 Pa * m^3/mol) of the ionised form ensure that PFBS will remain in the water phase. PFBS will only to a low degree be "filtered" out when water moves through different layers of sediment, see Section 3.2. Based on today's knowledge, there is no natural "sink" that will remove PFBS from the water phase, and therefore PFBS has the potential to reach remote and pristine areas, as well as ground water and drinking water sources.

Currently available methods to remove PFBS from water are expensive and not commonplace, see Section 3.3. Consequently, it is difficult to control emissions of PFBS from industry, purify wastewater or remove PFBS in drinking water production facilities. Suitable purification techniques may potentially become available in the future. However, their practical and economical applicability is as of yet uncertain. In light of this information and remaining uncertainties, past emissions to the environment are considered irreversible, leading to an irreversible exposure of the environment, as well as of man via the environment in practice.

Hence, the very high persistence of PFBS in combination with its high mobility in the aqueous phase leads to irreversible and potentially increasing contamination of the environment.

6.3.2.3. PFBS contamination worldwide, including in remote regions

The concerns expressed in Sections 6.3.2.1 and 6.3.2.2 lead to the concern for a worldwide spread of PFBS. The presence of PFBS at background concentrations in the low ng/L range in the global environment is already being reported and supports this concern. Due to the global water cycle and the fact that the aqueous compartments are all well connected, the very high persistence and the high mobility of PFBS lead to long distance transport processes in the environment (estimated CTD: 17616 km, see Section 3.4.2).

PFBS will with time gradually spread to the entire aquatic environment, mainly via sea currents and sea spray aerosols, see Section 3.4. Contamination of remote areas has been demonstrated by field analyses. Monitoring data confirm the uncontrollable spread of PFBS in the worldwide aqueous environment. PFBS has been detected in surface water, snow, ice, air and marine water from remote areas in the Arctic and the Antarctic, as well as in biota such as polar bears, killer whales, northern fulmars and thick-billed murres.

Furthermore, long-range transport of volatile PFBS-related substances may take place via atmospheric transport. Subsequent degradation will release PFBS and contribute to the overall environmental concentrations of the substance. Thus, PFBS may affect humans and the environment far away from its points of emission into the environment and vulnerable populations and ecosystems in remote regions are affected. The detection of PFBS in remote areas, like the Arctic, is taken as evidence of the very high persistence and high mobility of PFBS, see Section 3.4.3.

6.3.2.4. Continuous presence in water results in continuous bioavailability

The intrinsic properties of PFBS lead to the concern for a continuous bioavailability of the substance worldwide. Information available on PFBS in different aqueous compartments demonstrate that PFBS is ubiquitously present in water samples at background concentrations, or higher concentrations in the vicinity of release sources, see Section

3.2.5. Section 3.5.3 shows that PFBS is found in marine biota, in fresh water biota and in other biota, while Section 4.1.2 shows that PFBS is taken up in humans. Biota levels are higher near point sources where the external concentrations are higher. PFBS is also enriched in plants, see Section 3.7. These monitoring data support that PFBS can be bioavailable and that exposure may take place via food and drinking water. Because PFBS stays in the water phase, the substance will be continuously bioavailable to organisms that live in water, organisms that drink water, plants that extract water from soil, organisms that eat plants and eventually humans who will be exposed e.g. through food and via drinking water. In the absence of any natural degradation processes or sink, PFBS will remain bioavailable over multiple generations and hence can lead to inter-generational effects. A similar concern is also described for highly soluble and highly polar substances by Reemtsma et al. (2016).

It is of particular concern that PFBS has been detected in the human body - in blood, breast milk, lung, bone, kidney, urine, hair and cord blood. Concentrations are generally low, but in a few studies concentrations up to 5 ng/mL have been observed and in persons with known elevated exposure even higher levels have been measured, see Section 4.3.

The bioavailability, in combination with the concerns expressed in Sections 6.3.2.1, 6.3.2.2 and 6.3.2.3, lead to concern for increasing internal exposures in wildlife and humans over time, which can be expected to trigger effects.

6.3.2.5. PFBS may enter biota and humans via several routes

PFBS enters the biosphere as a result of manufacturing and industrial emissions and effluents, and through the use of PFBS or precursor compounds in mixtures and articles. PFBS and its precursors have a wide dispersive use, including in consumer products, e.g. for the surface treatment of textiles and porous hard surfaces.

Humans and animals are exposed to PFBS in water and plants through the diet, see Sections 3.2.5 and 3.7. Near point sources, local drinking water, homegrown vegetables and eggs from domestic hens may have elevated PFBS levels, see Section 3.9. It has been shown that PFBS enriches in the edible parts of agricultural products and feed. PFBS is frequently found in surface water, tap water, bottled water and produced drinking water, with the highest concentrations found in areas with high PFBS contamination. Humans are also exposed to PFBS through the indoor environment and household dust, see Section 4.2.

Occurence of PFBS in animal- and plant-derived protein supplement feed may increase as a result of increasing PFBS concentrations in the environment and the compound's ability to bind to proteins and enrich in plants. Since these supplemental feeds might be the main feed source for livestock and poultry, human exposure through consumption of livestock and poultry together with PFBS contaminated drinking water and plant derived food, might lead to long-term continuous elevated human exposure.

Continuous exposure is accompanied by a continuous bioavailability and constantly elevated levels of PFBS within the body. If releases of PFBS to the environment are not minimised, concentrations in vegetables and drinking water relevant for human nutrition will increase further.

Lam et al. (2016) observed a significant increasing temporal shifting trend from PFOS to PFBS in dolphin liver from the South China Sea in the period 2002 – 2014. This pattern was assigned to the substitution of PFOS by PFBS as an alternative in various applications. The observation supports that increased use of PFBS and its precursors may lead to increased levels of PFBS in biota.

6.3.2.6. Weight of evidence evaluation of concern for adverse effects on human health and the environment

Chapters 4 and 5 and their summaries in Section 6.3.1.8 and 6.3.1.9, respectively, suggest that PFBS has the potential to cause serious effects to humans and the environment. PFBS or its salts do not currently have a harmonised classification under 1272/2008. However, self-classifications do exist for e.g. PFBS and KPFBS.

Human health

PFBS bioaccumulates differently compared to the classic lipophilic substances, due to the binding to serum albumin and other proteins in blood and tissues, see Sections 3.5, 3.6 and 4.1. The protein binding and atypical distribution influence the distribution in the human body. Moreover, due to the differences in half-lives observed for PFBS in humans, around one month (up to 46 days), compared to rats and mice (less than 1 day), the toxicity observed in animal studies may underestimate the toxicity in humans. In particular, long-term effects cannot be sufficiently assessed, and a precautionary approach should be taken when considering the risks related to the substance.

PFBS is bioavailable and has been detected in human blood, breast milk, lung, bone, kidney, urine and hair. It has also been detected in human cord blood which is of particular concern since the foetus is highly vulnerable to exposure to toxic substances.

Toxicological data obtained for PFBS point towards similar health effects as documented for some long-chain PFAAs, although the potency seems to be lower. Effects on the liver, kidneys and the hematological system have been observed in rats, see Chapter 4 and summary in Section 6.3.1.8.

It is of particular concern that studies have found that PFBS may affect thyroid hormones together with deficits in growth and development, see Chapter 4. PFBS thereby has a potential for causing adverse effects in both humans and wildlife. Effect on thyroid hormones has been shown both for rats and mice.

The very high persistence, high mobility and bioavailability of PFBS leads to continuous exposure, and this adds to the concern for the observed effects, see Sections 6.3.2.1 to 6.3.2.5. It may therefore not be possible to reverse the exposure, and effects on human health and the environment are thereby to be considered irreversible. With a continuous and irreversible exposure, even effects that would be reversible in standard toxicity tests upon removal of exposure, would be irreversible if the environmental concentrations are allowed to reach levels that could trigger effects. Furthermore, there is a concern for currently unknown effects of PFBS in humans that may not be observed in standard toxicity tests or may only develop after life-long exposure.

Environment

PFBS has been detected in different species of wildlife, including endangered or vulnerable populations and in sensitive life stages, see Sections 3.4.3.3 and 3.5.3.1.

Low acute ecotoxicity has been measured for PFBS.

Exposure of wheat seedlings to PFBS/PFBA led to a reduced chlorophyll a content of 35% and reduced shoot biomass of 22.5%. PFCAs and PFSAs can cause a decrease in biomass in wheat and oxidative stress, indicating their potential for phytotoxicity, see Section 5.2.2.

Effects on mRNA expression of hormone receptors and genes associated with the thyroid pathway have been shown in tadpoles and in avian neuronal cells, respectively.

The ecotoxicological data showing effects on reproduction in F0 marine medaka fish (lowered GSI, delayed oogenesis and reduced fecundity) at 9.5 µg/L PFBS fulfils the T criteria for the environment of Annex XIII of REACH (i.e. NOEC or EC10 for marine or freshwater organisms less than 10 µg/L). Thyroid hormonal disturbances have also been seen in the exposed marine medaka.

In addition, there is a concern for currently unknown effects of PFBS in the environment that may not be observed in standard toxicity tests or may only develop after life-long exposure. This concern originates from the fact that PFBS will remain in the environment for so long that there is a high probability that effects in the environment that are not known today may be discovered in coming years, which is similar to what is described in the context of unknown effects for human health.

Concern for sensitive life stages, vulnerable species and future generations

PFBS has been measured in different species of wildlife, including green turtles and polar bears, which are both listed on the IUCN red list of threatened species. PFBS was also detected in marine mammals like dolphins and killer whales. In the whale the substance was shown to transfer from mother to foetus. Likewise, PFBS has been found to transfer from mother to eggs in birds, see Section 3.4.3.3.

It is of concern that PFBS is transferred from mother to offspring in humans, whales and birds. The foetus is highly vulnerable and sensitive to exposure to toxic substances. Future generations are exposed to PFBS at this sensitive development stage, including endangered species.

The weight-of-evidence for potential effects on human health and the environment can be characterised by two types of effects:

1. Observed adverse effects: concern for serious effects observed in the available studies that can be assessed based on the available data with (high) probability for human health and the environment, see paragraphs on human health and the environment earlier in this section.

2. Yet unknown effects: there is concern for possible effects that may only become apparent after life-long exposure that are normally not tested for in standard toxicity tests. This concern is inherent to the very high persistence of the substance and its continuous bioavailability.

Unexpected effects from life-long exposure to PFBS represent a concern when taken together with the findings that PFBS transfers from mother to foetus in humans and in animals and may affect sensitive life stages and future generations.

Possibility to remedy effects

Following the concerns expressed in Sections 6.3.2.1 to 6.3.2.5, exposures may occur with a delay, as measured from the moment of emission, which complicates management or prevention of effects. Methods to remove PFBS from water are expensive and not commonplace, see Section 3.3. Control of emissions of PFBS from industry, purification of wastewater or removal of PFBS during drinking water production may therefore be challenging. Suitable purification techniques may potentially become available in the future. However, their practical and economical applicability is highly uncertain, and such remediation techniques may come with high societal costs. Degradation of precursor compounds with formation of PFBS represents a future source of PFBS, even if the emissions of such precursors are stopped immediately. Consequently, once effects become apparent, it may be too late to take measures to protect humans and the environment from exposure.

Brendel et al. (2018) point out that the lack of appropriate water treatment technologies results in everlasting background concentrations in the environment, and thus, organisms are permanently and poorly reversibly exposed. Considering such permanent exposure, it is very difficult to estimate long-term adverse effects in organisms.

Concern related to co-exposure

The concerns brought forward in Sections 6.3.2.1 to 6.3.2.5 in combination represent a considerable concern deriving from PFBS itself. However, additional to this is the concern for co-exposure and combined effects from PFAAs of different chain lengths with similar effects on human health or the environment. Often more than one PFAAs can be identified in environmental samples, suggesting that PFAAs are likely to co-occur in soil, the aqueous environment and drinking water. For example, several PFCAs (e.g. PFOA) and PFSAs (e.g. PFOS) are often found in single monitoring sample. Gebbink et al. (2017) measured C4-C10 PFCAs and C4 and C6-C8 PFSAs (including PFBS) in river water nearby an industrial production plant in the Netherlands and found all substances in samples both upstream and downstream of the factory. Co-exposure is further substantiated by human biomonitoring data, as multiple PFAAs can be found in the blood plasma of an individual (Fromme et al., 2017; Calafat et al., 2019), see Section 4.12. This is one example of an endpoint for which additive effects among closely related PFASs may be expected. However, in general the current knowledge about combination effects of PFASs is limited. Due to high persistence of these substances, co-exposure may last for a very long time.

Summary of the overall assessment of effects on human health and the environment

Toxicological data obtained for PFBS point towards similar health effects as documented for some long-chain PFAAs, although the potency seems to be lower. Due to the differences in half-lives observed for PFBS in humans, around one month (up to 46 days), compared to rats and mice (less than 1 day), the toxic effects observed in animal studies may underestimate the toxicity in humans. PFBS has been found to affect thyroid hormones in mice together with deficits in perinatal growth, pubertal onset and reproductive organ development. Effects on liver, kidneys and the hematological system has been observed in rats.

Ecotoxicological studies, supported by in vivo studies in rodents and in vitro studies, provide evidence for adverse effects. Reduced chlorophyll a content and shoot biomass was observed when wheat seedlings were exposed to PFBS/PFBA. PFCAs and PFSAs can cause a decrease in biomass in wheat and oxidative stress, indicating their potential for phytotoxicity. PFBS has been found to affect mRNA expression of genes associated with hormonal pathways in tadpoles and in avian neuronal cells, while thyroid hormonal disturbances have been observed in marine medaka fish exposed to the substance. The ecotoxicological data showing effects on reproduction in F0 marine medaka fish (lowered GSI, delayed oogenesis and reduced fecundity) at 9.5 µg/L PFBS fulfils the T criteria for the environment of Annex XIII of REACH (i.e. NOEC or EC10 for marine or freshwater organisms less than 10 µg/L).

PFBS has been detected in human blood, breast milk, cord blood, lung, bone, kidney, urine and hair. Furthermore, PFBS has been found to transfers from mother to foetus in humans and in animals and may affect sensitive life stages and future generations.

6.3.2.7. Derivation of limit values is highly uncertain

Due to the inherent properties of PFBS (e.g. very persistent and highly mobile in the environment), it is difficult, maybe even impossible, to reliably estimate current and future environmental concentrations and thereby exposure to animals and humans, see Section

6.3.2.1. Potential adverse effects due to life-long exposure are largely unknown. Considering the poor reversibility of the environmental contaminations and the potential for continuously increasing background levels, estimation of acceptable releases is not possible. Hence, the only way to adequately control the risk is through minimisation of emissions.

Furthermore, the toxicity observed in animal studies may underestimate the toxicity in humans due to the differences in half-lives observed for PFBS in humans, around one month (up to 46 days), compared to rats and mice (less than 1 day).

Estimation of future environmental concentrations and exposure via the environment is further complicated by the use and emissions of precursor compounds that may degrade to PFBS. There is considerable use of such precursor compounds. The degradation time for the precursors depends on their specific identity, see Section 1.3.3.

PFBS is only one member of the large group of PFASs that humans and the environment are exposed to, see Section 1.4. The number of PFASs in products encountered in daily life has expanded, and in the last count by OECD, 4730 PFAS-related CAS-numbers were found. We are exposed to an increasing number of new and unidentified fluorinated products, some of which may share common mechanisms of actions and have additive effects. Often more than one PFAS can be identified in environmental samples, suggesting that PFASs are likely to co-occur in soil and the aqueous environment. In general the current knowledge about combinatorial effects of PFASs is limited. However, there are examples of studies that show that the effect of closely related PFASs add up for some endpoints, see Section 4.12 and Zeilmaker et al. (2018).

Long-term exposure cannot be reliably estimated. Hence, the concerns outlined in this document cannot be readily quantified with a sufficient level of certainty using the currently available information. Previous emissions that already have happened would need to be accounted for together with current and future releases. In addition, the degradation of precursors with formation of PFBS would constitute a secondary source of PFBS, and the PFBS levels in the environment might continue to increase even if emissions are stopped. Consequently, the establishment of a 'safe concentration' for PFBS in the environment is not possible.

6.3.2.8. Comparison of above concerns to concerns of PBT/vPvB substances

The ECHA Guidance for PBT/vPvB assessment (Chapter R.11) (ECHA (2017a) states:

"*Experience with PBT/vPvB substances has shown that they can give rise to specific concerns that may arise due to their potential to accumulate in parts of the environment and*

- *that the effects of such accumulation are unpredictable in the long-term;*
- *such accumulation is in practice difficult to reverse as cessation of emission will not necessarily result in a reduction in substance concentration*."

"Furthermore, PBT or vPvB substances may have the potential to contaminate remote areas that should be protected from further contamination by hazardous substances resulting from human activity because the intrinsic value of pristine environments should be protected" (ECHA Guidance R.11)."

The concerns expressed in Sections 6.3.2.1 to 6.3.2.7 are the same as these three key concerns for PBT/vPvB substances. PFBS with its very high persistence, high mobility and long-range transport potential matches very well with the guidance description above. Cousins et al. (2019) reflect on this type of concern in a more general manner for

compounds that are highly persistent and poorly adsorb to organic matter and sediments. They even demonstrate that if a chemical is highly persistent, its continuous release will lead to continuously increasing contamination irrespective of the chemical's other physical–chemical properties. It is argued that the increasing concentrations will result in increasing probabilities of the occurrence of known and as yet unknown effects and that, once adverse effects are identified, it will take decades, centuries or even longer to reverse contamination and therefore effects.

Section 4.0.1 of REACH Annex I explains that a hazard assessment addressing all the long-term effects and the estimation of the long-term exposure of humans and the environment cannot be carried out with sufficient reliability for PBT/vPvB-substances. This is also the case for PFBS and the concerns are of similar nature. Long-term exposure estimations for PFBS will be highly uncertain because of the very high persistence, the irreversibility of contaminations and the formation of PFBS from precursor compounds. Effects of PFBS on human health and the environment have been documented, and this is accompanied by the uncertainty of effects arising at longer time scales.

The environmental concentrations of PFBS will increase if the use and emissions continue. Because of its high long-range transport potential, due to the very high persistence and high mobility in water, remote areas and pristine environments will also experience increasing PFBS levels. The environmental abundance of PFBS is considered most likely impossible to reverse as there are no practically available methods for the removal of PFBS from the environment. Degradation of precursor substances to PFBS will result in continuous increase in the environmental concentrations and will further complicate the environmental distribution and potential clean-up. Hence, exposure of humans and the environment will likely continue for generations even after cessation of use. See also Section 6.3.2.1 and Cousins et al. (2019).

Continued manufacture, use and release of PFBS will lead to higher levels of PFBS in the environment as there is no natural sink or way of removal for PFBS. Hence, all the mass of PFBS that gets emitted will be bioavailable. The exposure will increase, together with the levels of PFBS in humans and biota. How the concentrations will compare to PBT/vPvB-substances depend on many factors and is difficult to predict. However, common for both PFBS and classic PBT/vPvB-substances is that once harmful levels in the environment have been reached, it is in practice difficult to remove the exposure and thereby reverse the harm.

Cousins et al. (2019) used model calculations to show the importance of persistence on environmental concentrations. The results indicated that concentrations of very persistent chemicals (e.g. with a degradation half-life in the order of 2000 days) may continue to increase for a certain time even after the emissions start to reduce. After the stop of emissions, the decreasing concentrations show a long tail that extends for many years.

A further similarity between PFBS and PBT/vPvB substances is the concern that irreversible or poorly reversible internal exposures to a substance can potentially lead to toxic effects that are not already known, as it appeared in several historical PBT/vPvB cases. This same concern is expressed for PFBS in Section 6.3.2.4. Both highly persistent and bioavailable substances, as well as PBT/vPvB substances, lead to unpredictable and uncontrollable internal exposures in the organism. PFBS is less readily adsorbed to soil, sediment and suspended matter compared to less mobile substances due to its properties. Therefore, it is likely that a higher proportion of the emitted substance will be bioavailable for organisms.

Regarding vPvB substances, ECHA guidance (ECHA 2017a) states:

"*In the case of vPvB substances, there is concern that even if no toxicity is demonstrated in laboratory testing, long-term effects might be possible since high but unpredictable levels may be reached in man or the environment over extended time periods*"

PFBS as a very persistent and highly mobile contaminant behaves differently in the environment as compared to a classic PBT/vPvB-substance that has a higher bioaccumulation potential and a lower mobility due to higher adsorption potential and lower aqueous solubility. The mobility of PFBS in soil and along waterways and the potential for long-range transport with sea currents will be higher. On the other hand, a low bioaccumulation potential may lead to less accumulation in the food-chain. If the half-life of excretion is lower, the levels of the substance in the body will decrease faster if the exposure is removed. On the other hand, calculations show that with an assumed half-life for PFBS in water of 10 years and a reported BAF in crab of 110 (Naile et al., 2013), the concentrations of PFBS in aquatic biota may be expected to exceed the biota concentrations for a persistent and bioaccumulative substance over time, see Section 3.5.2. Persistent and bioavailable substances, if either mobile or bioaccumulative, would seem to share the same concern for the development of high internal concentrations which may trigger effects. The continuous exposure, high mobility in water, no removal of PFBS from the environment and the enrichment in plants may ensure a continuous exposure via drinking water and diet.

6.3.3. **Overall concern and assessment of the level of concern**

PFBS is considered to be of equivalent level of concern to substances meeting the criteria laid down in Article 57 (a) to (e) according to Article 57 (f) of the REACH Regulation ((EC) 1907/2006) because of the overall concern arising from the concern elements described in Section 6.3.2. The elements which are used in this case for assessing the level of concern and description of how PFBS compares to those elements are listed in Table 38.

PFBS has due to its very high persistence, high mobility, potential for long-range transport, observed effects to human health and environment, contamination of water and accumulation in plants, a very high potential to cause effects in wildlife and in man via the environment. The very high persistence together with low adsorption potential (and therefore difficulty for end-of-pipe treatment) and high mobility imply a very high potential for increasing pollution stock. Outside of PFBS hotspots, irreversible, bioavailable, increasing exposures of both wildlife and man via the environment will be maintained as long as emissions continue, and even if emissions are stopped, the background concentrations will not start to decrease immediately. The consequence is a high potential for irreversible effects once effect levels have been reached, and an increasing seriousness of effects while exposures keep increasing. However, locally, close to emission sources, PFBS concentrations will decrease upon cessation of releases due to diffusion. The substance has due to its intrinsic properties a high potential to cause widespread exposures. Due to the challenging decontamination of drinking water sources, as well as the large variety of exposure routes via drinking water and food intake, there are no possibilities to avoid the continuous and increasing exposure in any human populations. Neither will any wildlife populations be protected from the whole released mass of the substance. It follows that both environment and humans are susceptible to impairment at large.

Table 38: Overview of the qualitative components relevant for assessing the level of concern

Irreversibility of the exposure of wildlife and man via the environment	PFBS has high potential to cause irreversible exposures. Based on experimental results from screening tests on KPFBS, read-across to similar measurements for the corresponding C1 and C8 sulfonic acids, as well as supportive evidence from BIOWIN predictions, it can be concluded that the

	degradation potential of PFBS in all environmental compartments is very low or negligible. Its very high persistence implies that PFBS will remain in the environment much longer than most other substances that are identified as exhibiting P or vP properties. PFBS by far fulfils the limits for being P and vP of Annex XIII of the REACH regulation. Exposures are not expected to decrease upon cessation of releases because of the very high persistence of the substance. Degradation of precursors with formation of PFBS constitutes a secondary source of PFBS, and the PFBS levels in the environment might continue to increase even if emissions of PFBS are stopped. The high potential to cause very long-term exposures causes a difficulty to quantify exposures with sufficient certainty.
Potential for rapid and wide geographic scale contamination	Due to the global water cycle and the fact that the aqueous compartments are all well connected, the very high persistence and the high mobility of PFBS lead to long distance transport processes in the environment. PFBS has already been detected in samples of surface water, snow, ice, air, marine water and biota from remote areas such as the Arctic and the Antarctic. Wide dispersive use of PFBS and its precursors in various applications further facilitates the global distribution of PFBS.
Potential to continuous increase of exposures	PFBS has a very high potential to cause an increasing pollution stock due to the combination of very high persistence and difficulty of using end-of-pipe emission reduction measures (as a result of low adsorption potential, negligible degradation and high water solubility). Furthermore, as a highly mobile substance, there are no local or intermittent sinks for the pollution stock and therefore the substance has high potential to cause continuous increase of exposure of wildlife. Additionally, due to the difficulty and costs of decontamination and remediation techniques, PFBS has a high potential to cause continuously increasing exposure of humans via the environment. It is very difficult to remove PFBS from water. Adsorption of PFBS to soil, sediment and organic matter is poor. Available techniques suggest that remediation of PFBS-containing water comes with high costs for society and the generation of serious amounts of waste from the water purification process. Removing PFBS from the water compartment of the environment is impossible in practice due to the widespread

	presence in the aqueous environment and the high mobility of the substance.
Potential for causing serious effects although those would not be observed in standard tests	The continuously increasing pollution stock (see above), will lead to increasing internal exposures and a high likelihood of reaching levels which would cause effects. The whole released mass of the substance will be continuously bioavailable to organisms that live in water, organisms that drink water, plants that extract water from soil, animals that drink water and eat plants or water living organisms and humans who will be exposed e.g. through food and via drinking water. With time serious effects even for endpoints where the substance would show a low or moderate intrinsic toxicity based on standard tests, may be expected. Wildlife feeding on e.g. water and plants which enrich PFBS may be susceptible to reaching effect levels.
Potential for causing serious effects on human health (known and unknown), and the environment (including the potential for irreversible effects)	For human health, the concerns relate to thyroid hormone disturbances observed in mice, together with developmental effects. Effects have also been observed on thyroid hormones and on the liver, kidney and haematological system in rats. The effects on thyroid hormones are of particular concern since the foetus is dependent on maternal production of thyroid hormones. Together with the data showing a moderate bioaccumulation potential in humans, these health effects are of concern. The ecotoxicological data showing effects on reproduction in F0 marine medaka fish (lowered GSI, delayed oogenesis and reduced fecundity) at 9.5 µg/L PFBS fulfils the T criteria for the environment of Annex XIII of REACH (i.e. NOEC or EC10 for marine or freshwater organisms less than 10 µg/L). Reduced chlorophyll a content and shoot biomass was observed when wheat seedlings were exposed to PFBS/PFBA. PFCAs and PFSAs can cause a decrease in biomass in wheat and oxidative stress, indicating their potential for phytotoxicity. PFBS has been found to cause effects on mRNA expression of hormone receptors in tadpoles and in genes associated with the thyroid pathway in avian neuronal cells, while thyroid hormonal disturbances have been observed in marine medaka fish exposed to the substance. The continuous long-term and increasing exposure could eventually trigger effects that could be considered irreversible, even if they are normally reversible in standard toxicity studies

	upon the removal of exposure.
Delay of effects	The highly mobile character of PFBS together with its very high persistence mean that exposures may occur with a delay, as measured from the moment of emission. It can be argued for PFBS that it is equally difficult to manage or prevent effects when exposure could occur with a delay, as it is difficult to manage or prevent effects that could occur after a long-term exposure. Degradation of precursor substances with formation of PFBS represents a long-term source of PFBS to the environment that will contribute long after emissions are ceased.
Potential to cause combined effects (co-exposure)	Humans and the environment are exposed to a mixture of PFASs, including PFBS, in a cumulative exposure. Co-exposure has been demonstrated by monitoring studies when several PFASs have been detected in the same sample. For example, multiple PFCAs (e.g. PFOA) and PFSAs (e.g. PFOS) are often found in environmental samples. Co-exposure may lead to additive effects and may last for a very long time as degradation of PFBS and other PFASs in the environment is likely to be very low or negligible.
Uncertainties in deriving safe concentration limits	The potential increasing and irreversible exposures may lead to PFBS causing yet unknown effects for human health and the environment. With time, effects may be discovered that may lead to more stringent safety levels for PFBS. The derivation of safe exposure levels may therefore be possible in principle but is considered not to be sufficiently reliable.
Possibility to remedy effects	Reversing effects may hardly be possible due to the difficulty to reduce exposure. Consequently, for PFBS prevention of emissions is preferred over remediation of releases and treatment of effects. Monitoring data have demonstrated the global transport of PFBS as the substance has been detected in samples of surface water, snow, ice, air and marine water from remote areas such as the Arctic and the Antarctic.
Uncertainties in quantifying exposures with sufficient certainty	Due to its very high persistence and high mobility, and the degradation of precursors as a long-term source of PFBS, quantifications of future exposure to PFBS are highly uncertain. No currently available exposure estimation tools would reliably

	predict exposures from such substances.
Potential to impair humans and the environment at large	There are no natural barriers or environmental sinks that may reduce environmental concentrations of PFBS, and hence exposure. There is a concern that the high potential for wide geographical scale contamination, in combination with its very high persistence and adverse effects (as described above), may eventually lead to an unpredictable and uncontrollable risk for human health or the environment. PFBS has already been detected in human blood in studies from Europe, US and Asia. Furthermore, PFBS has been found in biota such as dolphins and whales, as well as green turtles and polar bears, which are both threatened species.
Intergenerational effects	PFBS persists in the environment for a very long time, and, possibly, effects of current emissions may be observed or only become apparent in future generations. PFBS has a moderate bioaccumulation potential in humans and has also been detected in human cord blood. PFBS has been demonstrated to be transferred from mother to foetus in killer whales and to eggs in birds.
Societal concern	Art. 7.3 of the Water Framework Directive (2000/60/EC) stipulates that *"Member States shall ensure the necessary protection for the bodies of water identified with the aim of avoiding deterioration in their quality of water to reduce the level of purification treatment required in the production of drinking water."* PFBS is frequently found in surface water, tap water, bottled water and produced drinking water. Highest concentrations are found in areas with high PFBS contamination. Decontamination can only be achieved at high societal costs, if at all. Consequently, there is societal concern for the presence of PFBS in drinking water that requires immediate action.

6.3.4. Conclusion on the hazard properties and equivalent level of concern assessment

Perfluorobutane sulfonic acid (PFBS) and its salts are identified as substances of very high concern in accordance with Article 57(f) of Regulation (EC) 1907/2006 (REACH) as there is scientific evidence of probable serious effects to the environment and human health which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of REACH.

Substance identification

PFBS-salts are fully indistinguishable from PFBS in the environment as the salts exist in their dissociated anionic sulfonate form, just like PFBS itself, and they are all a part of an acid-base equilibrium in water. Hence, all conclusions on different end-points apply to any and all salt forms, as well as PFBS itself.

Intrinsic properties of PFBS

PFBS is very persistent in the environment. Based on the available data abiotic or biotic degradation of PFBS at relevant environmental conditions is expected to be very slow or negliglible. This is supported by read-across to perfluoroalkane sulfonic acids with both shorter and longer chain lengths, which also have a very low degradability. PFBS shows a preference for distribution to the aqueous phase due to its high solubility in water (52.6 g/L at 22.5-24 °C for the potassium salt), its low sorption potential (log K_{OC} 1.2 to 2.7) and it is considered highly mobile in the environment.

The very high persistence, together with low adsorption potential and high mobility, imply a very high potential for increasing environmental concentrations and potential irreversible exposures of wildlife and of humans via the environment. Long-term, low dose exposure may potentially lead to currently unexpected or even still unknown effects. In particular, endocrine disturbances may be of relevance when considering such exposure. PFBS is bioavailable via the aqueous environment. Together, these environmental fate properties lead to a high potential for irreversible effects. Furthermore, there are high costs and technical challenges related to the removal of PFBS using end-of-pipe treatment.

The high global transport potential (characteristic travel distance, CTD 17616 km, P_{OV} = 220 days), is demonstrated by detection of PFBS in samples of surface water, snow, ice, air and marine water from remote areas such as the Arctic and the Antarctic. This is supported by scientific assessments of the mobility of PFBS, together with QSAR modelling data. PFBS has also been found in biota like dolphins and whales, as well as green turtles and polar bears, which are both threatened species. This shows that PFBS is bioavailable, and that exposure may occur throughout the food chain and via drinking water and that this is already taking place worldwide.

Toxicological data relevant for human health assessment include effects on thyroid hormone disturbances observed in both rats and mice. These effects are serious and of particular concern since the developing foetus is dependent on maternal production of thyroid hormones. Evidence of effect on development and delay in pubertal onset was observed in mice and disturbed estrus cyclicity was observed in mice and rats. In addition, effects on liver, kidney and haematological system were observed in rats.

A serum elimination half-life of around one month (up to 46 days) has been measured in humans, which is considerably longer than the half-lives measured for rodents (less than 1 day). The limited data show that PFBS has at least a moderate bioaccumulation potential in humans. In pigs an average half-life of 43 days has been estimated.

The ecotoxicological data showing effects on reproduction in F0 marine medaka fish (lowered GSI, delayed oogenesis and reduced fecundity) at 9.5 µg/L PFBS fulfils the T criteria for the environment of Annex XIII of REACH (i.e. NOEC or EC10 for marine or freshwater organisms less than 10 µg/L). Exposure of wheat seedlings to PFBS resulted in reduced chlorophyll a content and shoot biomass together with a decrease in biomass and oxidative stress, which indicates a potential for phytotoxicity. Furthermore, PFBS has been found to cause effects on mRNA expression of hormone receptors in tadpoles and in genes associated with the thyroid pathway in avian neuronal cells, while thyroid hormonal disturbances have been observed in exposed marine medaka fish. Ecotoxicological studies, supported by *in vivo* studies in rodents and in vitro studies, provide evidence for adverse effects.

Overall, PFBS has a high potential to cause effects in wildlife and in humans exposed via the environment worldwide, due to its very high persistence, high mobility, potential for long-range transport, and observed adverse effects that are relevant for human health and the environment, and exposure via drinking water and food. The continuous and increasing exposure in human populations cannot be avoided if releases are not minimised. Similarly, wildlife populations cannot be protected from the total quantity of the substance released.

In addition, the potential for combined exposure to similar PFAAs substances is considered a supportive concern.

Scientific evidence of probable serious effects to human health and the environment is as follows:

- a moderate bioaccumulation potential in humans
- thyroid hormonal disturbances in rodents
- reproductive development deficiencies in mice
- disturbed estrus cyclicity in rodents
- effects on liver, kidney and haematological system in rats
- effects on reproduction in marine medaka (Environmental T)
- thyroid hormonal disturbances in marine medaka
- effects on mRNA expression of hormone receptors in tadpoles

The effects on thyroid hormones are serious since the foetus is dependent on maternal production of thyroid hormones important for e.g. growth, metabolism, reproductive organ and brain development. PFBS is also transferred to the foetus. The developmental effects are serious because they affect the embryos.

Based on the reported effects on reproduction in marine medaka PFBS fulfils the T criteria for the environment of Annex XIII of REACH (i.e. NOEC or EC10 for marine or freshwater organisms less than 10 µg/L). Thyroid hormonal disturbances in marine medaka are also observed. These environmental effects are supported by *in vivo* studies in rodents and *in vitro* studies, and provide evidence for adverse effects. In addition potential effects on hormone receptor expression in tadpoles are reported.

All these human health and environmental effects are serious because, in conjunction with environmental fate properties of PFBS (e.g. very high persistence, high mobility and long-range transport potential), they are potentially irreversible.

Equivalent level of concern

The level of concern is considered very high in particular due to the combination of the following concern elements:

- Potential for irreversible and increasing presence in the environment
- Potential for irreversible and increasing contamination of surface water, marine water and groundwater
- Continuous presence in water results in continuous bioavailability

- Worldwide occurrence
- PFBS enters the biosphere via several routes
- Intergenerational effects, observed mother-to-offspring transfer
- Potential for delay of effects
- Potential for causing serious effects although those would not be observed in standard tests
- Derivation of future exposure levels and safe concentration limits will be highly uncertain
- High societal concern for the presence of PFBS in drinking water sources

PFBS has been detected in humans worldwide and in different species of wildlife, including in endangered species and in remote areas. The substance has been found to transfer from mother to offspring in humans, whales and in birds and may disturb development at sensitive life stages and in vulnerable populations. It may be difficult in practice to manage exposures due to the high mobility of PFBS and the fact that exposures may take place at a different location than where releases occurred and at a different moment in time.

The very high persistence and high mobility of PFBS together lead to a concern for co-exposure with other contaminants with similar effects on human health and the environment. It may be expected that PFAAs cause similar effects, and hence that their individual contributions add up to the total effect. Co-exposure may lead to additive effects and may last for a very long time, because natural degradation processes for these substances are slow or negligible. This is brought into the weight-of-evidence as supportive information.

Limitations of the available remediation techniques raise a concern that the removal of PFBS from drinking water may only be possible with high societal costs. Remediation of environmental pollution may even be practically impossible due to the high mobility of the substance. Furthermore, PFBS will quickly diffuse from contaminated sites.

In conclusion

The combined intrinsic properties justifying the inclusion as a substance for which there is scientific evidence of probable serious effects to human health and the environment which give rise to an equivalent level of concern are the following: very high persistence, high mobility in water and soil, high potential for long-range transport, and difficulty of remediation and water purification as well as moderate bioaccumulation in humans. The observed probable serious effects for human health and the environment are thyroid hormonal disturbances and reproductive toxicity seen in rodents, and effects on liver, kidney and haematological system in rats, hormonal disturbances and effects on reproduction in marine medaka fish and effects on expression of hormone receptors in tadpoles. Together, these elements lead to a very high potential for irreversible effects.

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Annex I: Physical-chemical properties of substances used in read-across

Table 39 presents physical-chemical properties of the sulfonic acids trifluoromethane sulfonic acid, perfluorobutane sulfonic acid (PFBS) and perfluorooctane sulfonic acid (PFOS) collected from the OECD QSAR toolbox v4.2. These substances are compared in a read-across approach in the assessment of the persistence of PFBS.

Table 39: Physical-chemical properties of substances used in read-across (from OECD QSAR toolbox v4.2)

	Trifluoromethane sulfonic acid	**PFBS**	**PFOS**
C-chain length	C1	C4	C8
CAS No	1493-13-6	375-73-5	1763-23-1
SMILES	OS(=O)(=O)C(F)(F)F	OS(=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F	OS(=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F
pK_a	-3.43	-3.31	-3.32
Log K_{OW}	-0.49	1.82	4.49
Log K_{OA}	4.90	5.05	4.84
Log K_{OC} (log K_{OW} method)	0.65	1.93	3.41
Water solubility (mg/L)	1000000	8860	0.050
Vapour pressure (mm Hg)	0.545	0.0518	0.0064
Boiling point (°C)	166	211	NA

Annex II: Modelling nominal biota concentration development of PFBS according to the Crookes and Fisk model

Crookes and Fisk (2018) investigated how the concentrations of mobile and persistent chemicals in the environment develop over time and compared their findings with the bioaccumulation criteria of the Stockholm Convention. They found that substances that are both persistent and mobile in the environment have the potential to be transported long distances from the point of emission. If such substances accumulate over time in remote regions, they can reach levels that may have effects on both ecosystems and human health.

Crookes and Fisk (2018) modelled the expected time-trend for concentrations in biota of substances with a certain combination of half-life in water and and bioconcentration factors, see Chapter 4 and Figure 3 in their report and reproduction in Figure 5 below. Persistence in this model relates to the life-time in the relevant compartment, water, and degradation as well as sedimentation and other processes that remove the substance from the compartment. The authors found that for a given mass emission rate, as the half-life increases, the steady state concentration predicted in biota for substances with relatively low BCF values can, over extended periods of time, approach that of a substance that is considered to have a high bioaccumulation potential, and the steady state concentration ultimately reached is a function of both the persistence and BCF.

Figure 5 is a reproduction of Figure 3 in Crookes and Fisk (2018) showing time trends to accumulation in biota. The model shows that the nominal biota concentration for a substance with half-life 365 days and BCF 800 will approach the biota concentration for a substance with half-life 60 days and BCF 5000, after 2000 days.

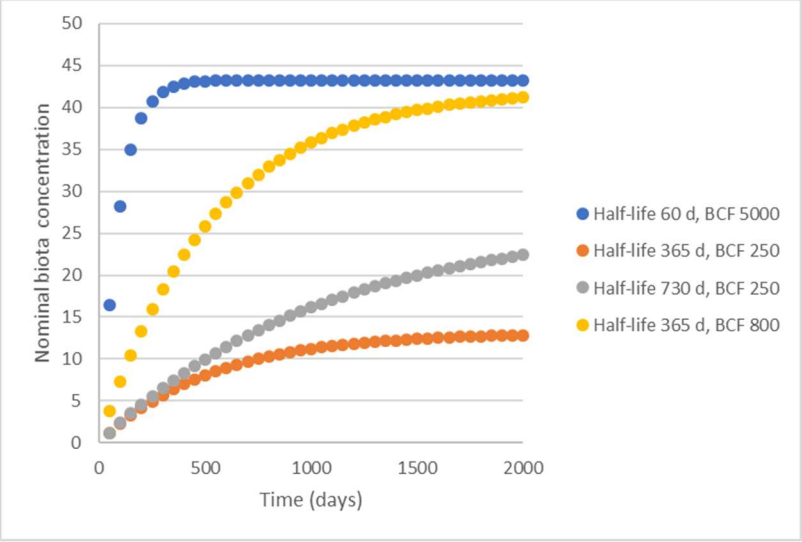

Figure 5: Reproduction of model for biota concentration development from Crookes and Fisk (2018)

The nominal biota concentration calculations were repeated for PFBS and compared with some relevant model substances. A half-life in water of 10 years for PFBS was assumed, representing a best-guess estimate in the absence of any measured half-life, and the calculations performed for the bioaccumulation values reported in this dossier: BCF Fish: 23.5 (Chen et al., 2016); BAF crab 110 (Naile et al., 2013) and BAF fish 1736 (Campo et al., 2015). The two latter studies report the bioaccumulation factor as field-based

bioconcentration factors (BCFs). The outcome of the modelling of development of biota concentrations for PFBS over time is shown in Figure 6. The model substances (A, B, C and D) have combinations of half-life and BCF as shown in the figure.

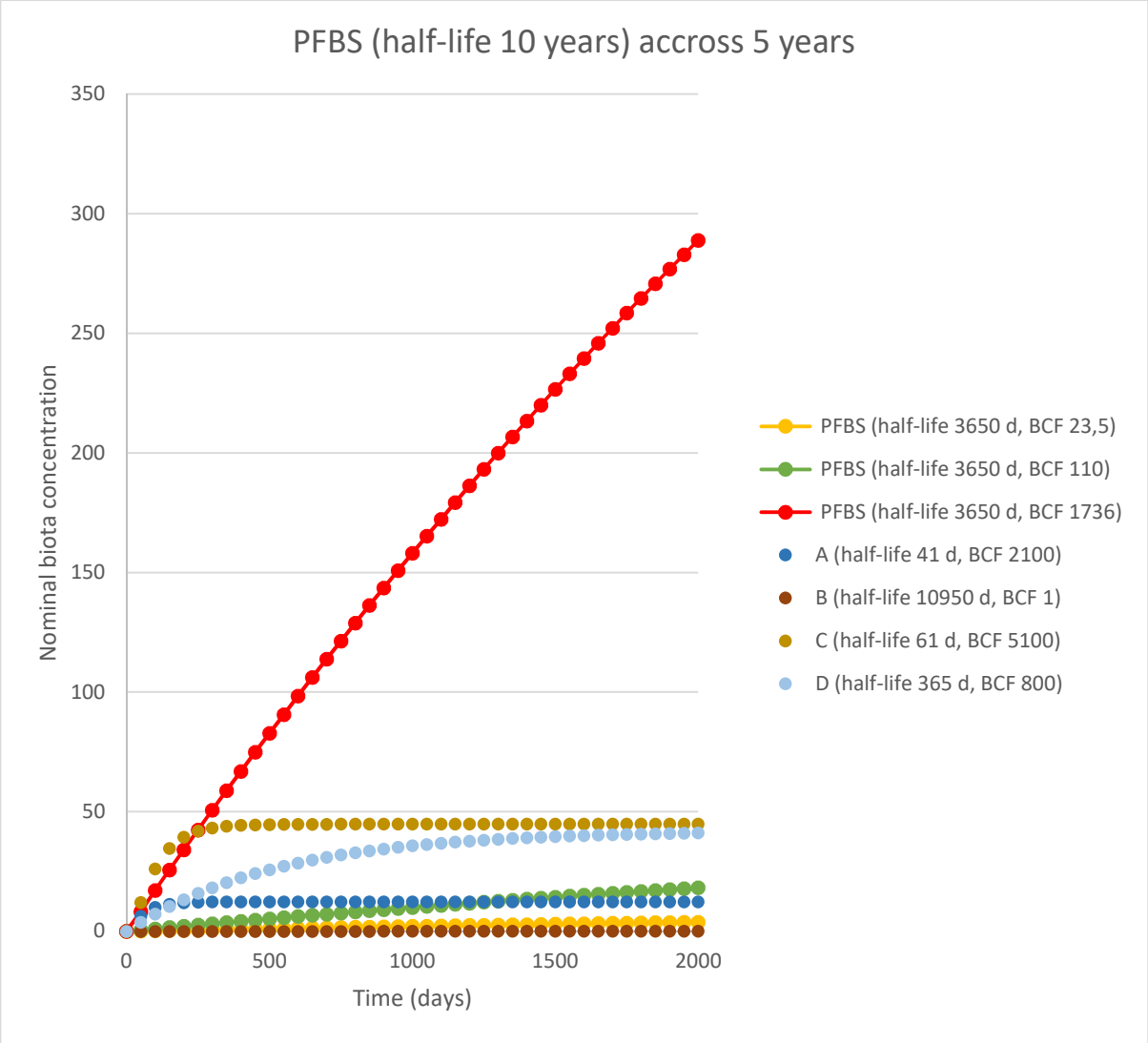

Figure 6: Modelling of development of nominal biota concentrations for PFBS over time

It is evident from Figure 6 that when considering an appropriate time scale, a long half-life for a substance may lead to high nominal biota concentrations, even when the BCF is only moderate. The red line represents a BAF/BCF for PFBS of 1736 reported in fish (Campo et al., 2015) and demonstrates the effect of a long half-life in combination with a relatively high BAF/BCF. However, as is outlined in Section 3.5.3 we consider this BAF/BCF to be an overestimate, and the red line is disregarded in this evaluation. The green line represents a BAF/BCF of 110 measured in crab (Naile et al., 2013). The graph in Figure 6 shows that this moderate BCF in combination with a half-life of 10 years, may lead to very high concentrations in biota over time. The green line even crosses the dark blue line, representing a substance with half-life in water of 41 days and a BAF/BCF of 2100, i.e. a substance just exceeding the P and B criteria in REACH Annex XIII.

Hence, when the model from the Crookes and Fisk (2018) report is used for PFBS with an assumed half-life in water of 10 years and a reported BAF/BCF in crab of 110, the concentrations of PFBS in biota may be expected to exceed the biota concentrations for a persistent and bioaccumulative substance over time.