Environmental risk evaluation report: Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate [F-53B] (CAS no. 73606-19-6)

Chief Scientist's Group report

April 2023

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Dr Robert Bradburne
Chief Scientist

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Executive summary

There is growing regulatory concern at international level about the emissions of per- and polyfluoroalkyl substances (PFAS) to the environment. This is due to their extreme persistence, which could lead to long-term exposure of both people and wildlife. High levels of exposure to certain PFAS has also been shown to cause harmful effects in humans and some have been declared to be 'Persistent Organic Pollutants' (POPs) under the United Nations Environment Programme (UNEP) Stockholm Convention.

The UK Government is developing an action plan to address the concerns arising from PFAS. As a contribution to this work, the Environment Agency informally reviewed several PFAS that are made or used at two UK production facilities and one additional substance that has been detected in the UK environment: 6:2 chlorinated polyfluorinated ether sulfonate (CAS no. 73606-19-6), which is also known as F-53B.

F-53B is a PFAS that belongs to the group of perfluoroether sulfonic acids. It is not registered under EU or UK REACH. There is currently no information about the actual quantities on the UK market (including from imported goods). However, F-53B may be a replacement for the POP perfluorooctane sulfonate (PFOS) given their structural similarity.

The Environment Agency has identified publicly available information on the regulatory status, uses, physico-chemical properties, environmental fate and (eco)toxicity of F-53B and has reviewed this information for reliability. The data have then been used to conduct an environmental hazard and risk assessment. Human health hazards have only been reviewed in so far as they are relevant for the environmental assessment. Potential risks to people following environmental exposure have not been addressed.

F-53B is not readily biodegradable and there is no evidence that it degrades significantly via abiotic mechanisms. In addition, there is no information on degradation rates or half-lives available from simulation studies. F-53B is therefore potentially persistent or very persistent (P/vP) according to Annex 13 of the REACH Regulation. However, the strength of the carbon-fluorine chemical bond, and laboratory and field data for similar PFAS such as PFOS suggests that the substance is likely to be extremely persistent. It is therefore precautionary to conclude that the substance likely meets the vP criterion in one or more environmental compartments. Non-standard data on bioconcentration in fish and data from monitoring studies suggest that F-53B is likely to be bioaccumulative or very bioaccumulative (B/vB). It is not possible to draw definitive conclusions on the bioaccumulation potential of F-53B in air-breathing organisms, but the available data suggests that F-53B may be at least as bioaccumulative as PFOS. Chronic aquatic ecotoxicity data indicates that F-53B is toxic (T). F-53B is therefore likely to meet both PBT and vPvB criteria.

Draft criteria have also been proposed to identify chemicals that are persistent, mobile and toxic (PMT) or very persistent and very mobile (vPvM). F-53B is likely to meet the draft PMT/vPvM criteria, suggesting that it might be hazardous for groundwaters and has the potential for long distance transport.

Based on the available *in vitro* and *in vivo* data, F-53B has the potential to be a thyroid disrupting compound. None of the studies were conducted to standard guidelines, so further testing would be needed to reach a definitive conclusion.

An indicative exposure assessment has been carried out by the Environment Agency based on an assumed use as a mist suppressant in chrome plating (an ongoing use of PFOS that is being phased out). No risks have been identified for any environmental compartments or life cycle stages based on risk characterisation ratios. However, if the PBT/vPvB properties of F-53B are confirmed, emission minimisation would need to be considered.

A number of recommendations are made to improve the dataset to allow a more robust assessment of the environmental hazards and risks posed by F-53B. Any future UK importer or REACH Registrant of F-53B may wish to consider these recommendations. This report, along with others in this series, will be used by the Environment Agency to inform the UK Government action plan on PFAS and the PFAS Regulatory Management Options Analysis (RMOA) being conducted under the UK REACH Regulation.

Introduction

There is growing international concern about the emissions of per- and polyfluoroalkyl substances (PFAS) to the environment. This is principally due to their extreme persistence, which could lead to long-term irreversible exposure of both people and wildlife. High levels of exposure to certain PFAS has also been shown to cause harmful effects in humans and some have been declared to be 'Persistent Organic Pollutants' (POPs) under the United Nations Environment Programme (UNEP) Stockholm Convention.

The UK Government is developing an action plan to address the concerns arising from PFAS. As a contribution to this work, the Environment Agency has informally reviewed several substances that are made or used at two known production facilities in the UK, namely AGC Chemicals Europe Ltd of Thornton Cleveleys, Lancashire and F2 Chemicals Ltd of Preston, Lancashire. Based on information provided by these companies, a provisional list of PFAS for further consideration was drawn up. This was narrowed down to the following eight substances which were, at the time, registered at more than 1 tonne per year under the EU REACH Regulation[1] and subsequently also under UK REACH. Additionally a potential unregistered substitute for perfluoroctanesulfonic acid (PFOS, which is a known POP) was included that had been identified from UK surface water monitoring. All of the substances chosen for further evaluation are listed below, initially using their EU-registered name:

- Ammonium difluoro[1,1,2,2-tetrafluoro-2-(pentafluoroethoxy)ethoxy]acetate - also known as perfluoro(2-ethoxy-2-fluoroethoxy)acetic acid ammonium salt or EEA-NH_4 (CAS no. 908020-52-0)
- Trideca-1,1,1,2,2,3,3,4,4,5,5,6,6-fluorohexane - also known as 1H-perfluorohexane or 1H-PFHx (CAS no. 355-37-3)
- 3,3,4,4,5,5,6,6,6-Nonafluorohexene - also known as perfluorobutylethylene or PFBE (CAS no. 19430-93-4)
- 1,1,1,2,2,3,3-Heptafluoro-3-[(trifluorovinyl)oxy]propane - also known as perfluoro(propyl vinyl ether) or PPVE (CAS no. 1623-05-8)
- 1,1,1,2,2,3,3,4,5,5,5-Undecafluoro-4-(trifluoromethyl)pentane - also known as perfluoroisohexane or PFiHx (CAS no. 355-04-4)
- Perflunafene - also known as perfluorodecalin or PFD (CAS no. 306-94-5)
- Hexafluoropropene or HFP (CAS no. 116-15-4)

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[1] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) - see: https://ec.europa.eu/environment/chemicals/reach/reach_en.htm

- Octafluoropropane - also known as perfluoropropane or PFP (CAS no. 76-19-7)

The additional unregistered substance also being considered here is:

- **6:2 Chlorinated polyfluorinated ether sulfonate - also known as 'F-53B' (CAS no. 73606-19-6)**

This report summarises the evaluation of the substance highlighted above in **bold** (i.e. F- 53B), to address the following questions:

- What data are currently available, and are they sufficiently reliable to assess the environmental hazards and risks from this substance?
- Can we establish numerical exposure limits for assessing environmental impacts (e.g. for use under permitting regimes)?
- Is this substance potentially able to reach remote environments and what is its groundwater contamination potential?
- Is this substance a potential candidate for future risk management?
- What information gaps remain and, if required, what is the most appropriate way of obtaining this information?

F-53B is not currently registered under the EU or UK REACH Regulations, so there is no registration dossier to consult for detailed information. The Environment Agency has therefore performed a literature review on the substance (Appendix A: Literature search) and information on its properties and uses is based on publicly available sources.

This report describes the substance and its structural analogues, its analytical chemistry, manufacture and use, regulatory status and then various environmentally relevant properties. This is followed by an environmental hazard assessment in Section 9, and an exposure and risk assessment in Sections 10 and 11. The final section (Section 12) summarises our findings. Although the focus of this evaluation is on environmental hazards and risks, there is a summary of mammalian toxicology information, where available and relevant to the environmental assessment. However, this report is not intended to provide a full consideration of hazards, exposure and risks to human health. This is not a formal UK REACH Evaluation.

1 Substance Identity

1.1 Name and other identifiers

Public name: 6:2 Chlorinated polyfluorinated ether sulfonate

IUPAC name: Potassium; 2-(6-chloro-1,1,2,2,3,3,4,4,5,5,6,6-dodecafluorohexoxy)-1,1,2,2-tetrafluoroethanesulfonate

CAS name: Not listed

EC number: Not listed

CAS number: 73606-19-6

Index number in Annex VI of the CLP Regulation: Not listed

Molecular formula: $C_8ClF_{16}KO_4S$

Molecular weight: 570.67 g/mol

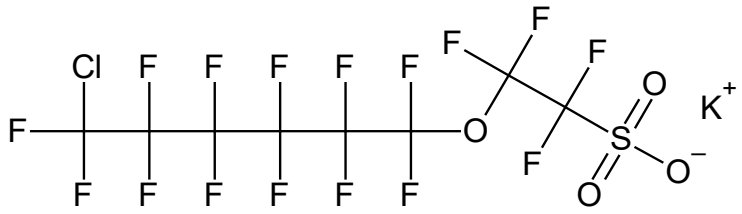
Smiles code: C(C(C(C(F)(F)Cl)(F)F)(F)F)(C(C(OC(C(F)(F)S(=O)(=O)[O-])(F)F)(F)F)(F)F)(F)F.[K+]

Synonyms: 2-(6-Chlorododecafluorohexyloxy)-1,1,2,2-tetrafluoroethanesulfonic acid potassium salt, 6:2 Cl-PFESA, 9Cl-PF3ONS, F-53B, Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate

Type of substance: Mono-constituent.

Wang *et al*. (2013) suggest that this substance is manufactured in a relatively pure form (e.g. a purity of > 98% was reported by Shanghai Synica Co., Ltd., Shanghai, China). However, other authors consider that F-53B is a mixture of 6:2 and 8:2 Cl-PFESA (see Section 1.2). For example, Liu *et al*. (2018) conducted a study using F-53B with a purity of "98%", which is stated to contain 91% C_8, 7% C_{10} and 0.3% C_{12} chlorinated polyfluorinated ether sulfonate. It is not always clear what the composition of the test material is in academic publications.

Figure 1.1 Structural formula of F-53B representing the atoms and how they are bonded to each other.



The substance will be referred to as F-53B for the purposes of this report. The chlorine atom is always reported to be located on the terminal carbon atom (Figure 1.1), although the possibility of it being present at other locations on the chain is unknown.

1.2 Structurally related substances

F-53B belongs to the group of perfluoroether sulfonic acids (PFESA).

The most relevant structural analogues are presented in Table 1.1.

- The parent acid of F-53B is perfluoro(2-((6-chlorohexyl)oxy)ethanesulfonic acid, which is expected to behave in an identical way to the potassium salt in the environment as it will dissociate to the same anion. Any conclusions for F-53B will therefore be applicable to the acid form.

- Potassium-11-chloroeicosafluoro-3-oxaundecane-1-sulfonate (also known as 8:2 Cl-PFESA) has two extra -CF_2- groups in the chain compared to F-53B. It is therefore a longer molecule with a higher molecular mass. This substance may possibly be a component of commercial F-53B products (see Section 1.1).

- F-53B is structurally similar to perfluorooctane sulfonic acid (PFOS). Both have a sulfonic acid group attached to a long hydrophobic (and oleophobic) alkyl chain containing eight fluorinated carbon atoms. The key structural difference is that F-53B also has an oxygen atom (ether group) between two of the fluorinated carbon atoms and one of the terminal fluorine atoms is replaced by a chlorine atom. F-53B therefore has a slightly higher molecular mass than PFOS (570.67 compared to 538 g/mol). The chlorine and oxygen atoms also confer different molecular dimensions, which may affect its ability to permeate membranes.

  The oxygen atom has two lone pairs of electrons, making the F-53B molecule susceptible to hydrogen bonding. Together with the chlorine atom, it is potentially more open to chemical attack than PFOS, and may possibly be a little less hydrophobic and oleophobic. However, fluorine atoms are highly electronegative and so will draw charge away from the lone pairs and chlorine atom. This is likely to make them less available for reaction than other alkyl ethers or chlorinated hydrocarbons, which are in any case relatively stable substances.

PFOS is an important analogue of F-53B because it has been studied extensively and in 2009 PFOS, its salts and the closely related substance perfluorooctane sulfonyl fluoride (PFOSF) were listed as Persistent Organic Pollutants (POPs) in Annex B to the Stockholm Convention. A detailed review of PFOS was previously carried out by the Environment Agency (2004).

Note: PFOS was produced using an electrochemical fluorination method that produced a range of chain lengths and around 30% by weight consisted of branched isomers (i.e. the commercial product was not the simple linear C_8 compound implied by the chemical name). The Environment Agency has not ascertained whether F-53B is also isomeric.

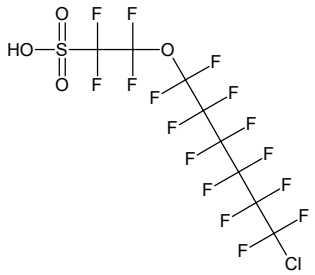
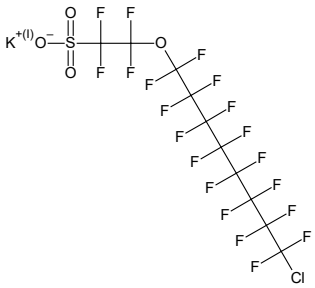
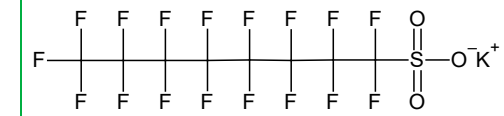
The US EPA CompTox Chemicals database (USEPA, 2020a) highlighted five additional structural analogues of F-53B, which are summarised in Appendix B. This includes F-53 (CAS no. 68136-88-9), which is identical to F-53B except that the chlorine atom is replaced by fluorine. F-53 was first synthesised in 1975, but F-53B was developed to reduce production costs and became the more important commercial product (Wang *et al.,* 2013).

A search of the European Chemicals Agency's (ECHA) public dissemination site (https://echa.europa.eu accessed October 2020) indicates that (with the exception of PFOS) none of these analogues (nor any derivatives) are registered under the EU REACH Regulation. EC numbers have not been assigned, so they do not appear to have been on the EU market prior to the introduction of REACH either.

1.3 Transformation products

As discussed in Section 6.1, F-53B is not readily biodegradable but achieved 43.5% degradation after 28 days in one study. The Environment Agency notes that this degree of mineralisation is surprising and that F-53B is probably an ultimate transformation product (a so-called "arrowhead") as it is highly fluorinated so likely to be highly persistent (see Section 6.1).

Table 1.1 Substance identifiers for close analogues of F-53B

Public name	Perfluoro(2-((6-chlorohexyl)oxy)-ethanesulfonic acid	Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	Perfluorooctane sulphonate, potassium salt
IUPAC name	2-(6-chloro-1,1,2,2,3,3,4,4,5,5,6,6-dodecafluorohexoxy)-1,1,2,2-tetrafluoroethanesulfonic acid	Potassium;2-(8-chloro-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-hexadecafluorooctoxy)-1,1,2,2-tetrafluoroethanesulfonate	Potassium;1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctane-1-sulfonate
CAS number	756426-58-1	83329-89-9	2795-39-3
Structural formula			
Molecular formula	$C_8ClF_{16}KO_4S$	$C_{10}ClF_{20}KO_4S$	$C_8F_{17}SO_3^- K^+$
Molecular weight	532.58 g/mol	670.69 g/mol	538 g/mol
SMILES code	OS(=O)(=O)C(F)(F)C(F)(F)OC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)Cl	C(C(C(C(C(F)(F)Cl)(F)F)(F)F)(F)F)(F)F)(C(C(C(OC(C(F)(F)S(=O)(=O)[O-])(F)F)(F)F)(F)F)(F)F)(F)F.[K+]	C(C(C(C(C(F)(F)S(=O)(=O)[O-])(F)F)(F)F)(F)F)(C(C(C(F)(F)F)(F)F)(F)F)(F)F)(F)F.[K+]

Synonyms	2-[(6-Chloro-1,1,2,2,3,3,4,4,5,5,6,6-dodecafluorohexyl)oxy]-1,1,2,2-tetrafluoroethane-1-sulfonic acid	2-[(8-chloro-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-hexadecafluoroctyl)oxyl]-1,1,2,2-tetrafluoro-ethanesulfonic acid, potassium salt 8:2 Cl-PFESA; 11Cl-PF3OUdS	Potassium PFOS; [Parent acid, CAS No. 1763-23-1; diethanolamine salt, CAS No. 70225-39-5; ammonium salt, CAS No. 29081-56-9; lithium salt, CAS No. 29457-72-5]
Source	US EPA (2020d)	US EPA (2020e)	US EPA (2020b); Environment Agency (2004)

Note: The above substances are not registered in EU or UK REACH.

2 Analytical chemistry

2.1 Regulatory and academic methods

The Environment Agency searched the academic literature for analytical methods for the detection of F-53B in the following environmental matrices: biota; water; fresh and marine; soil; sediment; sewage sludge; and air. The analytical method type, media and references are presented in Tables E.1 to E.10 and the most common analytical methods cited centred on liquid chromatography with mass spectrometric detection.

Only the anionic form of F-53B is expected in water and other media due to dissociation of the molecule. Three studies have analysed for F-53B (and related substances) in the UK environment, and a brief discussion of the methods is presented below:

- Pan *et al*. (2018) analysed for F-53B (6:2 Cl-PFESAs), its known impurities (4:2 and 8:2 Cl-PFESA) and the associated transformation product (6:2 H-PFESA) in river water samples. Briefly, a subsample of 200 mL of water (without filtration) was spiked isotopically mass-labelled recovery/internal standards (ISTDs) and then extracted by a weak anion-exchange cartridge. Target compounds were eluted with basic methanol, evaporated to dryness, and finally reconstituted with 200 µL of pure methanol. Substances were quantified with an Acquity I-Class ultra-performance liquid chromatograph (UPLC) coupled to a Xevo TQ-S mass spectrometer (Waters, Milford, MA, U.S.A.). Chromatographic separation was achieved using an Acquity BEH C18 column (1.7 µm, 2.1 × 75 mm, Waters, Milford, MA, U.S.A.).
- Barber *et al*. (2021) analysed for F-53B (6:2 Cl-PFESA) and its known impurities (4:2 and 8:2 Cl-PFESA) in UK sediment samples. Sediments were homogenised before extraction. Samples of 1 g were spiked with 20 µL of a mixture of isotopically mass-labelled ISTDs in methanol containing 0.2 ng/µL of each ISTD. Samples were solvent extracted and concentrated. Substances were quantified with an Acquity UPLC coupled to a Xevo TQ mass spectrometer with an electro spray ionisation probe (Waters, Milford, MA, U.S.A.). Chromatographic separation was achieved using a BEH C18 column (1.7 µm, 2.1 × 50 mm, Waters, Milford, MA, U.S.A.). For quality assurance purposes, a blank and reference material sample were analysed with every 10 samples;
- O'Rourke *et al*. (2022) analysed for F-53B (6:2 Cl-PFESA) and its known major impurity (8:2 Cl-PFESA) in samples of otter liver. Homogenised liver samples of 1 g were spiked with 20 µL of a mixture of ISTDs in methanol containing 0.2 ng/µL of each ISTD. Samples were solvent extracted and concentrated. The analysis was performed using an UPLC using a BEH C18 analytical column (50 mm x 2.1 mm and 1.7 µm particle size) and a TQ MS Xevo triple quadrupole mass spectrometer. For quality assurance purposes, a blank and reference material sample (NMCAG-RM1 spiked muscle tissue) were analysed with every 10 samples.

The Environment Agency considers that the description of a robust analytical method will typically include the following details:

- Instruments and consumables including chromatographic column, temperature, mobile phase composition, flow rates, gradient or isocratic separation and the detector optimisation and configuration.
- Certified reference standards, internal reference standards, calibration curve and range, accuracy, repeatability, sensitivity, limit of detection, limit of quantification, column recoveries, stability and reproducibility.
- The use of procedural blanks and control samples in both sample preparation and analysis.
- Sample preparation including clean-up consumables, concentration techniques and use of internal standards (plus justification for choice) for, quantification validation and recoveries, etc.
- Identification and discussion of technical limitations.
- Clear data handling and statistical analysis (including goodness of fit and handling of outliers)

The methods above have reported the required details that allow the generated data to be evaluated by the Environment Agency as robust and fit-for-purpose. Pan *et al*. (2018) and Barber *et al*. (2021) omitted descriptions with regards to calibration curves and the subsequent statistics. However, the Environment Agency considers the methods to be robust, due to similarities with O'Rourke *et al.* (2022).

3 Import, manufacture and uses

Although the UK left the European Union (EU) at the end of January 2020, European legislation already in place by December 2020 has been retained and transposed in to UK law. The European Chemicals Agency (ECHA) public databases are still a relevant source of information about industrial chemicals on the UK market at the time of writing.

F-53B has been detected in the UK environment (see Section 10.1.4) but is not registered under the EU or UK REACH Regulations. Like some other PFAS, F-53B could also be a degradation product of derivatives, but a search of ECHA's public dissemination site (https://echa.europa.eu accessed October 2020) indicates that no derivatives are currently REACH-registered.

It is also possible that the substance is (or was) present in articles (such as plastic items, clothing, etc.) imported from outside the EU, since there is no registration obligation if release of the substance from these articles in use is unintentional

It is therefore presumably not currently manufactured or imported by individual companies as either the substance itself or in the form of derivatives in quantities of 1 tonne/year or above in the UK or EU markets. There is currently no information about the actual quantities on the UK market (including in imported goods). Its use pattern (if any) is also unknown. However, if several companies are importing small amounts, the collective supplied tonnage could exceed 1 tonne/year without triggering any REACH obligations. It is also feasible that there is no actual use in the UK and that its presence here arises from long-range transport or imported articles, or historical applications (see Section 6.2.4).

F-53B may be a PFOS replacement given their structural similarity. For example, F-53B has reportedly been used in China as a mist suppressant for metal surface treatment for approximately 30 years (Wang *et al.*, 2013). The function of the substance is to lower the surface tension of metal plating solutions to prevent the formation of mists containing harmful components (e.g. chromium (VI) substances) from the baths that are used in the metal treatment process (Environment Agency, 2004). It is estimated that about 20 to 30 tonnes of F-53B were used in the Chinese metal plating industry (for both decorative and hard metal plating) in 2009 (Wang, Cousins, Scheringe and Hungerbühler, 2015).

Jun Huang of Tsinghua University (co-author of Wang *et al.* 2013) states that Shanghai Synica Co., Ltd ceased production of F-53B in 2020 due to more stringent environmental protection requirements in Shanghai. The production technology was transferred to another company (Shandong HUAFU Chemical Co., Ltd) although production had not yet started and F-53B was in short supply on the Chinese market, as the historical stockpile is very limited (personal correspondence, 7 December 2020).

The amount of PFOS derivatives (including salts) used in metal treatment in the UK was estimated to be below 0.5 tonnes/year in the early 2000s, before the substance was regulated as a POP (Environment Agency, 2004). Once the restriction of PFOS entered into force in July 2008, the amount of PFOS sold in the UK as a mist suppressant declined

to 150 kg/year (Danish Ministry of the Environment, 2011). PFOS-based mist suppressants can still be used for chrome plating, anodising and acid pickling within the UK, although many companies have replaced it with alternatives (Surface Treatment Association, 2018). It is therefore feasible that F-53B may also be used in the UK for this application, at least in trials of PFOS alternatives.

PFOS was used for a variety of other applications historically, although most of these uses are no longer permitted following its designation as a POP. Examples included (Environment Agency, 2004):

- metal plating;
- semi-conductors;
- photographic;
- aviation;
- fire-fighting foams stock;
- carpets;
- leather/apparel;
- textiles/upholstery;
- paper and packaging; and
- coatings and coating additives.

It is possible that F-53B has been used in some of these applications, although there is no evidence that this is the case in Europe.

For the purposes of this evaluation, the Environment Agency has assumed that F-53B is used as a mist suppressant in chrome plating with a UK market volume of 0.5 tonnes/year (as described in Environment Agency, 2004). The relevant life cycle stages are listed in Table 3.1. This level of supply is below that which triggers regulatory obligations under REACH. It is an illustrative worst-case assessment, because other PFAS (based on C6 fluorotelomer chemistry) are known to be used as PFOS replacements by many UK chrome plating companies. However, since perfluorohexanoic acid (PFHxA) and its derivatives are currently subject to a restriction proposal in the EU, it is feasible that companies could be looking for alternatives like F-53B, so it could take a larger market share in future.

An alternative approach would have been to assume an approximate (low) tonnage split across all of the uses previously known for PFOS. Since F-53B is clearly not a general replacement for PFOS (as it would otherwise be registered under REACH), this was considered unrealistic. A refinement of the exposure assessment could be considered if it is subsequently established which companies have actually used this substance.

Table 3.1 Overview of assumed uses of F-53B based on previously described uses of PFOS (Environment Agency, 2004)

Life cycle stage	Use(s)
Manufacture	No manufacture is assumed Imported into UK market at <1 tonnes/year (assumed)
Formulation	None identified
Uses at industrial sites	ERC4: Use of non-reactive processing aid at industrial site (no inclusion into or onto article) ERC6b: Use of reactive processing aid at industrial site (no inclusion into or onto article) ERC7: Use of functional fluid at industrial site
Uses by professional workers	Not considered (assessment targeted on environment)
Consumer uses	None identified
Article service life	None identified

4 Summary of relevant regulatory activities

4.1 Europe

4.1.1 European Chemicals Agency (ECHA)

The Public Activities Co-ordination Tool (PACT) (https://echa.europa.eu/pact, accessed July 2020) provides an overview of the substance-specific activities that EU regulatory authorities are working on under the EU REACH and CLP Regulations. F-53B is not currently included on PACT, and neither is it listed on the Community Rolling Action Plan (CoRAP) (https://echa.europa.eu/information-on-chemicals/evaluation/community-rolling-action-plan/corap-Table, accessed July 2020). This is not surprising, as it is not registered.

Between May and July 2020, the national authorities of Germany, the Netherlands, Norway, Sweden and Denmark invited interested parties to send in evidence and information on the use of PFAS in preparation for a joint EU REACH restriction proposal. The current scope of the work is wide and includes all substances that contain at least one aliphatic $-CF_2-$ or $-CF_3$ element (ECHA, 2021). F-53B is therefore within scope of this initiative.

4.1.2 European Food Safety Agency (EFSA)

EFSA provides scientific advice on safety of food additives, enzymes, flavourings, processing aids and other substances intentionally added to food; safety of food packing and other food contact materials.

A search of EFSA (http://www.efsa.europa.eu/, accessed July 2020) did not identify F-53B as being evaluated or noted in any published scientific opinions.

4.1.3 Oslo and Paris Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR)

The Oslo and Paris Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) is a mechanism by which 15 national governments and the EU co-operate to protect marine resources. Much of OSPAR's work on chemicals is now being addressed by REACH activities.

F-53B is not on the OSPAR List of Substances of Possible Concern https://www.ospar.org/work-areas/hasec/hazardous-substances/possible-concern (accessed July 2020), nor on the list of Chemicals for Priority Action adopted in 2002 https://www.ospar.org/work-areas/hasec/hazardous-substances/priority-action (accessed July 2020).

4.2 Regulatory activity outside of Europe

4.2.1 United States of America

The US Environmental Protection Agency (USEPA) is planning to carry out tiered toxicity and toxicokinetic testing for a range of PFAS in the near future (Patlewicz, Richard, Williams, Grulke, Sams, Lambert, Noyes, DeVito, Hines, Strynar, Guiseppi-Elie and Thomas, 2019). F-53B is not listed as being under investigation.

At the time of writing, F-53B is not listed as one of the substances undergoing risk evaluation as part of US EPA's existing chemical initiative under the Toxic Substances Control Act (TSCA) to determine whether they present an unreasonable risk to public health or the environment under the conditions of use (US EPA, 2020).

4.2.2 Canada

A search did not identify F-53B as being under assessment under the Prohibition of Certain Toxic Substances Regulations, 2012 (https://www.canada.ca/en/environment-climate-change/services/canadian-environmental-protection-act-registry/substances-list/toxic.html, accessed July 2020).

4.2.3 Australia

A search did not identify F-53B as being under assessment under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) (https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-assessments, accessed July 2020).

4.2.4 New Zealand

A search did not identify F-53B as being under assessment under the Hazardous Substances and New Organisms Act 1996 (https://www.epa.govt.nz/industry-areas/hazardous-substances/, accessed July 2020; https://www.epa.govt.nz/industry-areas/hazardous-substances/chemical-reassessment-programme/screened-chemicals-list/, accessed July 2020).

4.2.5 Japan

Industrial chemicals are managed under the Chemical Substances Control Law (CSCL), most recently amended in 2009 (https://www.nite.go.jp/chem/jcheck/list3.action?category=141&request_locale=en, accessed July 2020). Under the Act there are 3 lists:

- Class I Specified Chemicals - 28 substances (persistent, bioaccumulative, toxic)
- Class II Specified Chemicals - 23 substances (toxic and high risk)
- Priority Assessment Chemical Substance (PACS), currently 226 substances

F-53B is not on any of the above lists.

4.3 Other international agreements

4.3.1 United Nations Stockholm Convention on Persistent Organic Pollutants (POPs)

F-53B is not identified as a POP, and is not currently under evaluation (http://chm.pops.int/TheConvention/ThePOPs/AllPOPs/tabid/2509/Default.aspx, accessed July 2020).

The structural analogue PFOS (Section 1.2) was listed as a persistent organic pollutant (POP) in Annex B (restriction) of the UNEP Stockholm Convention on POPs in 2009.

4.3.2 Greenhouse gases

Fluorinated gases ('F-gases') may contribute to climate change due to their global warming potential (EC, n.d.) and they are often used as substitutes for ozone-depleting substances, because they do not damage the atmospheric ozone layer (EC, n.d.). F-gases are regulated under the Ozone-Depleting Substances and Fluorinated Greenhouse Gases (Amendment etc.) (EU Exit) Regulations (2019) which aims to reduce the emission of these gases into the environment.

F-53B is not included in the listed F-gases subject to the Regulation as it is not a gas.

5 Physico-chemical properties

This evaluation focusses on vapour pressure, water solubility and n-octanol-water partition coefficient, because they are the key physico-chemical end points for the environmental assessment of most organic chemicals. Surface tension and dissociation constant are also considered. The available information is discussed in this section and a conclusion drawn about which value the Environment Agency considers most suitable for the further evaluation of this substance.

Since there is no REACH registration, the assessment relies on data reported in the academic literature, supplemented where necessary with information from analogues (see Section 1.2) and openly available *in silico* quantitative structure-activity relationship (QSAR) models. QSAR models are generally considered to be a screening-level tool and measured values are preferable, provided that they are sufficiently reliable. Further information on the models used in this assessment is provided in Appendix C.

The Environment Agency has assigned reliability scores in accordance with the ECHA R.4. Guidance Document (ECHA, 2011). Detailed evaluations have not been possible for any physico-chemical endpoint reported in the academic literature, due to the lack of supporting information.

An overview of physico-chemical data is presented in Table 5.1. Key data selected for the exposure assessment are presented in Section 10.

5.1 Vapour pressure

5.1.1 Measured data

No experimentally derived vapour pressure value was reported in the publicly available information.

5.1.2 Predicted data

A sub-cooled vapour pressure of 0.047 kPa (0.35 mmHg) was predicted by Gomis *et al*. (2015) using the COSMOtherm model. The reliability of this prediction is unknown. The Environment Agency converted this value to a vapour pressure at 25 °C (9.12×10^{-6} kPa) using an assumed melting point of 400 °C based on the potassium salt of PFOS (see Section 5.1.3) and Equation R.16-3 of the REACH R.16 Technical Guidance (ECHA, 2016) as reproduced in Figure 5.1.

Table 5.1 Summary of physico-chemical properties for F-53B.

Property	Value(s)	Reliability	Reference
Physical state at 20 °C and 101.3 kPa	Solid	-	-
Melting / freezing point	206 °C (software prediction using MPBPWIN v1.43) 72.1 °C (software prediction using OPERA)	4 – not assignable	Gomis *et al.*, 2015; US EPA, 2020a
Boiling point	486 °C (software prediction using MPBPWIN v1.43) 224 °C (software prediction using OPERA)	4 – not assignable	Gomis *et al.*, 2015; US EPA, 2020a
Relative density	-	-	-
Vapour pressure	9.12×10^{-6} kPa at 25 °C (software prediction using COSMOtherm) 1×10^{-10} kPa at 25 °C (software prediction using MPBPWIN v1.43) 3×10^{-7} kPa at 25 °C (software prediction using OPERA)	4 – not assignable	Gomis *et al.*, 2015; US EPA, 2020a
Surface tension	None identified	-	-
Water solubility	0.57 mg/L (software prediction using WSKOWWIN v1.43) 7.5×10^{-6} mg/L (software prediction using OPERA) 2.49×10^{-6} mg/L (software prediction using COSMOtherm)	4 – not assignable	Gomis *et al.*, 2015; US EPA, 2020a
n-Octanol/water partition coefficient (log K_{OW})	3.1 (software prediction using KOWWIN v1.68) 3.39 (software prediction using OPERA) 7.03 (software prediction using COSMOtherm)	4 – not assignable	Gomis *et al.*, 2015; US EPA, 2020a
Particle size distribution	-	-	-
Stability in organic solvents	-	-	-
Dissociation constant	pKa = 0.14	4 – not assignable	Gomis *et al.*, 2015

Figure 5.1 Vapour pressure conversion calculation, Equation R.16-3 (ECHA, 2016)

$$VP_L = \frac{VP}{\exp\left[6.79\left(1-\frac{TEMPmelt}{TEMP}\right)\right]}$$

VP_L sub-cooled liquid vapour pressure [Pa]
VP vapour pressure [Pa]
$TEMP_{melt}$ melting point of substance [K]
TEMP environmental temperature [K]

The Environment Agency estimated the vapour pressure of F-53B using the EPISuite™ MPBPWIN (v1.43) model, based on the ionic SMILES code (US EPA, 2020c). The US EPA CompTox dashboard also contained predicted vapour pressures for F-53B generated from OPERA software (US EPA, 2020a). These values are presented in Table 5.2 (the Environment Agency has converted the values from mmHg to kPa). The ChemSpider database did not contain any predicted vapour pressures for F-53B (RSC, 2020a).

Table 5.2 Predicted vapour pressure for F-53B

Source	Details	Vapour pressure at 25 °C
COSMOtherm	Sub-cooled vapour pressure of 0.047 kPa [0.35 mmHg]	9.12 x 10^{-6} kPa [6.84 x 10^{-5} mmHg]
EPISuite™ MPBPVP v1.43	Mean of Antoine and Grain Methods BP = 486 °C (estimated within the model) MP = 206 °C (estimated within the model)	1 x 10^{-10} kPa [7.51 x 10^{-10} mmHg]
OPERA	Global applicability domain: Outside Local applicability domain index: 0.367 Confidence interval 0.383	3 x 10^{-7} kPa [2.25 x 10^{-6} mmHg]

In silico predicted values should always be treated with caution where substances in the training set and external test set are not visible.

- Guidance provided with the MPBPWIN model indicated that the relationship between the experimental and predicted vapour pressure values for a test set of 1 642 compounds was good, with an R^2 of 0.949, standard deviation of 0.59 and an average deviation of 0.32. The training set contained several perfluorocarbons (see Appendix C) but no close structural analogues to F-53B and it is unlikely that the predicted value for F-53B falls within the applicability domain of the model.
- For the OPERA model, no structural analogues of F-53B were included in the training set or external test sets. F-53B is considered outside the global applicability

domain and has a low local applicability domain index (< 0.4). This prediction is not considered reliable based on the OPERA model applicability domain criteria.

5.1.3 Data from structural analogues

The Environment Agency has sought data for the analogue PFOS. Both F-53B and PFOS are ionic solids, although F-53B has a slightly higher molecular weight and more complex linear chain, which will influence its crystal structure. The Environment Agency considers that the two substances are likely to have similar vapour pressures.

The potassium salt of PFOS has a reported melting point of >400 °C and a measured vapour pressure of 3.31 x 10^{-7} kPa using the spinning rotor method (GLP compliance was not specified; no reference temperature stated but it is assumed to be at 25 °C). The result may be due to volatile impurities in the substance (Environment Agency, 2004).

For comparison, the predicted vapour pressure for the potassium salt of PFOS generated from the MPBPVP v1.41 model in EPISuite™ was reported in Environment Agency (2004) as 1 x 10^{-12} kPa (7.5 x 10^{-12} mmHg) at 25 °C (mean of Antoine and Grain methods). The US EPA CompTox dashboard contained predicted vapour pressures for PFOS generated from the OPERA software (US EPA, 2020b). The median predicted value is 3.3 x 10^{-7} kPa (2.5 x 10^{-6} mmHg) at 25 °C (global applicability domain: inside; local applicability domain index: 0.999, confidence Interval 0.972).

5.1.4 Recommended value

A measured vapour pressure is not available.

In silico predicted values range from 9 x 10^{-6} kPa to 1 x 10^{-10} kPa at 25°C (US EPA, 2020a; US EPA, 2020c). The reliability of these predictions is highly uncertain. The measured vapour pressure of the potassium salt of PFOS is 3.31 x 10^{-7} kPa around room temperature, and the OPERA model appears to perform well for that substance. The equivalent OPERA prediction for F-53B is likely to be reasonably good even though the substance itself is outside the model's global applicability domain.

Ideally a vapour pressure should be measured using a standard method. In the absence of this information, the Environment Agency considers that the vapour pressure of F-53B is likely to be around **3 x 10^{-7} kPa at 25 °C** for the purposes of this evaluation based on the structural analogue PFOS. This is within the range of *in silico* derived values from the models explored. The value suggests that the substance is of low volatility.

5.2 Surface tension

5.2.1 Measured data

No experimentally derived data for surface tension of F-53B was identified in the literature search.

5.2.2 Predicted data

The US EPA CompTox dashboard includes predictions of surface tension for the substance itself, rather than an aqueous solution, which are not considered further.

5.2.3 Data from structural analogues

The Environment Agency has sought data for the analogue PFOS. Both F-53B and PFOS share the same hydrophilic sulfonic acid group and have hydrophobic chains. F-53B has one oxygen atom in its chain, which might provide some opportunity for hydrogen bonding and so its chain might not be quite as hydrophobic as PFOS. Nevertheless, the Environment Agency considers that the two substances are likely to have similar effects on surface tension in aqueous solution.

Environment Agency (2004) reports that PFOS is surface active, although no further details were given.

5.2.4 Recommended value

The Environment Agency considers that F-53B is likely to be surface active, although an exact value for surface tension is not available. This conclusion is supported by its reported use as a mist suppressant in metal treatment baths (see Section 3).

Ideally surface tension in aqueous solution should be measured using a standard method.

5.3 Water solubility

5.3.1 Measured data

No experimentally derived water solubility value was identified in the literature search for F-53B. Since it is likely to be surface active (see Section 5.2), any measurement would need to take account of the critical micelle concentration.

In aquatic toxicity tests, the highest nominal stock solution concentration prepared was 200 mg/L in culture medium, with a highest nominal test concentration of 64 mg/L (see Section 7.1). This suggests that the water solubility is significantly in excess of 50 mg/L.

5.3.2 Predicted data

Gomis *et al.* (2015) predicted a water solubility of 2.49 x 10^{-6} mg/L at 25 °C using the COSMOtherm model. The reliability of this prediction is unknown.

The Environment Agency estimated the water solubility for F-53B using the EPISuite™ models based on the ionic SMILES code (US EPA, 2020a). The US EPA CompTox dashboard contained predicted water solubility endpoint values for F-53B generated from OPERA and ACD/Labs software (US EPA, 2020a). Values were converted by the

Environment Agency from mol/L to mg/L using a molecular weight of 570.67 g/mol, and are presented in Table 5.3: Predicted water solubility for F-53B.

Table 5.3 Predicted water solubility for F-53B

Source		Details	Water solubility
COSMOtherm		-	2.49 x 10^{-6} mg/L at 25 °C
EPISuite™ MPBPVP v1.43	log K_{OW} method	log K_{OW} used: 3.1 (estimated)	0.57 mg/L at 25 °C
	Fragment method	-	1.1 x 10^{-4} mg/L at 25 °C
ACD/Labs		-	3.06 mg/L
OPERA		Global applicability domain: Outside	7.13 mg/L [1.25 x 10^{-5} mol/L]

In silico predicted values should always be treated with caution where substances in the training set and external test set are not visible.

- Guidance provided with the WSKOWWIN model indicates that the relationship between the experimental and predicted values for a training set of 1 450 compounds was good, with an R^2 of 0.97, standard deviation of 0.409 and an average deviation of 0.313. The validation set contained several perfluorocarbons (see Appendix C) although not close structural analogues of F-53B and it is unlikely that the predicted value for F-53B falls within the applicability domain of the model.
- For the OPERA model, no structural analogues of F-53B were included in the training set or external test sets. F-53B is considered outside the global applicability domain and has a low local applicability domain index (< 0.4). This prediction is not considered reliable based on the OPERA model applicability domain criteria.

5.3.3 Data from structural analogues

The Environment Agency has sought data for the analogue PFOS. Both F-53B and PFOS share the same hydrophilic sulfonic acid group and have hydrophobic chains. F-53B has a slightly higher molecular weight and its chain might not be quite as hydrophobic as PFOS due to the presence of an oxygen atom (which might provide some opportunity for hydrogen bonding) and a chlorine atom. Nevertheless, the Environment Agency considers that the two substances are likely to have a similar level of water solubility in terms of the order of magnitude.

PFOS has a measured water solubility of 517 mg/L at 20 °C (Environment Agency, 2004). It is not known whether precautions were taken to avoid exceeding the critical micelle concentration.

For comparison, the US EPA CompTox dashboard contained predicted water solubility endpoint values for PFOS generated from EPISuite™, TEST and ACD/Labs software (US

EPA, 2020b). Values were converted by the Environment Agency from mol/L to mg/L using a molecular weight of 538.22 g/mol.

Table 5.4 Predicted water solubility for PFOS

Source	Details	Water Solubility
EPISuite™ water solubility estimate from fragments (v1.01 est.)	Predicted value: 2.25 x 10^{-7} mol/L	0.121 mg/L
TEST	Predicted value: 4.72 x 10^{-6} mol/L	2.54 mg/L
ACD/Labs	-	2.27 mg/L
OPERA	Global applicability domain: Inside Local applicability domain index: 1.0 Confidence interval 0.887	440 mg/L [8.17 x 10^{-4} mol/L]

The OPERA model appears to provide the best estimate of the water solubility value that was used in Environment Agency (2004).

5.3.4 Recommended value

A measured water solubility value is not available.

In silico predictions for the water solubility of F-53B were between 2.5 x 10^{-6} and 7.13 mg/L (US EPA, 2020; USEPA, 2020a). This is a very wide range, and their reliability is unclear. They are much lower than the measured water solubility of the analogue PFOS, which was 517 mg/L at 20 °C (Environment Agency, 2004). Of the various models that have been investigated, only the OPERA model appears to provide a reasonably good match to this measured value, although it is a slight under-prediction (440 mg/L). This suggests that the water solubility of F-53B might be above 7 mg/L as predicted using the same model, although there is significant uncertainty. The concentrations achieved in aquatic ecotoxicity tests suggest a concentration up to at least 200 mg/L.

Ideally, the water solubility should be measured using a standard method, taking care to avoid exceeding the critical micelle concentration. In the absence of this information, the Environment Agency considers that the water solubility is approximately **500 mg/L** at 20 °C for the purposes of this evaluation (based on the value used for PFOS).

5.4 Partition co-efficient (n-octanol/water; log K_{OW})

5.4.1 Measured data

No experimentally derived log K_{OW} value was identified in the literature search for F-53B. The Environment Agency notes that log K_{OW} measurements for PFAS are intrinsically difficult because of the problems caused by surface activity (which can hinder phase

separation). An attempt could be made to estimate the log K_{OW} using the ratio of solubility in water and in n-octanol.

5.4.2 Predicted data

Gomis *et al.* (2015) predicted a log K_{OW} of 7.03 using the COSMOtherm model. The reliability of this prediction is unknown.

The Environment Agency also estimated a log K_{OW} value for F-53B using the EPISuite™ KOWWIN v1.68 model, based on the ionic SMILES code. The US EPA CompTox dashboard contained estimated log K_{OW} values for F-53B from ACD/Labs, ACD/Labs consensus and OPERA software (US EPA, 2020a). Values are presented in Table 5.5.

Table 5.5 Predicted log K_{OW} for F-53B

Source	Details	log K_{OW}
COSMOtherm	Not available	7.03
EPISuite™	KOWWIN v1.68 estimate	3.10
ACD/Labs	Not available	9.72
ACD/Labs consensus	Not available	5.71
OPERA	Global applicability domain: Inside Local applicability domain index: 0.308 Confidence interval 0.330	-0.0299

In silico predicted values should always be treated with caution where substances in the training set and external test set are not visible.

- Guidance provided with the KOWWIN model indicates that the relationship between the experimental and predicted values for a validation set of 10 331 compounds was good, with an R^2 of 0.94 and standard deviation of 0.47. The training set contained several perfluorocarbons (see Appendix C) although no close structural analogues to F-53B and it is unlikely that the predicted value for F-53B falls within the applicability domain of the model.
- For the OPERA model, a structural analogue of F-53B was included in the training set and external test sets (PFOS). F-53B is considered to lie inside the global applicability domain but has a low local applicability domain index (< 0.4). This prediction is not considered reliable based on the OPERA model applicability domain criteria.

Measured organic carbon-water partition coefficients (K_{OC}) and fish bioconcentration factors (BCFs) are available for this substance (see Sections 6.2.1 and 6.3.1). There is a relationship between these values and the K_{OW} for neutral hydrophobic substances that could allow a tentative K_{OW} value to be back-calculated. F-53B is ionised at environmental pH (see Section 5.6) so these relationships might not be the same. However, since some

models use a K_{OW} value as an input, the Environment Agency has estimated an equivalent K_{OW} value from the available K_{OC} data using the equation in EUSES (v2.03) (ECHA, 2020) for predominantly hydrophobics (log K_{OC} = 0.81 log K_{OW} + 0.10).

- A log K_{OW} of 4.38 to 4.79 (mean of 4.63) can be calculated from the range of log K_{OC} values in soil of 3.65 to 3.98 (mean of 3.85) (Section 7.2.1.4);
- A log K_{OW} of 5.42 can be calculated from a log K_{OC} value of 4.49 in sediment (Section 7.2.1.4).

Although the applicability of the equation is uncertain for a substance that is both ionic and contains hydrophobic structural features, the range of calculated log K_{OW} values derived from the soil K_{OC} are similar to the log K_{OW} of 4.8 for structural analogue PFOS as discussed below (Section 5.4.3).

5.4.3 Data from structural analogues

The Environment Agency has sought data for the analogue PFOS. Both F-53B and PFOS share the same hydrophilic sulfonic acid group and have hydrophobic chains. F-53B has a slightly higher molecular weight and its chain might not be quite as hydrophobic as PFOS due to the presence of an oxygen atom (which might provide some opportunity for hydrogen bonding). Nevertheless, the Environment Agency considers that the two substances are likely to have a similar log K_{OW} value in terms of the order of magnitude.

A calculated log K_{OW} value of -1.08 was reported for PFOS in Environment Agency (2004), but this was not considered realistic due to the difficulties in measuring the solubility in n-octanol and in water. In the absence of any reliable measured values, Environment Agency (2004) assumed an “equivalent” log K_{OW} value of 4.8, which was estimated by back-calculation from a measured fish bioconcentration factor (2 796 L/kg wet weight (ww)). For comparison, the US EPA CompTox dashboard contained estimated log K_{OW} values for PFOS from EPISuite™, ACD/Labs, ACD/Labs consensus and OPERA software (US EPA, 2020a). These values are presented in Table 5.6.

Table 5.6 Predicted log K_{OW} for PFOS

Source	Calculation Details	log K_{OW}
EPISuite™ KOWWIN v1.68	Based on the SMILES code	4.13
ACD/Labs	Not available	7.03
ACD/Labs consensus	Not available	4.17
OPERA	Global applicability domain: Inside Local Applicability domain index: 0.998 Confidence Interval 0.652	-1.08

The EPISuite™ KOWWIN v1.68 and ACD/Labs consensus models appear to provide the best estimate of the log K_{OW} value that was preferred in Environment Agency (2004).

5.4.4 Recommended value

A measured log K_{OW} value is not available, and is unlikely to be technically feasible for F-53B.

Predicted log K_{OW} values span a very large range, from -0.03 to 9.72. The reliability of these values is unknown, but comparison of the model outputs with a back-calculated value for PFOS suggests that an equivalent log K_{OW} in the range 3.1 to 5.7 might be appropriate for F-53B.

The log K_{OW} value is used in environmental hazard and risk assessment to provide an indication of partitioning behaviour (including bioaccumulation potential) and ecotoxicity. Given the uncertainties in the available information, the Environment Agency considers that direct measurements of bioaccumulation, partitioning to solid phases and ecotoxicity are necessary for this type of substance. For those models for which a K_{OW} value is necessary, the Environment Agency has selected a log K_{OW} of 4.63 based on a back-calculation from the available measured K_{OC} data from soil. Although this might not be precise, it is derived from measured data. A log K_{OW} range of 4.38 to 4.79 can be used for sensitivity analysis in exposure modelling i.e. as derived from the upper and lower values of measured K_{OC} from soil; the mean of these values is **4.63 at 25 °C**, which can be used for primary modelling purposes. This value is preferred to the log K_{OW} of 5.42 that can be calculated from a log K_{OC} value of 4.49 in sediment. This is because the soil derived K_{OW} values are similar to the value selected for PFOS (4.8) in Environment Agency (2004).

5.5 Octanol-air partition coefficient (K_{OA})

5.5.1 Measured data

No experimentally derived K_{OA} value was identified in the literature search for F-53B.

5.5.2 Predicted data

Gomis *et al.* (2015) predicted a log K_{OA} of 8.4 using the COSMOtherm model. The reliability of this prediction is unknown.

The Environment Agency has estimated an n-octanol-air partition coefficient (K_{OA}) using the dimensionless HLC (K_{AW}) of -6.84 (see Section 6.2.4) and a log K_{OW} value of 4.63 (Section 5.4) ($K_{OA} = K_{OW}/K_{AW}$). The resulting log K_{OA} is 11.47.

As noted in Section 5.4, the Environment Agency recommends that the uncertainty in the K_{OW} value should be addressed using sensitivity analysis.

As there is uncertainty in the HLC (K_{AW}), the reliability of these derived K_{OA} values is unknown.

The US EPA CompTox dashboard contained predicted K_{OA} values for F-53B generated from Cosmotherm and OPERA software (US EPA, 2020a). These values are presented in Table 5.7.

Table 5.7 Predicted log K_{OA} for F-53B

Source	Details	log K_{OA}
OPERA	Global applicability domain: Inside Local Applicability domain index: 0.834 Confidence Interval: 0.613	6.33
COSMOtherm	Not available	8.4
Calculated	Calculated from log K_{AW} of -6.84 and a log K_{OW} value of 4.63 (K_{OA} = K_{OW}/K_{AW})	11.47

In silico predicted values should always be treated cautiously where substances in the training set and external test set are not visible.

- For the OPERA model, no close structural analogues of F-53B were included in the training and external test sets. F-53B is considered inside the global applicability domain and has a high local applicability domain index (> 0.6), therefore the prediction is considered reliable based on the OPERA model applicability domain criteria.

These values can be used in the assessment of bioaccumulation in air-breathing organisms (Section 6.3.2).

5.6 Dissociation constant

5.6.1 Measured data

No experimentally derived pKa value was identified in the literature search for F-53B.

5.6.2 Predicted data

Gomis *et al.* (2015) used the SPARC model to predict a pKa of 0.14. The authors noted that the pKa values of perfluoroalkyl substances are likely to be underestimated by this model. For example, the experimentally measured pKa for GEN-X® reported by Gomis *et al.* (2015) (without specifying a CAS number) is 3.8, which is higher than the estimated value of −0.06 using SPARC.

5.6.3 Data from structural analogues

PFOS has a calculated pKa of -3.27 (Environment Agency, 2004).

5.6.4 Recommended value

Given the uncertainty in predicting a pKa value for this type of substance, the Environment Agency considers that an exact value cannot be derived. However, the sulfonate group is strongly acidic, and so for the purposes of this assessment, F-53B is assumed to exist in an ionised state at environmentally relevant pH values (4 to 9).

6 Environmental fate properties

The same comments about sources of data, reliability scoring and use of supplemental information apply as for Section 5.

6.1 Degradation

6.1.1 Abiotic degradation

6.1.1.1 *Hydrolysis*

No relevant information was identified during the literature search. Although the carbon-chlorine bond is potentially labile, based on the structure of F-53B the Environment Agency would not expect hydrolysis to be a significant degradation pathway in the environment.

6.1.1.2 *Phototransformation in air*

No experimental data on phototransformation in air was identified during the literature review.

Gomis *et al*. (2015) investigated F-53B using the AOPWIN model in EPISuite™ (US EPA, 2012). This estimates the degradation half-life in air based on molecular structures and an assumed hydroxyl radical concentration of 5 x 10^5 molecules/cm^3, which is the same as the standard hydroxyl radical concentration given in ECHA (2016). A half-life of 114 days was estimated for the neutral form of F-53B. The authors note that the predictive power of AOPWIN for PFAS is limited, although some fluorinated substances are included in the training set. Based on comparisons of predicted and measured photodegradation rates for three fluorinated substances and four legacy PFAS, Gomis *et al*. (2015) expect that AOPWIN may underestimate the true half-life in air for this type of substance.

The US EPA CompTox dashboard contains a predicted atmospheric hydroxylation rate for F-53B generated from the OPERA software (US EPA, 2020a). The predicted value is 2.33 x 10^{-15} cm^3/molecule/sec. However, F-53B is not considered by CompTox to be within the applicability domain of the model, so this value is not used in this assessment.

F-53B is not volatile, and so is not expected to partition significantly to the atmosphere (see Section 6.2.2). The Environment Agency therefore considers that photodegradation in air is unlikely to be a significant degradation pathway.

6.1.1.3 *Phototransformation in water*

A single study on phototransformation in water was identified by the Environment Agency during the literature search. Wang *et al*. (2013) investigated the photochemical decomposition of F-53B in water with various catalysts. A solution of 45 mg/L F-53B in ultrapure water was treated with UV light (high pressure mercury lamp at 220 V, 300 W)

alone or with UV light in the presence of hydrogen peroxide (to generate hydroxyl radicals). After 2 hours under these conditions, no change in F-53B concentration was observed in the vessel treated with UV light. Less than 5% degradation was observed in the test vessel treated with UV light in the presence of hydrogen peroxide. The authors concluded that F-53B is resistant to degradation under these conditions, and so F-53B would be expected to be stable to phototransformation in the environment.

This study investigated the direct and indirect photodegradation of F-53B over a period of 2 hours. Although the method used does not follow a standard guideline and was only conducted over a very short time period, it does indicate that rapid photodegradation in water would not be anticipated and is considered suitable for use as supporting information.

6.1.1.4 *Phototransformation in soil*

No relevant information was identified during the literature search.

6.1.1.5 *Data from structural analogues*

PFOS is considered to be extremely persistent. It has not been found to undergo any significant abiotic degradation in water and estimates of abiotic degradation in air are slow (UNEP, 2006; Environment Agency, 2004).

6.1.2 Biodegradation in water

6.1.2.1 *Measured data*

Table 6.1 Summary of screening biodegradation studies

Method	Results	Reliability	Reference
OECD TG 301D (closed bottle) Not to GLP	Not readily biodegradable 43.5% degradation after 28 days (ThOD)	1 (key study)	Wang *et al*. (2013)

A biodegradation screening study is reported in Wang *et al*. (2013). It was said to follow OECD Test Guideline (TG) 301D (closed bottle test) but was not conducted in accordance with the principles of Good Laboratory Practice (GLP). The test was conducted using F-53B with a purity of >98%. The inoculum used in the study was from a sewage treatment plant (although it is not stated whether this was a municipal or industrial plant). The sludge was not pre-adapted to the test material. Ten test vessels with test compound and inoculum were set up, and 2 of these vessels were sampled on days 0, 7, 14, 21 and 28. Ten blank controls and ten positive control vessels were also included. Sodium benzoate was used as the positive reference substance. The test was carried out at a concentration of 3 mg/L F-53B or 2 mg/L sodium benzoate over 28 days. Degradation was monitored by measuring the dissolved oxygen concentration and calculating the Biological Oxygen

Demand (BOD) and Theoretical Oxygen Demand (ThOD). Degradation of the test substance was found to be 23.8% after 21 days and 43.5% after 28 days, showing that the substance did not meet the criteria to be considered readily biodegradable.

Following a review of the paper, the Environment Agency considers that this study followed the standards of OECD TG 301D and that the relevant validity criteria were met (less than 20% variation between replicates at the end of the test and reference substance degradation met the threshold by 14 days - reaching >85%). This guideline is considered suitable for testing substances which are poorly water soluble or volatile and sufficient raw data were provided to allow the reported degradation rates to be re-calculated. The data indicate that the substance does not meet the criteria to be considered readily biodegradable.

6.1.2.2 *Predicted data*

Gomis *et al*. (2015) estimated the degradation half-lives of F-53B using the models in EPISuite™ (US EPA, 2012). Outputs from BIOWIN3, which estimates the biodegradation rate based on molecular structures, were converted into half-lives using the conversion scheme proposed by Aronson, Boethling, Howard and Stiteler (2006). The half-lives were estimated for the neutral form of F-53B. Half-lives of 720 days were estimated for both water and soil. However, the authors note that the predictive power of BIOWIN3 for PFAS is limited due to a lack of data for these substances in the training set and that there is insufficient empirical data to determine the effect of ionisation on degradation rate.

The US EPA CompTox dashboard contains a predicted biodegradation half-life of 4.62 days generated from the OPERA software (US EPA, 2020a), but notes that F-53B is outside of the applicability domain of this model, so the prediction is not used here.

6.1.2.3 *Data from structural analogues*

PFOS is considered to be extremely persistent. It has not been found to undergo any significant biodegradation in aerobic activated sewage sludge, sediment or soil or in anaerobic sewage sludge (UNEP, 2006; Environment Agency, 2004).

6.1.2.4 *Discussion*

The experimental data indicate that the substance does not meet the criteria to be considered readily biodegradable when using a standard test method. The level of degradation observed in this screening test (43.5% after 28 days) is somewhat surprising given the known properties of other PFAS, which are generally assumed to be very persistent.

6.1.3 Biodegradation in sediment

No relevant information was identified during the literature search.

6.1.4 Biodegradation in soil

No relevant information was identified during the literature search.

6.1.5 Summary and discussion of degradation

Based on its chemical structure, F-53B is not expected to undergo significant hydrolysis under environmental conditions. F-53B has not been observed to undergo direct photolysis in water and was found to have low rates of indirect photodegradation in the presence of hydroxyl radicals in water. No experimental data were available for phototransformation rates in air, but based on evidence from other PFAS, a half-life significantly exceeding 2 days is likely.

A 28-day biodegradation screening study is available which concludes that F-53B is not readily biodegradable (achieving 43.5% degradation after 28 days). The Environment Agency notes that this degree of mineralisation for such a highly halogenated (and in particular, fluorinated) substance is surprising, but the test appears to be valid. The conclusion of *not readily biodegradable* will be used in the risk assessment.

There are no environmental simulation data so a realistic half-life in relevant media cannot be established. Highly fluorinated substances generally do not undergo significant abiotic or biotic degradation under relevant environmental conditions. The Environment Agency notes that the analogue PFOS is assumed to have a half-life significantly longer than 60 days in water and 180 days in sediments and soils (UNEP, 2006). These half-lives could be used as an approximation in the absence of more reliable data for the substance itself. However, given the unexpected level of degradation in the ready test, the Environment Agency recommends that further studies are performed to confirm the environmental half-life of F-53B, along with transformation products and pathways.

6.2 Environmental distribution

6.2.1 Adsorption/desorption

6.2.1.1 *Measured data*

The literature review identified five published studies that report partition coefficients for F-53B in relevant environmental media. A summary is provided in Table 6.2. The majority of studies identified reported Kd values, which are calculated by dividing the concentration of the substance in the solid phase by the concentration of the substance in the aqueous phase. Kd values are also referred to as Kp values (ECHA, 2016). Normalisation by the amount of organic carbon in the solid phase provides organic carbon-water partition coefficient (K_{OC}) values.

Wei *et al*. (2019) report on the sorption and desorption of F-53B (purity >98%) in soil maintained at a temperature of 25 °C. Six farmland soil samples (0 to 20 cm) were collected from different provinces in China. A range of soil types were included (sand 14 to

51%, silt 16 to 76%, clay 6 to 37%, organic carbon 0.87 to 2.71%, pH 4.3 to 7.9). Each sample was air dried and homogenized before being used for sorption and desorption experiments, which were all conducted in triplicate. Initially, the sorption of F-53B at a single concentration of 500 µg/L was investigated with samples collected at predetermined intervals from 0 to 48 hours. This allowed the investigators to determine that equilibrium was reached within this time. Then, a standard amount of each soil was suspended in F-53B solutions at concentrations of 10 to 600 µg/L for 48 hours. After centrifugation and filtration, the test solutions were analysed by HPLC-MS/MS to determine the kinetic sorption and the sorption isotherms. Desorption experiments were then conducted by removing the supernatant and drying the soil residues, before adding double de-ionised water to re-suspend the soil. Samples were shaken for 48 hours before the concentrations of F-53B in the test solutions were measured by HPLC-MS/MS after centrifugation and filtration.

Table 6.2 Summary of adsorption/desorption studies

Method	Results (L/kg)	Reliability	Reference
Non-guideline study (batch equilibrium)	log Kd 1.95-2.28 in 6 soils	1 (key study)	Wei *et al*. (2019)
Non-guideline study (observational)	Average log Kd 2.80 in marine sediment-sea water Average log Kd 2.94 in suspended particulate matter-sea water	2 (supporting study)	Liu *et al*. (2019a)
	Average log Kd 0.264 in marine sediment-sea water	2 (supporting study)	Wang *et al*. (2019)
	Average log Kd 4.11 ± 0.58 in suspended particulate matter-sea water Average log Kd 3.17 ± 0.76 in suspended particulate matter-river water	2 (supporting study)	Zhao *et al*. (2020)
	Average log K_{OC} 4.49 ± 0.64 in suspended particulate matter-river water and sediment-river water	2 (supporting study)	Li *et al*. (2020b)
	Average log Kd 1.5 ± 0.41 in sediment-pore water	2 (supporting study)	Feng *et al*. (2020)

Results from the sorption experiments indicated that steady-state was reached in all soils within 48 hours and that the measured data were well described by the pseudo-second order model used to determine the sorption kinetics. All 6 soils were found to have relatively high sorption capacities for F-53B, with the highest maximum sorption capacity reported to be 92.36 mg/kg. The authors report that the Kd values for the 6 soils range from 0.09 to 0.19 L/g (90 to 190 L/kg; equivalent log Kd 1.95 to 2.28). The desorption experiments indicate that desorption from these 6 soils was low, with 1.4 to 8.2% desorbed over 48 hours.

Although the Wei *et al*. (2019) study does not follow a standard guideline, a batch equilibrium method similar to OECD TG 106 was used with six soils of different types to estimate both the sorption and desorption of F-53B. The types of soil used are less variable than those recommended in OECD TG 106, with clay content of 6-37% and organic carbon 0.87-2.71% in the soils tested compared to <10 to 80% and <0.5 to >10% recommended in the guideline. The data presented demonstrate that steady state was reached within the sorption period and the authors note that the modelling methods used to determine the sorption and desorption fitted the data well.

In contrast, a series of monitoring studies have provided information about the distribution and partitioning behaviour of F-53B (among several other PFAS) in aquatic environments in China. Although some of the researchers are involved with more than one paper, the studies appear to be independent and all report separate datasets. The monitoring data are summarized in Section 10.1.4.

- Liu *et al*. (2019a) conducted a monitoring study in Bohai Bay, China. Sea water and sediment samples were taken from 20 sites and analysed F-53B concentrations in the surface water, suspended particulate matter (SPM) and sediment by HPLC-MS/MS. The measured concentrations were used by the authors to calculate the sediment-water and SPM-water partition coefficients (Kd). F-53B was detected in all 20 water and SPM samples, and in 17 of the sediment samples. Individual Kd values for each sample or ranges are not presented in the paper, although it is stated that they varied widely at different sampling sites. Instead, the average log Kd is reported as 2.8 L/kg for marine sediment-sea water and 2.94 L/kg for SPM-sea water.

- Wang *et al*. (2019) conducted a monitoring study in the Pearl River Delta, China. Surface water (1 metre below the surface), bottom water (1 metre above the seafloor) and sediment samples were collected in 2018. In total 250 water samples and 53 sediment samples were collected. Samples were analysed by ultra-performance liquid chromatography-mass spectrometry (UPLC-MS/MS). The measured concentrations were used by the authors to calculate the sediment-water partition coefficient (Kd). F-53B was detected in 97.1% of surface water, 82.9% of bottom water and 54.7% of sediment samples. It is not stated whether the surface or bottom water concentrations were used to calculate the Kd, and individual Kd values for each sample or ranges are not presented in the paper. Instead, the log Kd is reported as 0.264 L/kg for water-sediment.

- Zhao *et al*. (2020) conducted a monitoring study in Bohai Bay and its surrounding rivers, China. Fifty-two sea water samples and 35 river water samples were collected and analysed by HPLC-MS/MS for F-53B concentrations in surface water and SPM. The measured concentrations were used by the authors to calculate the SPM-water partition coefficients (Kd). Individual Kd values for each sample are not presented in the paper. Instead, the average log Kd is reported as 4.11 ± 0.58 L/kg for SPM-sea water and 3.17 ± 0.76 L/kg for SPM-river water.

- Li *et al*. (2020b) conducted a monitoring study in the Hai River basin, China. Surface water and sediment samples were collected in 2017. In total 40 water samples and 20 sediment samples were collected. Samples were analysed by UPLC-MS/MS. The

measured concentrations were used by the authors to calculate the sediment-water partition coefficient and SPM-water partition coefficient (Kd), which are then converted to organic carbon-water partition coefficient (K_{OC}) values based on the organic carbon content of each sample. The individual K_{OC} values for each sample are not presented in the paper, but the K_{OC} for sediment and SPM are combined to give an average log K_{OC} of 4.49 ± 0.64 L/kg.

- Feng *et al*. (2020) conducted a monitoring study in the Yellow Sea, China. Thirty sea water and sediment samples were collected and analysed by UPLC-MS/MS for F-53B concentrations in surface water and sediment (including pore water). The measured concentrations were used by the authors to calculate the sediment-pore water partition coefficients (Kd). Individual Kd values for each sample are not presented in the paper. Instead, the average log Kd is reported as 1.5 ± 0.41 L/kg.

Liu *et al*. (2019a), Wang *et al*. (2019), Zhao *et al*. (2020), Li *et al*. (2020b) and Feng *et al*. (2020) are observational studies that use monitoring data to estimate the Kd or K_{OC} of F-53B for sediment and suspended particulate matter. Unfortunately, the Kd is typically reported as an average value instead of as a range which would have been more informative for this assessment. However, these papers do still give some indication of the adsorption behaviour of F-53B in the aquatic environment.

6.2.1.2 *Predicted data*

Gomis *et al*. (2015) estimated the log K_{OC} of the anionic form of F-53B using a method proposed by Tülp *et al*. (2009). Initially, a log K_{OC} for the neutral form was calculated based on a predicted log K_{OW} from COSMOtherm. This was then adjusted to a log K_{OC} for the anionic form based on the ratio of measured log K_{OC} for the neutral and anionic forms of PFOA and PFOS. Gomis *et al*. (2015) calculate a log K_{OC} of 3.28 for anionic F-53B. As discussed in Section 5.4, the reliability of the predicted log K_{OW} value is unknown and consequently the reliability of the calculated log K_{OC} is also uncertain.

The US EPA CompTox dashboard contains a predicted K_{OC} of 329 (log K_{OC} 2.52) generated from the OPERA software (US EPA, 2020a), but notes that F-53B is outside of the applicability domain of this model, so the prediction is not used here.

6.2.1.3 *Data from structural analogues*

PFOS is reported to have log Kd values of 0.99 to 1.55 in soil and 0.87 in sediment (Environment Agency, 2004). The organic carbon content of the soils and sediment tested are not reported in Environment Agency (2004). However, assuming a standard soil with 2% organic carbon and a standard sediment with 5% organic carbon (ECHA, 2016) log Koc values of 2.69 to 3.25 for soil and 2.17 for sediment have been calculated by the Environment Agency.

Several of the observational studies described for F-53B also report Kd values for PFOS. Liu *et al*. (2019a) report average log Kd of 2.64 L/kg for sediment-water and 3.04 L/kg for SPM-water for PFOS and Wang *et al*. (2019) report a log Kd of 0.285 L/kg for PFOS, which are very similar to the values reported for F-53B in those two papers. Zhao *et al*.

(2020) report the average log Kd as 3.6 ± 0.43 L/kg for SPM-sea water and 2.9 ± 0.88 L/kg for SPM-river water for PFOS. Li *et al*. (2020b) combined the K_{OC} for sediment and SPM to give an average log K_{OC} of 3.75 ± 0.61 L/kg for PFOS, which was statistically significantly lower than that of F-53B. Feng *et al*. (2020) calculate a log Kd for PFOS, but the value is not stated in their paper. However, they do note that the log Kd of PFOS was lower than that of F-53B and the Environment Agency estimates the value to be just below 1 from reading off a graph. These three papers all report that F-53B has a higher log Kd or log K_{OC} value than PFOS, indicating that F-53B is likely to partition more strongly to soil or sediment in the environment than PFOS.

6.2.1.4 *Recommended value*

The study by Wei *et al*. (2019) reports log Kd in 6 soils ranging from 1.95-2.28 in a non-GLP study that approximates to OECD TG 106. Unfortunately, Wei *et al*. (2019) do not link the soil Kd value and the specific soil tested, so these values cannot be converted to a K_{OC}. However, the organic carbon range of the soils tested is similar to that of the standard soil with 2% organic carbon (ECHA, 2016), so the log Kd values have been converted to log K_{OC} by the Environment Agency. The log K_{OC} values based on a soil with 2% organic carbon would be 3.65 to 3.98. As this is a relatively narrow range, the Environment Agency has used the mean value (log K_{OC} 3.85) for the purposes of this assessment. This is a similar order of magnitude as the range of predicted values, even though the reliability of these data is uncertain.

Monitoring studies provide supporting information to suggest that F-53B is moderately or strongly adsorptive in aquatic environments. For example, an average log K_{OC} of 4.49 ± 0.64 was derived for sediment and suspended particulate matter in one study (Li *et al*., 2020b).

6.2.2 Volatilisation

The vapour pressure of F-53B is assumed to be around 3 x 10^{-7} kPa at 25°C (see Section 5.1) and the water solubility is assumed to be around 500 mg/L at 20°C (see Section 5.3). Based on these values and the molecular weight, the Henrys Law constant can be calculated using Equation R.16-4 of ECHA (2016) as 3.4 x 10^{-4} Pa/m^3.mol. This value indicates that F-53B would not be expected to be volatile.

6.2.3 Distribution modelling

To estimate the distribution of F-53B in the environment, the Environment Agency has run the EQC v3.0 Mackay Level III fugacity model using the input parameters indicated in Table 6.3. The model was run twice. In scenario 1 all emissions were assumed to be released to water. In scenario 2 emissions were split equally between air, water and soil.

Table 6.3 Estimated distribution of F-53B

Input Parameter	Value	
Molecular mass	570.67 g/mol	
Water solubility	500 mg/L	
Vapour pressure	0.0003 Pa	
Henry's Law constant	0.00034 Pa m^3/mol	
Log K_{OW}	4.63	
Half-life in air (hours)	2.4 x 10^{41}	
Half-life in water (hours)[a]	2.4 x 10^{41}	
Half-life in soil (hours)	2.4 x 10^{41}	
Half-life in sediment (hours)	2.4 x 10^{41}	
Model output	Scenario 1	Scenario 2
Air %	0	0.04
Water %	99.8	56.8
Soil %	0.004	43.0
Sediment %	0.2	0.11

Note: a -The upper bound value for biodegradation of a non-readily biodegradable substance in EUSES is 1 x 10^{40} days to represent infinity (equivalent to 2.4 x 10^{41} hours)

On the basis of these input parameters and the assumption that F-53B does not degrade in the environment, the model predicts that if released to water then nearly all the substance would remain in the water compartment. If released to air, water and soil equally then F-53B would be expected to partition to the water and soil compartments. Changing the log K_{OW} to 4.38 and 4.79 did not alter the estimated environmental distribution of F-53B.

The Environment Agency has used the SimpleTreat model in EUSES (v2.03) to predict the following partitioning of F-53B in a wastewater treatment plant. The sensitivity of changing the log K_{OC} value is summarised in Table 6.4.

Table 6.4 Predicted partitioning of F-53B in a wastewater treatment plant

Fraction of emission to compartment / degraded	Log K_{OC} 3.65	3.85	3.98
Air	< 0.1%	< 0.1%	< 0.1%
Water	65.3 %	54.7 %	47.9 %
Sludge	34.7 %	45.3 %	52.1 %
Biodegradation	0.0 %	0.0 %	0.0 %

This model predicts that a significant fraction will partition to sludge, with a large fraction emitted to effluent. The reliability of this prediction for this type of substance is unknown, and the uncertainties in the physico-chemical input parameters also mean that this distribution might not be fully reliable.

The Environment Agency considers that although some caution is required as most of the input data is predicted, the results support the idea that both sludge and surface water are the key environmental compartments for F-53B. This is mainly because the model results are driven by the sorption value and water solubility.

6.2.4 Long-range transport potential

The OECD has produced a decision support tool for estimating the long-range transport potential (LRTP) of organic chemicals at a screening level (Wegmann *et al*., 2009). It is a steady state non-equilibrium model in a standardised evaluative environment and predicts two characteristics that can be used to provide an indication of the LRTP of a substance (Characteristic Travel Distance, Transfer Efficiency), together with overall persistence (P_{OV}).

Gomis *et al*. (2015) used the LRTP screening tool to estimate the potential distribution of F-53B. The input parameters needed to run the model are log K_{OW}, log K_{AW} (air-water partition coefficient), and the half-lives in air, water and soil. Gomis *et al*. (2015) estimated the physico-chemical parameters for the neutral form of F-53B using SPARC or COSMOtherm, so these were adjusted to account for the proportion expected to be ionised. The degradation half-lives were those predicted by the US EPA EPISuite for the neutral form of F-53B, as there was insufficient empirical data to justify any adjustment. Degradation half-lives of 2 751 hours in air and 17 280 hours in water and soil were used for F-53B. Three emission scenarios were modelled, with emissions to air, water or soil only. The results presented in Gomis *et al*. (2015) were the highest overall persistence (1 039 days), characteristic travel distance (1 741 km) and transfer efficiency (0.024%). Given the uncertainty in the input parameters, the Environment Agency considers the modelling by Gomis *et al*. (2015) to be highly uncertain.

To estimate the LRTP of F-53B, the Environment Agency has performed calculations using the input parameters indicated in Table 6.5.

The sensitivity of changing the log K_{OW} value was investigated but due to the very low degradation rate used in the input parameters for air, water and soil compartments, negligible change in the output was recorded from changing the log K_{OW} value over the range 4.38 to 4.79.

Table 6.5 Estimated long-range transport potential of F-53B using OECD LRTP

Input Parameter	Value		
Molecular mass	570.67 g/mol		
Log K_{AW} [a]	-6.84		
Half-life in air (hours)	2.4×10^{41}		
Half-life in water (hours)[b]	2.4×10^{41}		
Half-life in soil (hours)	2.4×10^{41}		
LRTP output parameter	Log K_{OW}		
	4.38	4.63	4.79
Characteristic Travel Distance (km)	45 643	37 576	31 857
Transfer Efficiency (%)	1.3	1.8	2.3
P_{OV} (days)	1.44×10^{40}	1.44×10^{40}	1.44×10^{40}

Note: a - This is the log of the dimensionless HLC calculated using Equation R.16-5 of ECHA R16 – see Section 6.2.2.

b -The upper bound value for biodegradation of a non-readily biodegradable substance in EUSES is 1×10^{40} days to represent infinity (equivalent to 2.4×10^{41} hours).

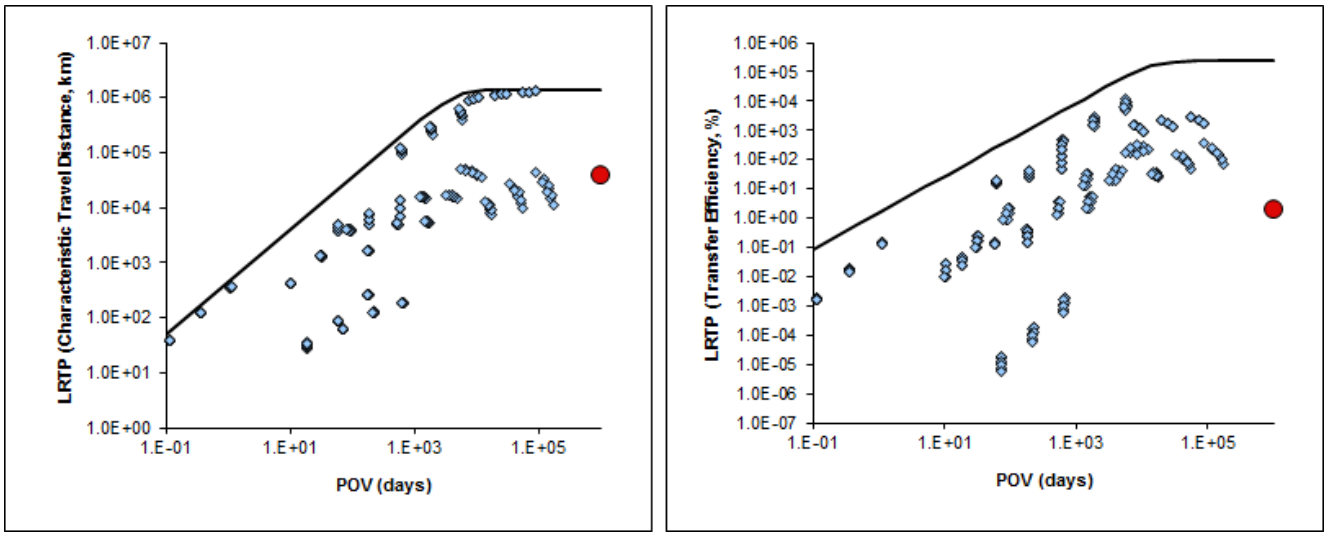
The OECD LRTP screening tool predicts the following outputs:

- Overall persistence (Pov).
- Characteristic Travel Distance (CTD): a transport-oriented LRTP indicator. It quantifies the distance from the point of release to the point at which the concentration has dropped to 1/e, or about 37% of its initial value; and
- Transfer Efficiency (TE): is a target-oriented LRTP indicator originally applied to quantify the deposition of chemicals transported from different regions to the North American Great Lakes.

The OECD LRTP screening tool allows comparisons of these three characteristics for a range of substances, provided in Figure 6.1.

Based on this screening tool, it appears that F-53B may be capable of long-range transport. Evidence of occurrence (or not) of F-53B in the Arctic and other remote regions would also need to be taken into account (noting the proximity of industrial activity and population centres). This is beyond the scope of this evaluation.

Figure 6.1 Long-range transport potential of F-53B (red dot) compared to the long-range transport potential of other POPs (blue dots) generated in the OECD LRTP screening tool



Note: In the left hand graph the x axis is overall persistence in days (Pov) and the y axis is the Characteristic Travel Distance (km). In the right hand graph the x axis is overall persistence in days (Pov) and the y axis is the Transfer Efficiency (%).

Ti *et al*. (2018) modelled the long-range transport potential of F-53B using the Globo-POP tool (Wania and Mackay, 1995). The physico-chemical and fate parameters needed to run the model were modelled using QSAR or taken from other publications which had generated calculated values. Due to the lack of measured degradation data, three scenarios assuming different degradation rates were modelled, with half-lives ranging from 4 320 hours (~0.5 years) to 1 x 10^{15} hours (to represent an assumption of no degradation). Globo-POP is a non-steady state model that can calculate an Arctic Contamination Potential (ACP) to provide an indication of whether a chemical is likely to be transported over large distances to remote environments within set time periods. Ti *et al*. (2018) ran the Globo-POP model assuming that all emissions of F-53B would be to freshwater.

Two types of ACP are reported by Ti *et al*. (2018). The absolute ACP is defined as “the fraction of F-53B mass present in Arctic surface compartments relative to the cumulative global emissions over a period”. After 1 year the absolute ACP was 0.01-0.02% and after 10 years the absolute ACP was 0.05-1%. The relative ACP is defined as “the fraction of the total global mass in the environment present in the surface media (all media except the atmosphere) of the northernmost latitudinal band of the model after a certain time period”. After 1 year the relative ACP was 0.2 to 0.25% and after 10 years the relative ACP was 0.55 to 1.05%. The authors state that these modelling results indicate that F-53B will reach remote areas although F-53B is likely to take longer than 1 year to reach the Arctic via ocean currents. The model predicts that the major reservoirs of F-53B will be freshwater and surface oceanic waters, with only a small proportion being transferred to the deep sea and burial by sediment.

6.3 Bioaccumulation

6.3.1 Bioaccumulation in aquatic organisms

6.3.1.1 *Measured data*

The available studies are summarised in Table 6.6. Further monitoring studies that only report on the quantification of F-53B in biota are presented in Section 10.1.4.

The literature review identified nine laboratory studies that reported on the bioaccumulation, and in some cases the depuration, behaviour of F-53B. The four papers by Shi *et al*. (2019a, 2019b, 2018, 2017) are all conducted by the same research laboratory, with the later three papers reporting different aspects of the same experiment. The three studies by Wu *et al*. (2019a, 2019b, 2019c) and Tu *et al*. (2019) were all conducted by the same research laboratory, but report four independent experiments.

In addition, four monitoring studies were identified that reported on the measured concentrations of F-53B in aquatic organisms and calculated either bioaccumulation factors or trophic magnification factors.

Laboratory fish studies

- Shi *et al*. (2017) exposed 6-hour-post-fertilisation (hpf) Zebrafish (*Danio rerio*) embryos individually in well plates to F-53B with an analytical purity of >96% at a nominal concentration of 4 mg/L until 96 hpf to investigate the uptake of F-53B. An experiment reported in the same paper demonstrated that the 96-hour survival of the embryos was not significantly different to that of the control at this exposure concentration. The exposure concentration was prepared with the use of a solvent (dimethyl sulfoxide, DMSO) and the final DMSO concentration in each test vessel and the solvent control was 0.1% v/v. Test media were renewed every 24 hours. Fifteen embryos were sampled at 24, 48, 72 and 96 hours and washed in deionised water. Samples of test media were taken at the start of the exposure and after 24 hours, just before test media renewal.

  Concentrations at 24 hours were slightly lower than those at 0 hours, with a reduction of up to 11.6% which the authors attribute to uptake by the embryo. Results are reported based on the initial measured concentration of 3 mg/L. F-53B was found to accumulate rapidly in the Zebrafish embryos reaching a maximum of 123.14 ng/embryo at 96 hpf, but did not achieve steady state over the 96-hour uptake period. An unspecified number of exposed embryos were then transferred to clean water for a 24-hour depuration period. At 120 hpf, 8.8% of the accumulated F-53B had cleared from the fish. The authors conclude that this demonstrates that the Zebrafish embryos were unable to significantly eliminate F-53B. Bioconcentration factors (BCF) are not calculated in this paper, and the data are not presented in a way that allows the Environment Agency to calculate a BCF.

Table 6.6 Summary of bioaccumulation studies for F-53B

Method	Results	Reliability	Reference
Non-guideline study (embryo uptake and depuration) semi-static	Zebrafish BCF not calculated. Rapid accumulation over 96 hours, steady state not reached. 8.8% depurated after 24 hours.	2 (supporting study)	Shi *et al*. (2017)
Non-guideline study (multi-generation uptake) flow-through	Zebrafish BCF not calculated. Sex-specific accumulation in blood and gonad in F0 generation. Also detected in F1 generation which had not been directly exposed.	2 (supporting study)	Shi *et al*. (2018)
Non-guideline study (multi-generation uptake) flow-through	Zebrafish BCF not calculated. Sex-specific accumulation in liver in F0 generation	2 (supporting study)	Shi *et al*. (2019a)
Non-guideline study (multi-generation uptake and depuration) flow-through	Zebrafish BCF not calculated. Accumulation in F1 and F2 embryos which had not been directly exposed. Minimal clearance after 5 days' depuration.	2 (supporting study)	Shi *et al*. (2019b)
Non-guideline study (embryo uptake) semi-static	Zebrafish BCF not calculated. Accumulation in exposed embryos.	2 (supporting study)	Wu *et al*. (2019a)
Non-guideline study (adult uptake and depuration) semi-static	Zebrafish Tissue-specific BCF_k 228 to 2 212 in females and 473 to 4 425 in males. Tissue-specific elimination half-lives 169.6 to 358.5 h in females and 152.4 to 318.1 h in males.	2 (supporting study)	Wu *et al*. (2019b)
Non-guideline study (larval uptake and depuration) semi-static	Zebrafish BCF_k 3 612 to 3 615 Elimination half-lives 241.5 to 258.6 h.	2 (supporting study)	Wu *et al*. (2019c)
Non-guideline study (embryo uptake) semi-static	Zebrafish BCF 125 to 358	3 (unreliable)	Tu *et al*. (2019)

Non-guideline study (algal uptake) static	Alga *Scenedesmus obliquus* BAF 27 040	3 (unreliable)	Liu *et al*. (2018)
Non-guideline study (observational)	Carp Tissue-specific median BAF: 6 310 to 169 824 Whole body median BAF: 13 183 to 20 893	2 (supporting study)	Shi *et al*. (2015)
	Frog *Pelophylax nigromaculatus* Whole body mean BAF 1 304	2 (supporting study)	Cui *et al*. (2018a)
	Marine food web TMF: 3.43 to 4.32	2 (supporting study)	Liu *et al*. (2017a)
	BAF for various marine species: 155 to 24 547 Marine food web TMF: 3.37	2 (supporting study)	Chen *et al*. (2018)

- A series of related papers present the results from a subsequent multi-generational exposure experiment (Shi *et al*. 2019a, 2019b, 2018). Adult Zebrafish were exposed to F-53B in a flow-through system for 180 days before transferring the F1 eggs to clean water and investigating a range of chronic endpoints in the F0, F1 and F2 generations (see Sections 7.1.1 and 9.2). One hundred and twenty adult Zebrafish were exposed in single sex groups of 30 fish to F-53B (purity >99.5%) at each nominal exposure concentration (0.005, 0.05 and 0.5 mg/L) for 180 days. Exposure concentrations were prepared with the use of a solvent (DMSO) and the final DMSO concentration in each test vessel and the solvent control was 0.004% v/v. Fish were fed twice daily and maintained at 28 °C and a 14:10 hour light: dark cycle. Ten pairs of fish from each exposure group were randomly selected to spawn in clean water each week. After 180 days the F0 fish were anaesthetised and F-53B quantified in the blood, gonads and liver. Whole fish measurements were not made for the F0 generation. F1 and F2 fish were reared in clean water, and were also analysed for F-53B. Three samples of test media from each exposure concentration were taken at the start of the exposure and after 180 days.

  The mean measured concentrations were within 10% of nominal, so results are reported based on the nominal exposure concentrations. For an experiment with an exposure period of 180 days a larger number of sampling points would have increased the confidence that the exposure concentrations were maintained throughout the study, although the measured concentrations at day 0 and day 180 were close to nominal. F-53B was not detected in the fish sampled from the control.

  The results are reported in several different papers and the concentrations of F-53B in different biological samples are reported in Table 6.7. Shi *et al*. (2018) report the concentrations of F-53B detected in F0 blood and gonads from six replicate fish. Male blood concentrations of F-53B were 1.6-2.4 times higher than those of female fish, which was found to be statistically significantly different. Shi *et al*. (2019a)

report the concentrations of F-53B detected in F0 livers. The authors note that the concentrations detected in liver were higher than those detected in gonads in Shi *et al*. (2018). Shi *et al*. (2018) conclude that sex-specific bioaccumulation was demonstrated, and suggest that the high concentrations in ovaries could be due to binding to the high levels of protein found there.

F-53B was measured in 180-day old F1 adults in blood and gonads from six replicate fish (Shi *et al*., 2018), indicating that F-53B was transferred from the F0 parents. BCFs are not calculated in this paper, and the data are not presented in a way that allows the Environment Agency to calculate them either (whole body concentrations are required).

Table 6.7 F-53B concentrations detected in fish samples from a multi-generational exposure study

Life stage	Tissue	Nominal F-53B exposure concentration (mg/L)			Reference
		0.005	0.05	0.5	
F0 adult male	blood (µg/mL)	12.85	63.58	362.79	Shi *et al*. (2018)
	testes (ng/mg)	2.12	11.63	33.9	Shi *et al*. (2018)
	liver (ng/mg)	12.65	111.41	not tested	Shi *et al*. (2019a)
F0 adult female	blood (µg/mL)	8.03	31.79	151.16	Shi *et al*. (2018) (calculated by Environment Agency)
	ovaries (ng/mg)	4.6	58.23	142.78	Shi *et al*. (2018)
	liver (ng/mg)	5.11	67.54	not tested	Shi *et al*. (2019a)
F1 embryos	whole body (ng/mg)	1.97	24.75	not tested	Shi *et al*. (2019b)
F1 adult male	blood (µg/mL)	0.31	not tested	not tested	Shi *et al*. (2018)
	testes (ng/mg)	0.1	not tested	not tested	Shi *et al*. (2018)
F1 adult female	blood (µg/mL)	0.37	not tested	not tested	Shi *et al*. (2018)
	ovaries (ng/mg)	0.12	not tested	not tested	Shi *et al*. (2018)
F2 embryos	whole body (ng/mg)	0.0054	not tested	not tested	Shi *et al*. (2019b)

Shi *et al*. (2019b) report F-53B concentrations in the whole bodies of the F1 embryos, and in larvae that were raised in clean water for 5 days. Three replicates of 30 embryos or larvae were pooled and analysed. The concentration of F-53B in F1 embryos whose parents were exposed to 0.005 mg/L exposure was 1.97 ng/mg, and after 5 days' depuration this had reduced to 1.91 ng/mg. The concentration of F-53B in F1 embryos whose parents were exposed to 0.05 mg/L exposure was 24.75 ng/mg, and after 5 days' depuration this had reduced to 23.63 ng/mg.

Concentrations of F-53B were also measured in F2 embryos and larvae following the same method. The concentration of F-53B in F2 embryos whose grandparents were exposed to 0.05 mg/L exposure was 0.0054 ng/mg and after 5 days depuration this had reduced to 0.0047 ng/mg. The authors conclude that F-53B can be transferred from parent to offspring and that F-53B is not significantly cleared from the embryo once exposure has stopped.

- Wu *et al*. (2019a) exposed approximately 250 2-hpf Zebrafish embryos to F-53B (>99% purity) at nominal concentrations of 0.5, 20 and 200 µg/L for five days.

Exposure concentrations were prepared with the use of a solvent (DMSO) and the final DMSO concentration in each test vessel and the solvent control was 0.01% v/v. Embryos were maintained at 27 °C and a 14:10 hour light:dark cycle. Each exposure concentration and control had three replicates and the exposure solutions were changed daily. The chemical analysis for this study is reported in Deng, Wu, Xu, Jin, He, Wan, Yu, Rao and Tu (2018). Samples were taken at the start of the exposure and after 24 hours. The chemical analysis indicated that exposure concentrations were lower than nominal but maintained over 24 hours. The authors suggest that this is due to rapid initial adsorption to the glass surfaces. As the exposure concentrations were not found to decrease over the renewal period the arithmetic mean of the initial and day 1 concentrations were used to report the results. These were 0.23, 12.5 and 135 µg/L for the 3 treatments. Wu *et al*. (2019a) report that the concentration of F-53B accumulated by the embryos was measured using UPLC-MS/MS. F-53B was not detected in the control group. Whole body concentrations of F-53B were 0.170, 8.299 and 52.671 ng/mg ww in the 0.5, 20 and 200 µg/L exposure groups, respectively. BCFs are not calculated in this paper and as there was only a single sampling point it is not possible to determine whether steady state was reached. However, based on the data in the report, a non-steady state BCF of 390 to 739 L/kg can be estimated.

- Wu *et al*. (2019b) used adult Zebrafish to determine the uptake and depuration of F-53B. Male and female Zebrafish were exposed separately to F-53B with a purity of >99% at nominal concentrations of 10 and 100 µg/L. Exposure concentrations were prepared with the use of a solvent (DMSO) and the final DMSO concentration in each test vessel and the solvent control was 0.01% v/v. All exposure concentrations and the solvent control were run in triplicate. Exposure solutions were renewed daily, and fish were fed twice daily with brine shrimps. At each sampling point (1, 3, 5 and 7 days during uptake and 8, 10 and 12 days during depuration) 3 fish were sampled from each tank. Liver, gill, gonad and brain were dissected and pooled by sex and treatment. F-53B was quantified in the water and fish samples by HPLC-MS/MS. BCFs and elimination half-lives were calculated by the authors. The authors report that the exposure concentrations were selected as 1% of the LC_{50} reported by Wang *et al*. (2013) and Shi *et al*. (2017) and that no abnormal behaviour or mortality was observed throughout the experiment.

  The measured exposure concentrations of F-53B in the 10 and 100 µg/L exposures were 7.24 to 7.66 µg/L and 73.95 to 74.92 µg/L, respectively. The authors state that F-53B water concentrations during the depuration phase were negligible. F-53B concentrations in liver, gill, gonad and brain were found to increase over the 7-day exposure period, and steady state was not reached. The accumulation rates were found to be sex- and tissue-specific. In females, accumulation was highest in the gonads and liver with no statistical difference between these two tissues, but was statistically lower in gill and brain. In males, accumulation was highest in liver and gills with no statistical difference between these two tissues, but was statistically lower in gonads and brain. Wu *et al*. (2019b) report the highest detected concentrations as 5.42 and 59.19 mg/kg in ovaries and 8.98 and 116.24 mg/kg in

male liver, for the two exposure groups. The Environment Agency notes that these are very similar to the concentrations reported by Shi *et al*. (2018) over a much longer exposure period.

BCFs were calculated for individual tissues using both a first-order kinetic model and assuming steady state. However, since steady state was not demonstrated to have been reached, the Environment Agency prefers the values from the kinetic model (BCF_k) in this assessment (since this should approximate to steady state). The kinetic model used is the same as that in OECD TG 305, but growth and lipid content were not adjusted for although these should not have altered much over this relatively short exposure and depuration period. Although it is not explicitly stated by the authors, it appears that the measured water concentrations have been used in the BCF calculations. The tissue-specific BCF_k values range from 228 to 2 212 for female Zebrafish, and 473 to 4 425 for male Zebrafish. The tissue-specific elimination half-lives ranged from 7.1 to 14.9 days for females and 6.4-13.3 days for males. Whole body BCF and elimination rates were not calculated by the authors as only tissue specific samples were analysed.

- Wu *et al*. (2019c) used 72-hpf Zebrafish larvae to determine the uptake and depuration of F-53B. Zebrafish were exposed to F-53B with a purity of >99% at nominal concentrations of 10 and 100 µg/L in 500 mL beakers of 200 larvae. Exposure concentrations were prepared with the use of a solvent (DMSO) and the final DMSO concentration in each test vessel and the solvent control was 0.005% v/v. Exposure solutions were renewed daily to maintain exposure concentrations. At each sampling point (2, 6, 21, 29, 45 and 48 hours during uptake and 50, 54, 69 and 72 hours during depuration) 15 larvae were sampled from each beaker and rinsed several times in clean exposure medium to remove any non-accumulated substance. F-53B was quantified in the water and larvae by HPLC-MS/MS. BCFs and elimination half-lives were calculated by the authors. The authors report that the exposure concentrations were selected as 1% of the LC_{50} reported by Wang *et al*. (2013) and Shi *et al*. (2017) but no information is provided on mortality levels observed during the experiment.

  The measured exposure concentrations of F-53B in the 10 and 100 µg/L exposures were 4.93 µg/L and 51.92 µg/L, respectively. The authors state that F-53B water concentrations during the depuration phase were negligible (0.54 - 0.181 µg/L). The whole body burden of F-53B increased rapidly over the 48-hour exposure period, but did not reach steady state. In fact, Figure 2 in Wu *et al*. (2019c) indicates that uptake was still linear after 48 hours' exposure. BCFs were calculated using both a first-order kinetic model and assuming steady state. However, since steady state was not demonstrated to have been reached, the Environment Agency prefers the BCF_k values in this assessment (since they should approximate to steady state). The kinetic model used is the same as that in OECD TG 305. Growth and lipid content were not adjusted for. Although it is not explicitly stated by the authors, it appears that the measured water concentrations have been used in the BCF calculations. The whole body BCF_k was 3 612 at the nominal 10 µg/L exposure, and

3 615 at the nominal 100 µg/L exposure. The elimination half-lives ranged from 241.5 – 258.6 hours, with 6.4 - 7.1% eliminated over the 24-hour depuration period. The Environment Agency considers that due to the short length of the depuration period these values should be considered uncertain, but that they do indicate that F-53B is slowly depurated.

- Tu *et al*. (2019) investigated the uptake of F-53B in Zebrafish embryos exposed from 3-hpf to 4-days post fertilisation (dpf). Fertilised embryos were exposed to nominal F-53B concentrations of 0.015, 0.15 and 1.5 mg/L or a blank control. Exposure solutions were renewed daily. The test vessels were maintained at 28.5 °C under a 12:12 hour light:dark cycle, and all treatments were run in triplicate. The exposure concentrations were analysed by HPLC-MS/MS at the beginning of the exposure (T0) and just before renewal (T24). Measured concentrations at T0 were 0.0073, 0.0798 and 1.0807 mg/L and at T24 were 0.0062, 0.0716 and 1.1432 mg/L for the low, medium and high exposure concentrations respectively. BCFs are calculated by the authors and range from 125 - 358 for F-53B. The Environment Agency does not consider that this paper is fully reliable. The method of BCF calculation is not reported (e.g. it is not clear whether the nominal or an average measured water concentration was used). It is also not stated whether any intermediate time points were sampled, or if steady state was reached. Finally, more than 15% mortality was observed in the 0.015 and 1.5 mg/L exposure groups. As these concentrations would not be expected to result in significant acute toxicity (see Section 7.1.1), this suggests that the test organisms were stressed in some other way. The Environment Agency considers that this study should not be used as part of the weight of evidence assessment.

Although a number of experimental studies are available that investigated the bioaccumulation of F-53B in fish, none were conducted to standard guidelines or to GLP and not all calculated a BCF. However, together, the studies demonstrate that F-53B has the potential to bioaccumulate in fish, with faster uptake rates than depuration rates. Two studies that report BCF are considered reliable by the Environment Agency. Wu *et al*. (2019c) report a BCF_k of 3 615 in Zebrafish larvae and Wu *et al*. (2019b) report a tissue-specific BCF_k of up to 4 425 in adult Zebrafish.

Laboratory algal studies

Liu *et al*. (2018) report an algal accumulation study and an algal growth inhibition study (see Section 7.1.3) using *Scenedesmus obliquus*. The test was conducted using F-53B with a purity of 98%, which is stated to contain 91% C_8, 7% C_{10} and 0.3% C_{12} chlorinated polyfluorinated ether sulfonate. A stock solution of 200 mg/L was made in culture medium and this was diluted to prepare nominal exposure concentrations of 0.05, 0.5 and 5 µg/L. It is stated that the results in the paper are based on measured concentrations and that samples were taken after 2 hours in vessels without algal cells, but the analytical results are not presented. Algal cells were exposed to F-53B and a sample taken after 1 minute as a measure of the amount of F-53B rapidly adsorbed to the surface of the algae. A second sample was taken after 2 hours' exposure, which the author's state was to avoid algal growth resulting in a dilution effect. The amount of F-53B accumulated within the

algal cell was calculated by subtracting these results. Concentrations were determined using HPLC-MS/MS.

The amount of F-53B accumulated by the algal cells in 2 hours increased with exposure concentration. Bioaccumulation factors (BAFs) were calculated by the authors, and they note that steady state may not have been reached over this short exposure period. The mean BAF for F-53B was 27 040.

The Environment Agency does not consider this study reliable for use in this assessment due to the very short exposure period and the difficultly in determining whether the F-53B had been accumulated within the algal cells or adsorbed onto them.

Field studies

- Shi *et al*. (2015) investigated the bioaccumulation of F-53B in wild Crucian Carp (*Carassius carassius*). Adult fish and surface water samples were collected from the Xiaoqing River (30 fish) and Tangxun Lake (13 fish) in China in July 2014 and analysed to determine the concentration of F-53B in water, blood, kidney, gonad, liver, heart, brain, swim bladder, gill, muscle and bile. Three water samples were collected from Xiaoqing River and 2 water samples from Tangxun Lake at the same time as the fish were caught. Samples were analysed by HPLC-MS/MS and quantified by comparison to a F-53B standard purified from a commercial F-53B product. The authors calculate a range of BAFs based on the concentration of individual tissues compared to the water concentration, and also the concentration in the whole fish body compared to the water concentration (whole fish concentrations were calculated on the basis of the proportion of each type of tissue in the fish, but the values are not reported).

  F-53B was detected above the limits of quantification in all tissue and surface water samples (see Section 10.1.4). The highest concentrations of F-53B in fish were found in the blood (34-43%), muscle (21-28%) and gonad (16-20%). The authors note that this finding suggests that F-53B binds to serum albumin. Tissue specific median log BAF ranged from 3.8-5.23 for Tangxun Lake and 3.92-5.01 for Xiaoqing River. The whole body median log BAFs were 4.32 for Tangxun Lake and 4.12 for Xiaoqing River, and the authors conclude that F-53B is highly bioaccumulative. The authors do not provide any discussion of the fact that concentrations in fish that represent bioaccumulation over an extended period have been compared to water samples from a single time point, nor how representative the water samples may have been.

- Cui *et al*. (2018a) report on the occurrence and tissue distribution of F-53B in wild Black-spotted Frog (*Pelophylax nigromaculatus*). Frogs were collected in 2016 from paddy fields from 4 sites in China. The Changshu and Huantai sites were near large scale fluorochemical sites, while the Quzhou and Zhoushan sites were not. Fifty-six frogs were collected in total and dissected to provide samples of liver, kidney, heart, lung, stomach, intestine, gonad, skin and muscle. Water samples were collected from the same sites in parallel. Samples were analysed by UPLC-MS. The whole body burden was calculated by the authors based on the assumed proportion of

each tissue type. The BAF was calculated as the concentration of F-53B in the whole body divided by the concentration in the corresponding water sample (whole body concentrations were calculated on the basis of the proportion of each type of tissue in the frogs, but the values are not reported).

F-53B was detected in all frogs and surface water samples (see Section 10.1.4), with higher concentrations observed in the sites nearer to fluorochemical plants. In male frogs, the largest amounts of F-53B were in the skin, liver and muscle, whereas in females the largest amounts of F-53B were in the ovaries. The mean BAF for F-53B across all sites was 1 304 (log BAF 3.12). The authors note that only a single water sample was taken at each site and that this may not have been representative of the exposure of the frogs throughout their lives. In addition, frogs do not spend all of their time in water, so the BAF might not reflect all potential routes of exposure.

- Liu *et al*. (2017a) conducted a monitoring study in the Bohai Sea, China to measure the concentrations of F-53B in marine organisms (114 biota samples across 2 algal species, 11 invertebrate species, 8 fish species) from 2012 to 2014. Samples were collected and then transported to the laboratory on ice before being washed in tap and Milli-Q water. Algae were analysed whole, but only the soft tissue of invertebrates and the flesh of fish was analysed. Sampled individuals were combined into the 114 composite samples before analysis by UPLC-MS. F-53B was detected in nearly all of the species collected, with a detection frequency of 50% (see Section 10.1.4).

  Samples were also analysed with a Thermo DELTA V isotope ratio mass spectrometer to determine the stable isotope of nitrogen and carbon. This information was used by the authors to elucidate the structure of the food web and the trophic level of the species sampled using the filter feeder zhikong scallop (*Chlamys farreri*) as the reference point. The authors calculated the trophic magnification factor (TMF) of F-53B using stable isotope analysis and three different regression methods after combining all samples from all locations from 2012 to 2014. The TMF ranged from 3.43 to 4.32. As biomagnification is indicated by a TMF value above 1, the authors conclude that F-53B does biomagnify. The authors note that the use of soft tissue instead of whole body concentrations and the fact that the fish may migrate to feed over larger areas than those sampled may have added uncertainty to the calculation of the TMF.

- Chen *et al*. (2018) also conducted a monitoring study in the Bohai Sea, China to measure the concentrations of F-53B in sea water and marine organisms (152 samples across 6 invertebrate species, 10 fish species, 1 seabird, 1 mammal). The date of sampling is not stated, although the authors indicate that this study was conducted after that of Liu *et al*. (2017a). No details on the location or number of sea water samples is given, so it is unclear whether these samples were taken at the same time and place as the biological samples. Biota samples were collected and transported to the laboratory on ice before analysis by HPLC-MS/MS. Only the soft tissue of invertebrates and the flesh of fish, birds and mammals was analysed.

F-53B was detected in all of the species collected, with a detection frequency of 81.3% (see Section 10.1.4). The lowest concentrations were found in an invertebrate and the highest in the Finless Porpoise (*Neophocaena* sp.). The authors calculated the tissue specific log BAF for each species (range 2.19 to 4.39, average 2.98). The highest BAF was for the Finless Porpoise.

Samples were also analysed with a Thermo DELTA V isotope ratio mass spectrometer to determine the stable isotope of nitrogen and carbon. This information was used by the authors to elucidate the structure of the food web and the trophic level of the species sampled using the filter feeding short-necked clam (*Ruditapes philippinarum*) as the reference point. The authors calculated the TMF of F-53B using stable isotope analysis and a linear regression method. The TMF was 3.37. As biomagnification is indicated by a TMF value above 1, the authors conclude that F-53B does biomagnify and noted that although this study included species at a higher trophic level than that of Liu *et al*. (2017a) similar TMFs were calculated. Although not discussed, the areas of uncertainty raised by Liu *et al*. (2017a would also apply to this study.

6.3.1.2 *Predicted data*

The US EPA CompTox dashboard contains predicted fish BCF for F-53B generated from the OPERA software (US EPA, 2020a). Although the calculated value is reported as a BCF on the US EPA CompTox dashboard, the QMRF for this model states that the output is a log BCF. The OPERA model predicts a BCF of 275 422 (log BCF of 5.44), but notes that F-53B is outside of the applicability domain of this model, so the prediction is not used here.

A BCF can also be calculated based on the log K_{OW} and the equation in ECHA (2017c). The Environment Agency has used an estimated log K_{OW} of 4.63 to calculate a BCF of 1 720. As noted in Section 5.4, the log K_{OW} value is uncertain and consequently the calculated BCF will be too. In addition, the assumption that hydrophobic and lipophilic interactions between compound and substrate (as modelled by the log K_{OW}) are the main mechanisms governing bioaccumulation behaviour may not be applicable for this type of substance due to the oleophobic repellency of the perfluorinated alkyl chain as well as the potential for protein binding (Section 8.1). The Environment Agency therefore considers this estimate to be highly uncertain.

6.3.1.3 *Monitoring data*

Several studies have investigated levels of F-53B in wildlife, without drawing conclusions about bioaccumulation behaviour (see Section 10.1.4 for further details).

For example, Shi *et al*. (2015) note that F-53B was detected in fish (10 samples) purchased from a Beijing food market which were used during analytical method development, and that the detection of F-53B in fish samples from 3 different areas of China suggests that it has a widespread distribution.

The substance has been found in predatory organisms at the top of the food chain. Within the UK, it has been detected up to 2.1 µg/kg ww in Eurasian Otter (*Lutra lutra*) liver samples (O'Rourke *et al*., 2022). Given the apparent low level of use in the UK, this is unexpected. O'Rourke *et al*. (2022) also report that 8:2 Cl-PFESA was detected in Eurasian Otter liver samples up to 0.214 µg/kg ww. The muscle concentration of F-53B in Finless Porpoise (*Neophocaena* sp.) collected from the Bohai Sea, China was also reported to be higher than muscle concentrations in 10 fish species by Chen *et al*. (2018).

6.3.1.4 *Data from structural analogues*

PFOS is reported to have a BCF_k value of 2 796 in a flow-through fish study on the Bluegill Sunfish (UNEP, 2006; Environment Agency, 2004). This is in the same order of magnitude as the F-53B BCF values reported by Wu *et al*. (2019b and 2019c).

The 4 observational studies that report on the bioaccumulation potential of F-53B also include data for PFOS. Shi *et al*. (2015) report tissue-specific median BAF of 589 to 19 055 and whole body median BAF of 1 905 to 2 692 for PFOS in carp, and note that the whole body BAF values were statistically significantly higher for F-53B than PFOS. Cui *et al*. (2018a) report a whole body mean BAF of 1 050 for PFOS in the frog, *Pelophylax nigromaculatus*. The authors note that the BAF values for F-53B were higher than those for PFOS. Liu *et al*. (2017a) state that the TMF values for PFOS of 3.83 to 3.88 were comparable with those they calculated for F-53B. Chen *et al*. (2018) report BAFs for various marine species of 170 to 16 218 for PFOS and notes that they are not statistically significantly different from those of F-53B for fish, but that for gastropods F-53B did have a statistically significantly higher BAF. Chen *et al*. (2018) also report a marine food web TMF of 3.94 for PFOS, which was similar to that of F-53B.

These studies indicate that F-53B has a similar or higher potential to bioaccumulate in aquatic organisms than PFOS.

6.3.2 Terrestrial bioaccumulation

6.3.2.1 *Measured data*

A single experimental study was identified during the literature search that investigated the bioaccumulation of F-53B in terrestrial plants.

Lin *et al*. (2020) investigated the accumulation and toxicity (see Section 7.2) of F-53B to wheat seedlings. F-53B with a purity of >98% was analysed as part of the study and found to consist of 90% F-53B and 10% 8:2 Cl-PFESA. Wheat seeds were germinated and grown for 11 days in nutrient media before being exposed to F-53B hydroponically in solution (nominal 50 and 100 µg/L) or a blank control. All exposures were carried out in triplicate. Seedlings were exposed for 7 days at 25/20 °C (day/night) under a 16 hour:8 hour light:dark cycle. After 7 days the seedlings were harvested. Roots were washed vigorously in water to remove any F-53B adsorbed to their surfaces. F-53B was then extracted twice from the root and shoot samples before analysis by ULPC-MS/MS.

Results for F-53B and 8:2 Cl-PFESA are reported separately and combined. No F-53B or 8:2 Cl-PFESA was detected in the roots or shoots of the blank controls. F-53B and 8:2 Cl-PFESA were detected in both roots and shoots of exposed plants, indicating that both compounds were taken up by the roots and translocated within the plant. Root concentrations were around an order of magnitude higher than concentrations in the shoots, and in both cases the concentration measured at the higher exposure concentration was significantly higher than that at the lower exposure concentration. BCF values were reported for the root and shoot at each exposure concentration. For F-53B, the BCF for the shoot ranged from 2.6 to 3.7 and the BCF for roots ranged from 126.5 to 158.7. For 8:2 Cl-PFESA, BCF for the shoot ranged from 0.75 to 1.25 and the BCF for roots ranged from 194.7 to 266.7. Lin *et al*. (2020) also report a translocation factor (TF, the ratio of shoot concentration to root concentration) to give an indication of how readily the test substances are translocated within the plant. The TF for F-53B was 0.024 to 0.025 and for 8:2 Cl-PFESA was 0.004 to 0.005.

This non-guideline study investigated the accumulation of F-53B in plants via an aqueous exposure route. As only a single time point was sampled it is not possible to determine whether a steady state was reached. However, this study does demonstrate that F-53B can be taken up by plant roots and translocated within the plant to the shoots, which would be expected for a relatively water soluble compound such as F-53B.

6.3.2.2 *Predicted data*

In terms of bioaccumulation in air-breathing organisms, the screening criteria are log K_{OW} > 2 and log K_{OA} > 5. The log K_{OW} of F-53B is around 4.63 as an approximation (Section 5.4). The K_{OA} has been estimated to be between 6.33 and 11.47 (Section 5.5). These values are highly uncertain, but suggest that F-53B has the potential to accumulate in air-breathing organisms.

The Environment Agency has predicted a BCF for F-53B for earthworms using the preferred log K_{OW} value of 4.63. This was done in EUSES v2.03 using the 'Predominantly hydrophobics" chemical class. The calculated BCF was 513 L/kg ww. If a log K_{OW} value of 4.79 is assumed, the BCF would be 724 L/kg ww. As noted in Section 5.4, the log K_{OW} value is uncertain and consequently the calculated BCF will be too. In addition, the assumption that hydrophobic and lipophilic interactions between compound and substrate (as modelled by the log K_{OW}) are the main mechanisms governing bioaccumulation behaviour may not be applicable for this type of substance due to the oleophobic repellency of the fluorinated alkyl chain as well as the potential for protein binding (Section 8.1). The Environment Agency therefore considers this estimate to be unreliable.

6.3.2.3 *Monitoring data*

Three monitoring studies were identified that investigated F-53B concentrations in terrestrial species.

- Briels *et al*. (2019a) used non-destructive sampling to determine the concentration of various legacy and emerging contaminants in Northern Goshawk (*Accipiter gentilis*) nestlings. Blood plasma and feather samples were collected from 61 nestlings in Norway from 2015 to 2016 and analysed using ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). Samples were collected from two areas: Trondelag (higher degree of urbanisation and agriculture) and Troms (lower degree of urbanisation and agriculture). F-53B was not detected in any of the samples (LOQ not stated).

- Cui *et al*. (2019) monitored the blood and food sources of two primate species, the Golden Snub-nosed Monkey (*Rhinopithecus roxellana*) and Francois' Leaf Monkey (*Trachypithecus francoisi*) for various PFAS. A total of 64 samples were taken from three locations with captive monkeys that are fed by humans (Wuzhou Breeding Centre, Tongling Zoo and Shanghai Wild Zoo), and one location with monkeys that mainly forage in the wild (Shennongjia nature reserve). All four sites were located in China. Samples were analysed using UPLC-MS/MS, and as the reference standard for F-53B could not be sourced commercially it was laboratory synthesised by the Shanghai Institute of Organic Chemistry.

  F-53B was detected in 89.1% of blood samples across all four sites, with the highest concentration (0.13 µg/L) detected in a monkey from the Wuzhou Breeding Centre. The site at which monkeys mainly foraged in the wild had the lowest concentrations of F-53B (up to 0.02 µg/L). For the Golden Snub-nosed Monkey from Shanghai Wild Zoo and for Francois' Leaf Monkeys at Wuzhou Breeding Centre, the concentration of F-53B in blood was found to be positively correlated with age. F-53B was also detected in at least one dietary source from each site. Tree leaf samples across all four sites had average F-53B concentrations of 0.06 to 0.32 ng/g dry weight (dw). Multigrain cake at two sites was found to contain F-53B at average concentrations of 0.01 to 0.03 ng/g dw and pumpkin at one site had an average F-53B concentration of 0.01 ng/g dw. Drinking water samples from all four sites contained F-53B at a concentration range of 0.025 to 0.341 ng/L. Cui *et al*. (2019) state that tree leaves were likely to be the major intake source for the monkeys, although they could not determine from their work whether the F-53B in leaves was from gas uptake, particle retention on the leaf surface or uptake from soil.

- Lan *et al*. (2020) monitored the concentration of F-53B in soil, maize and poplar leaves, and locusts. Three samples were taken from each of 3 sites in China, selected on the basis of high soil concentrations of F-53B from a previous monitoring campaign reported in the same study. Samples were analysed using HPLC-MS/MS. F-53B was detected in all 9 soil and locust samples, but only in

leaves from 1 site (see Section 10.1.4). Lan *et al*. (2020) report BAFs of 0.633 to 1.88 and transfer factors from maize leaves to locusts of 1.46 to 3.86 for the 1 site at which these could be calculated. The authors also note that F-53B was detected more frequently in locust samples than plant samples and suggest that locusts may accumulate F-53B directly from exposure to the soil, rather than via their food.

A discussion of the human biomonitoring data is included in Section 8.6. Although none of the studies attempt to derive a bioaccumulation rate in humans, the measured data indicate that F-53B is widely detected in both high exposure and low exposure groups in China, with positive detections in human blood, umbilical cord serum, urine, breast milk, semen, hair and nails.

6.3.2.4 *Data from structural analogues*

PFOS is reported to have a terrestrial BCF value of 2.5 in earthworms (Environment Agency, 2004). However, PFOS has been concluded to be bioaccumulative due to its detection at elevated concentrations in top predators (UNEP, 2006).

Lin *et al*. (2020) also report BCF and TF for PFOS. The BCF in shoots range from 3.08 to 4.18 and in roots from 136.7 to 139.8. The TF for PFOS was 0.023 to 0.029. There was no statistically significant difference between the F-53B and PFOS shoot or root BCF or TF. Although this non-guideline study is not considered to be fully relevant to the terrestrial bioaccumulation endpoint by the Environment Agency, the data do indicate that F-53B and PFOS showed similar levels of accumulation in this experiment.

6.3.3 Summary and discussion of bioaccumulation

F-53B has the potential to bioaccumulate in aquatic gill-breathing organisms, based on a measured fish BCF_k of 3 615 in Zebrafish larvae (Wu *et al*., 2019c). Although this is a non-guideline study, it is supported by a study on adult Zebrafish that reports a tissue-specific BCF_k of up to 4 425 (Wu *et al*., 2019b). Both these studies were conducted at a maximum exposure concentration of 100 µg/L, which the authors' state was selected as 1% of the acute fish LC_{50}, so should not have been influenced by toxicity. A number of other studies with the same species ranging from acute exposures of embryos to a multi-generational study also indicate that F-53B has the potential to accumulate rapidly into different tissues (including liver) and has a much slower depuration rate. There is also maternal transfer to eggs, which spans at least 2 generations.

However, there is no standard test guideline that reports whole body BCF's in juvenile or adult fish over a time frame of weeks or months. In addition, it is normal practice to consider lipid normalisation for most substances, and the lack of lipid content data prevents such adjustment. Growth correction may also be important for some fast growing species, although this might be less important for Zebrafish. Some PFAS are known to bind to proteins which may make lipid normalisation less relevant. There is some evidence (e.g. from Shi *et al*., 2015 and the papers summarised in Section 8.1) that protein binding may be relevant for F-53B.

Several observational studies have also detected F-53B in wild animals and some of these have also included the calculation of a BAF or TMF. Observational studies that investigate bioaccumulation can be difficult to interpret as the concentration in the organism reflects accumulation (and depuration) over an extended period of time, and water concentrations based on spot samples may not be representative of the whole exposure period. As such, BCFs obtained from experimental studies where steady state has been demonstrated are strongly preferred. However, whilst bearing this uncertainty in mind, the 4 observational studies that have been summarised (Chen *et al*., 2018; Cui *et al*., 2018a; Liu *et al*., 2017b; Shi *et al*., 2015) all report that F-53B is detected in a wide range of aquatic organisms and water samples collected from different areas of China. Tissue specific log BAFs from the four studies range from 2.19 to 5.23, and whole body log BAFs range from 3.12 to 4.32. The TMF calculated from 2 studies in the Bohai Sea (Chen *et al*., 2018; Liu *et al*., 2017b) ranges from 3.43 to 4.32. Monitoring studies also indicate accumulation in organisms at the top of the food chain, including at locations where use is likely to be limited. Taken together, these studies indicate that F-53B has the potential to bioaccumulate in aquatic species and are supportive of the data obtained from the experimental studies discussed above.

From the limited data on terrestrial bioaccumulation there are experimental and observational data to suggest that F-53B can be taken up and translocated within plants and that it has been detected in monkeys (with potential exposure from both their food and drinking water sources) and locusts (with potential exposure from both their food and directly from soil). Bioaccumulation in air-breathing organisms is a possibility based on screening data. A discussion of the available human biomonitoring data is included in Section 8.6. The measured data indicate that F-53B is widely detected in both high exposure and low exposure groups in China, with positive detections in human blood, umbilical cord serum, urine, breast milk, semen, hair and nails. F-53B can be distributed within the human body and transferred to the next generation across the placenta or in breast milk. A single study (Shi, Vestergren, Xu, Zhou, Li, Liang and Cai, 2016) calculates a renal elimination rate for F-53B that was found to be statistically significantly slower than the rate for PFOS calculated in the same study and a total elimination half-life that is higher than that of PFOS. A definitive conclusion on the bioaccumulation potential of F-53B in air-breathing organisms would require further data on the human clearance time via all clearance routes or better predictive methods, but the available information suggests that F-53B may be at least as bioaccumulative as PFOS.

7 Ecotoxicology

7.1 Aquatic compartment (including sediment)

7.1.1 Fish

7.1.1.1 *Short-term (acute) toxicity*

The literature search identified four published studies on the acute toxicity of F-53B to fish. An overview is provided in Table 7.1.

- Wang *et al*. (2013) report the results of a 96-hour semi-static acute fish toxicity study using adult Zebrafish that was stated to follow OECD TG 203. The test was conducted using F-53B with an analytical purity of >98%. Seven nominal exposure concentrations were tested (1, 1.7, 2.89, 4.91, 8.35, 14.26, 24.14 mg/L) together with a control, but no detail is provided on how these exposure concentrations were prepared. All exposure concentrations were confirmed to be >80% nominal at the start of the test, and the lowest and highest concentrations were analysed daily. The test media was renewed after 48 hours so both old and new media was analysed at that time point. Exposure concentrations in these two vessels were reported to remain within ± 20% of nominal, so results are reported based on the nominal concentrations. Seven fish were exposed to each test concentration and a blank control. The 96-hour LC_{50} was reported to be 15.5 mg/L based on nominal concentrations.

  The Environment Agency considers that the study is well reported and that the OECD validity criteria were met. There were some minor deviations from the guideline, as the test animals were only held for 7 days prior to testing and the loading rate was not stated. However, as no mortality was observed in the control or exposure vessels this suggests that the conditions of the test were appropriate. Only the lowest and highest exposure concentrations were confirmed by analysis every 24 hours, but these showed the exposure concentrations to be stable and within ± 20% of nominal.

- Shi *et al*. (2017) report the results of an acute fish toxicity study using Zebrafish embryos that were exposed from 6-hours post fertilisation (hpf) to 96-hpf as a range finding study prior to further experiments. The test was conducted using F-53B with an analytical purity of >96%. Seven nominal exposure concentrations (1, 2, 4, 8, 16, 32 and 64 mg/L) were prepared with the use of a solvent (DMSO). The DMSO concentration in each test vessel and the solvent control was 0.1% v/v. There was no analytical confirmation of the exposure concentrations. The 90-hour LC50 was reported to be 13.77 mg/L based on nominal concentrations. No further details are provided. The Environment Agency considers that the results of this range finder study should be used as supporting information only.

Table 7.1 Summary of acute toxicity to fish

Method	Species	Analytical method	Results	Reliability	Reference
OECD TG 203 (semi-static) Not to GLP	Zebrafish *Danio rerio* (adult)	LC-MS/MS	96- h LC_{50} 15.5 mg/L (nominal)	1 (key study)	Wang *et al*. (2013)
Non-guideline study similar to OECD TG 236 (semi-static)	Zebrafish *Danio rerio* (embryo)	UPLC	90-h LC_{50} >12 mg/L (initial measured) 126-h LC_{50} 6 mg/L (initial measured) 66-h NOEC (hatching) ≥12 mg/L (initial measured) 102-h NOEC (malformations) 1.5 mg/L (initial measured) 90-h NOEC (heart malformations, heart rate) 3 mg/L (initial measured)	2 (supporting studies)	Shi *et al*. (2017)
Non-guideline study similar to OECD TG 236 (semi-static) Single test concentration	Zebrafish *Danio rerio* (embryo)	UPLC	90-h LC_{50} >0.57 mg/L (nominal) 66-h NOEC (hatching, malformation) ≥0.57 mg/L (nominal) 66-h NOEC (liver histopathology, lipid profile) <0.57 mg/L (nominal)	2 (supporting studies)	Yi *et al*. (2019)
Non-guideline study (semi-static)	Zebrafish Danio rerio (adult)	HPLC	7-day LC50 >0.075 mg/L (mean measured)*	2 (supporting studies)	Wu et al. (2019b)
Non-guideline study (semi-static)	Zebrafish Danio rerio (adult)	None	28-day LC50 >0.1 mg/L (nominal)*	2 (supporting studies)	Wu et al. (2019b)

* Although these results are not from an acute time frame as the endpoint was lethality they are recorded here.

In the same study, Shi *et al*. (2017) used the LC50 they had derived in the range finder to set the exposure concentrations for a more detailed study of the effects of F-53B on Zebrafish embryos. This study followed a similar experimental design to that of OECD TG 236 but was terminated when the embryos were 132-hpf instead of 96-hpf. Thirty 6-hpf embryos were exposed individually in well plates to F-53B at

nominal concentrations of 2, 4, 8 and 16 mg/L until 132-hpf. Exposure concentrations were prepared with the use of a solvent (DMSO) and the final DMSO concentration in each test vessel and the solvent control was 0.1% v/v. Test media was renewed every 24 hours and the study was conducted in triplicate. Survival, malformation and hatching rate were observed every 12 hours and heart rate was measured at 72-hpf. Samples of test media were taken at the start of the exposure and after 24 hours, just before test media renewal. Concentrations at 24 hours were slightly lower than those at 0 hours, with a reduction of up to 11.6% which the authors attribute to uptake by the embryo. Results are reported based on the initial measured concentrations of 0, 1.5, 3, 6 and 12 mg/L, and as test media was renewed every 24 hours this means that concentrations remained within ±20% of these.

The 90-hour LC_{50} was reported to be >12 mg/L based on initial measured concentrations. The authors note that survival decreased rapidly between 96- and 132-hpf, such that by 132-hpf the LC_{50} was 6 mg/L based on initial measured concentrations. Hatching in the control group reached over 90% by 72-hpf and F-53B had no significant effect on hatching at this time point. However, hatching was significantly reduced at 48-hpf and 60-hpf indicating that F-53B delayed hatching when compared to the control but did not alter overall hatching success. Malformations were first observed from 84-hpf and included pericardial and yolk sac oedemas, bent spines, bent tails and uninflated swim bladders. At 108-hpf the NOEC (malformation) was 1.5 mg/L based on initial measured concentrations. Data on malformations at 132-hpf are not reported. Heart malformations were significantly increased and heart rate was significantly decreased after 96-hpf at the highest two exposure concentrations. Expression of some genes involved in normal cardiac development was also found to be significantly altered at all treatment levels after 72-hpf.

This study followed a similar method to OECD TG 236 and the validity criteria for that guideline have been used to assess the reliability. No information is provided in the paper on the fertilisation rate of the batch tested or on the experimental conditions (temperature, dissolved oxygen concentration) and no positive control was used. The concentration of solvent in the final exposure media was higher than the 0.01% permitted by the OECD TG. However, the overall survival and hatching rate in the controls met the validity criteria, which suggests that the conditions of the test were appropriate at 96-hpf. As independent feeding in Zebrafish typically occurs at around 5 days (JRC, 2014), the increase in mortality observed between 96- and 132-hpf may also have been related to the embryos having used up the yolk food supply, but not having an alternative food source. Despite the inclusion of a range finding study, under the conditions of the test the LC_{50} was not reached at 90 hours exposure (the endpoint most similar to that of OECD TG 236), which does limit the usefulness of this study. Although some sub-lethal endpoints are also reported, due to the short exposure duration these endpoints should be used as supporting information only.

- Yi *et al*. (2019) studied of the effects of F-53B at a single exposure concentration on Zebrafish embryos. This study followed a similar experimental design to that of OECD TG 236. Twenty 6-hpf embryos were exposed individually in well plates to F-53B with >98% purity at a nominal concentration of 1 µmol/L (equivalent to 0.57 mg/L) until 7-dpf. Exposure media were prepared with the use of a solvent (methanol) and the final methanol concentration in each test vessel and the solvent control was 1% v/v. Test media were renewed every 24 hours and the study was conducted in triplicate. Survival, malformation and hatching rate were observed at 6, 12, 24, 48, 54, 58, 72 and 96-hpf and 7-dpf. Samples of test media were taken before and after exposure, although it is not stated how many samples were analysed. The concentration before exposure is reported to be 0.88 µmol/L (equivalent to 0.502 mg/L) and the concentration after exposure 0.764 µmol/L (equivalent to 0.436 mg/L). Results are reported based on the nominal exposure concentration, although the Environment Agency notes that the measured values are 76.5-88.1% nominal, which could justify the use of a mean measured concentration.

  The authors state that exposure to 0.57 mg/L F-53B did not cause acute toxicity at 96-hpf. Apical results from 7-dpf are not reported. Hatching in the control group reached over 100% by 96-hpf and F-53B had no significant effect on hatching at this or any other previous time point. The malformation rate was below 2% in both the control and F-53B exposure group at 96-hpf. The livers of the fish were examined and hepatic cells in the F-53B exposed fish were found to have micro- and macrovacuolisation and enlarged intercellular spaces compared to those of the controls. Whole body total cholesterol and triglycerides concentrations were found to be significantly increased after F-53B exposure, whilst low density lipoprotein concentration was significantly decreased. Expression of some genes involved in normal lipid metabolism was also found to be significantly altered after 7 days' exposure.

  This study followed a similar method to OECD TG 236 and the validity criteria for that guideline have been used to assess the reliability. No information is provided in the paper on the fertilisation rate of the batch tested or on the experimental conditions (temperature, dissolved oxygen concentration) and no positive control was used. The concentration of solvent in the final exposure media was much higher than the 0.01% permitted by the OECD TG. However, the overall survival and hatching rate in the controls met the validity criteria, which suggests that the conditions of the test were appropriate. As only a single exposure concentration was tested and the LC_{50} was not reached, this does limit the usefulness of this study. Although some sub-lethal endpoints are also reported, due to the short exposure duration these endpoints should be used as supporting information only.

- Wu *et al*. (2019b) report the results from a bioaccumulation (uptake and depuration) study with F-53B using adult Zebrafish. Full details of the test system are provided in Section 6.3.1. The authors state that no abnormal behaviour or death occurred in any of the treatment groups throughout the experimental uptake phase. Therefore,

the 7-day LC50 is higher than the maximum mean measured exposure concentration of 0.075 mg/L. This was a non-standard acute toxicity study which used a longer exposure period than the standard OECD test guideline and included feeding. In addition, the exposure concentration was set at a level to investigate bioaccumulation, rather than lethality. However, the method was well described and the exposure concentrations confirmed by analysis every 2 days.

Wu *et al*. (2019b) also report a second experiment in which Zebrafish were exposed to F-53B for 28 days. The exposure concentrations were identical to those in the 7-day uptake experiment and were prepared as described for the previous study (see Section 6.3.1). Adult male and female fish were exposed separately at a density of 10 fish per tank, with three replicates at each concentration. There was no analytical confirmation of the exposure concentrations. The authors state that no deaths were observed in any treatment group, so the 28-day LC50 is > 0.1 mg/L based on nominal concentrations. This was a non-standard sub-acute toxicity study which used a much longer exposure period than the standard acute fish OECD test guideline and included feeding. In addition, the exposure concentration was set at a level to investigate bioaccumulation, rather than lethality. Although chemical analysis was not used to confirm the exposure concentrations the Environment Agency considers it likely that the exposure concentrations achieved were similar to those in the 7-day uptake study reported in the same paper as the same methods were used to prepare and maintain the exposure concentrations.

For this screening evaluation, the Environment Agency considers the study by Wang *et al*., (2013) that reported a **96-hour LC_{50} of 15.5 mg/L** to be the key acute fish study.

7.1.1.2 *Long-term (chronic) toxicity*

The literature search identified two published studies on the chronic toxicity of F-53B to fish. Both of these, Shi *et al*. (2018) and Shi *et al*. (2019a), report results from the same experiment. An overview is provided in Table 7.2.

Table 7.2 Summary of chronic toxicity to fish

Method	Species	Analytical method	Results	Reliability	Reference
Non-guideline study (flow-through)	Zebrafish *Danio rerio*	UPLC	180-day NOEC (F0 weight, length, condition factor, gonadosomatic index, female gonad histopathology) < 0.005 mg/L (nominal) 180-day NOEC (F0 male gonad histopathology) 0.005 mg/L (nominal) 177-day NOEC (F0 fecundity) < 0.005 mg/L (nominal) 177-day NOEC (F0 fertilisation) 0.05 mg/L (nominal) 177-day NOEC (F1 embryo malformation and survival) < 0.005 mg/L (nominal) 177-day NOEC (F1 weight, length, female gonadosomatic index) < 0.005 mg/L (nominal) 177-day NOEC (F1 condition factor, male gonadosomatic index, male and female gonad histopathology) 0.005 mg/L (nominal) 177-day NOEC (F1 fecundity) < 0.005 mg/L (nominal) 177-day NOEC (F1 fertilisation) 0.005 mg/L (nominal) 177-day NOEC (F2 embryo malformation and survival) < 0.005 mg/L (nominal)	2 (supporting study)	Shi *et al*. (2018)
Non-guideline study (flow-through)	Zebrafish *Danio rerio*	UPLC	180-day NOEC (F0 relative liver weight male and female) 0.005 mg/L (nominal) 180-day NOEC (F0 enlarged hepatocytes male and female) < 0.005 mg/L (nominal)	2 (supporting study)	Shi *et al*. (2019a)

Method	Species	Analytical method	Results	Reliability	Reference
			180-day NOEC (F0 triglyceride levels male) <0.005 mg/L (nominal) 180-day NOEC (F0 triglyceride levels female, total cholesterol levels male) 0.005 mg/L (nominal) 180-day NOEC (F0 total cholesterol levels female) 0.05 mg/L (nominal)		

Shi *et al*. (2018) exposed adult Zebrafish to F-53B in a flow-through system for 180 days before transferring the F1 eggs to clean water and investigating a range of chronic endpoints in the F0, F1 and F2 generations. One hundred and twenty adult Zebrafish were exposed in single sex groups of 30 fish to F-53B (purity >99.5%) at each nominal exposure concentration (0.005, 0.05 and 0.5 mg/L) for 180 days. Exposure concentrations were prepared with the use of a solvent (DMSO) and the final DMSO concentration in each test vessel and the solvent control was 0.004% v/v. Fish were fed twice daily and maintained at 28 °C and a 14:10 hour light:dark cycle. Ten pairs of fish from each exposure group were randomly selected to spawn in clean water each week. Reproductive endpoints (fecundity and fertilisation rate) were recorded after 177 days' exposure. All eggs were observed for fertilisation success and F1 embryos were observed for hatching rate, malformation and survival. After 180 days the F0 fish were anaesthetised and various morphological, histopathological, hormonal and gene expression endpoints measured. F1 fish were reared in clean water in tanks until they were transferred to a flow-through system at 60 days post fertilisation (dpf). Using the same method as for F0, the reproductive output of the F1 generation was measured. F2 embryos were also observed for hatching rate, malformation and survival. Three samples of test media from each exposure concentration were taken at the start of the exposure and after 180 days. The mean measured concentrations were within 10% of nominal, so results are reported based on the nominal exposure concentrations.

High levels of mortality were observed in the highest exposure concentration after 180 days, so results from this treatment group were excluded by the authors. The lower two treatment groups had less than 10% mortality and no observed abnormal behaviour. F1 larvae of adults exposed to 0.05 mg/L did not survive beyond 15-dpf. Therefore, only control and the 0.005 mg/L exposure groups were included in the F1 and F2 study.

For control fish, the fecundity of the F0 and F1 generations remained stable, with over 145 eggs/female/day recorded for both generations. The malformation and embryo mortality rates in the control F1 and F2 generations were <3.3%. After 180 days' exposure the F0 generation were found to have statistically significant changes in size, condition and histopathology at the lowest exposure concentration (0.005 mg/L) when compared with the

control. Although fertilisation rates were unaltered, fecundity of the F0 generation was also statistically significantly reduced at the lowest exposure concentration. Despite the F1 eggs being transferred to clean water, effects were also observed in the F1 and F2 generation, with embryo malformation significantly increased and survival and fecundity significantly reduced after F0 exposure to 0.005 mg/L.

Further results from this study are reported in Shi *et al*. (2019a), who investigated the impact of F-53B exposure on the liver. Relative liver weights were statistically significantly increased in both male and female fish after 180 days' exposure to 0.05 mg/L. In addition, histopathological changes were noted, including karyolysis and cytoplasmic vacuolation. F-53B exposure was also found to result in statistically significantly enlarged hepatocytes in both males and females at 0.005 mg/L when compared to the controls. Changes in triglyceride and total cholesterol levels also indicated that F-53B exposure disrupted lipid metabolism, with male fish showing statistically significant effects from 0.005 mg/L and female fish from 0.05 mg/L. Expression of some genes involved in normal liver function and the PPAR signalling pathway was also found to be significantly altered at 0.005 mg/L. Analysis of gene expression in 5-dpf F1 larvae demonstrated that some gene expression was also significantly altered in that generation.

Further endpoints relevant to potential endocrine effects are discussed in Section 9.2.

Both this study design and the draft OECD TG Zebrafish extended one generation reproduction test (ZEOGRT) report endpoints from three generations of fish. However, there are notable differences in the experimental design. In the ZEOGRT, spawning F0 adults are exposed for three weeks, and the F1 and F2 generation are also exposed to the test compound. In this study, adult F0 fish were exposed for a much longer period of time (180 days), but the F1 and F2 generations were raised in clean water.

Although this study followed a non-standard guideline it is well described. Adult fish were exposed for an extended period of time under flow-through conditions, with the exposure concentrations confirmed at the beginning and end of the experiment. For an experiment with an exposure period of 180 days a larger number of sampling points would have increased confidence that the exposure concentrations were maintained throughout the study, although the measured concentrations at day 0 and day 180 were close to nominal. A large number of typical apical endpoints are reported, together with a number of additional endpoints that may provide information on the mode of action of F-53B.

The studies by Shi *et al*. (2019a, 2018) demonstrate that F-53B has statistically significant effects on fecundity, embryo malformation and survival at the lowest exposure concentration tested (0.005 mg/L) over a period of 177 days. Various other effects on histopathology, gene expression and hormone levels were also observed at this exposure concentration, and effects were also seen in the F1 and F2 generations despite being reared in clean water. For the purposes of this screening evaluation, the Environment Agency considers that Shi *et al*. (2018) is the key study, with a **177-day NOEC (fecundity, embryo malformation and survival) of <0.005 mg/L,** although there is some uncertainty in the maintenance of the exposure concentration over this period as there were no regular checks by chemical analysis.

7.1.2 Aquatic invertebrates

No relevant information is available.

7.1.3 Algae and aquatic plants

The literature search identified two published studies on the toxicity of F-53B to algae, An overview is provided in Table 7.3.

Table 7.3 Summary of toxicity to algae

Method	Species	Analytical method	Results	Reliability	Reference
OECD TG 201 Not to GLP	*Scenedesmus obliquus*	HPLC	72-h E_rC_{50} 40.3 mg/L (measured) 72-h E_rC_{10} 24.1 mg/L (measured) 72-h NOE_rC 2.49 mg/L (measured)	2 (supporting study)	Liu *et al*. (2018)
Non-guideline (static)	*Chlorella* species (not defined)	None	6-day NOE_bC 0.001 mg/L (nominal) 8- to 14-day NOE_bC <0.00001 mg/L (nominal)	3 (not reliable)	Niu *et al*. (2019)

- Liu *et al*. (2018) report an algal accumulation study (see Section 6.3.1) and an algal growth inhibition study following OECD TG 201 using *Scenedesmus obliquus*. The test was conducted using F-53B with a purity of 98%, which is stated to contain 91% C_8, 7% C_{10} and 0.3% C_{12} chlorinated polyfluorinated ether sulfonate. A stock solution of 200 mg/L was made in culture medium and this was diluted to prepare five nominal exposure concentrations ranging from 5 to 60 mg/L (other concentrations not stated). The authors note that the highest test concentration had resulted in complete growth inhibition in a range finder study. It is stated that the results in the paper are based on measured concentrations but it is unclear at what time points the exposure concentrations were monitored. Control and exposure vessels were prepared in triplicate and the biomass was determined by fluorescence every 24 hours.

  A dose dependent effect on algal growth rate was observed with the measured 72-hour E_rC_{50} stated to be 40.3 mg/L, the 72-hour E_rC_{10} 24.1 mg/L and the 72-hour NOE_rC 2.49 mg/L.

  Some test conditions differ from those specified in the OECD test guideline (e.g. test vessels are shaken three times daily instead of being continuously shaken, and the vessels are kept under a 16:8 hour light:dark cycle instead of under continuous illumination) or are not reported in the paper and a non-standard algal species was used, although it is stated that the algal culture was in the exponential growth phase before exposure. Insufficient data are reported in the paper to determine whether the OECD TG validity criteria were met, and the authors do not make any statement on this. Despite these shortcomings, the Environment Agency considers that the results from this study can be used as supporting information in this assessment.

- Niu *et al*. (2019) report the results of a non-standard algal toxicity study using a marine microalga (*Chlorella* sp.). The test was conducted using F-53B (referred to as “6:2 Cl-PFAES”) with an analytical purity of >98%. Three nominal exposure

concentrations were tested (10, 100 and 1 000 ng/L) in standard F/2 enriched seawater media with a salinity of 30 (units not given), but no detail is provided on how these exposure media were prepared. There was no analytical confirmation of the exposure concentrations.

The algae were transferred to the test vessels at an initial density of 1.5×10^5 cells/mL when the initial culture was in the exponential growth phase. The test vessels had covers to prevent loss of the test material but allow gas exchange and were maintained at 22 °C and with a 12:12 hour light:dark cycle at 3000 lux. All exposure concentrations were tested in triplicate, together with blank controls. The algal biomass (cell count) was determined every 2 days until the experiment was concluded on day 14. The authors report that the results were analysed using an ANOVA to determine whether exposure to F-53B resulted in a difference in cell count when compared to the control. After 6 days' exposure there was no significant difference observed. However, from day 8 to 14, all three exposure concentrations had a statistically significant reduction in cell count when compared to the control in a dose-dependent manner. The authors note that on day 14 the reduction in cell count at 1 000 ng/L was 68.89% when compared to the control.

The Environment Agency considers that there were significant weaknesses in this non-standard study. The typical duration of a standard algal growth inhibition test is 72 hours. Studies that are longer than this cannot be assumed to maintain the control algae in the exponential growth phase (ECHA, 2017b). Indeed, Figure 1 in Niu *et al*. (2019) clearly demonstrates that the cell number in the control algae is not exponentially growing by the end of this test. However, as the authors report that there were no significant effects on biomass up to 6 days exposure the results for this shorter exposure period could be used.

In addition, the standard endpoint from algal studies is growth rate, rather than biomass. This is because the biomass endpoint is dependent on the growth rate of the test species, the duration of the study and the test design, whilst the growth rate is independent of the study design. ECHA (2017b) states that studies which only report the biomass endpoint and which do not contain sufficient raw data to allow re-analysis, should not be used.

The Environment Agency has therefore not used the results from Niu *et al*. (2019) in this screening assessment.

For the purposes of this screening evaluation, the Environment Agency considers that Liu *et al*. (2018) is the key study, reporting a **72-hour E_rC_{50} of 40.3 mg/L and a 72-hour E_rC_{10} of 24.1 mg/L**.

No toxicity data are available for higher plants.

7.1.4 Sediment organisms

No relevant information is available.

7.1.5 Other aquatic organisms

No relevant information is available

7.1.6 Data from structural analogues

Environment Agency (2004) and UNEP (2006) include a summary of the available acute and chronic aquatic toxicity data for PFOS. The lowest reported freshwater endpoints are:

- Fish 96-h LC_{50} 4.7 mg/L
- Invertebrate 48-h EC_{50} 27 mg/L
- Algal 96-h IC_{50} 48.2 mg/L
- Fish 42-d NOEC 0.3 mg/L
- Invertebrate 28-d NOEC 7 mg/L
- Algal 96-h NOEC 5.3 mg/L
- Aquatic plant 7-d NOEC 15.1 mg/L

These data indicate that fish are the most sensitive taxa in both the acute and chronic tests.

Some data for marine species are also available for PFOS:

- Fish 96-h LC_{50} 13.7 mg/L
- Invertebrate 96-h LC_{50} 3.6 mg/L
- Algal 96-h EC_{50} >3.2 mg/L
- Invertebrate 35-d NOEC 0.25 mg/L
- Algal 96-h NOEC >3.2 mg/L

These data indicate that marine invertebrates may be more sensitive to PFOS than freshwater invertebrates and have a chronic NOEC similar to freshwater fish.

In comparison, the available data indicate that F-53B is more acutely and chronically toxic to fish than algae (like PFOS), but no data are available for aquatic invertebrates. The data for PFOS give some reassurance that freshwater fish are likely to be a sensitive taxon for F-53B, but it should be noted that marine invertebrates may be of similar (or slightly greater) sensitivity.

No sediment data for PFOS are reported in Environment Agency (2004) or UNEP (2006).

7.2 Terrestrial compartment

7.2.1 Experimental data

The literature search identified two published studies on the terrestrial toxicity of F-53B. An overview is provided in Table 7.4.

Table 7.4 Summary of toxicity to terrestrial organisms

Method	Species	Analytical method	Results	Reliability	Reference
Non-guideline	Wheat *Triticum aestivum*	None	7-day NOEC (shoot weight, root weight) 100 mg/L (nominal) 7-day NOEC (root permeability) 50 mg/L (nominal) 7-day NOEC (chlorophyll α, chlorophyll β) <50 mg/L (nominal) 7-day NOEC (carotinoids) 100 mg/L (nominal)	2 (supporting study)	Lin *et al*. (2020)
Non-guideline (*in ovo*)	Chicken *Gallus gallus*	UHPLC-MS/MS	20-day NOEC (pipping, hatching, survival, body mass) 1 500 ng/g egg (nominal) 20-day NOEC (heart rate) <150 ng/g egg (nominal) 20-day NOEC (HSI) 150 ng/g egg (nominal)	2 (supporting study)	Briels *et al*. (2018, 2019b)

- Lin *et al*. (2020) investigated the accumulation and toxicity of F-53B to wheat *Triticum aestivum* seedlings. F-53B with a purity of >98% was analysed as part of the study and found to consist of 90% F-53B and 10% 8:2 Cl-PFESA. Wheat seeds were germinated and grown for 11 days in nutrient media before being exposed hydroponically to F-53B in solution (nominal 1, 10, 25, 50, 100 and 250 mg/L) or a blank control. All exposures were carried out in triplicate. Seedlings were exposed for 7 days at 25/20 °C (day/night) under a 16:8 hour light:dark cycle (for further description of the study, see Section 6.3.2).

  After 7 days' exposure shoot and root fresh weight were statistically significantly reduced when compared to the controls at 250 mg/L. Shoot weight was 48% reduced and root weight was 58% reduced. Root membrane permeability (an indicator of cell membrane damage) was significantly increased at a concentration of 100 mg/L and above. The content of chlorophyll α, chlorophyll β and carotenoids in wheat leaves was also reduced by exposure to F-53B at concentration of 50 mg/L and above.

  This non-guideline study investigated the toxicity to terrestrial plants via an aqueous exposure route that is of considered of limited relevance when assessing the toxicity of F-53B via the soil. Therefore, this study does not provide suitable information for use in a quantitative soil risk assessment. However, this information may be of relevance in an exposure scenario where river water is being used for

irrigation in hydroponic greenhouses. It also indicates that plants can take up F-53B, and that this causes toxicity.

- Briels *et al*. (2018) studied the developmental toxicity of F-53B alone or in combination with PFOS to the domestic chicken *Gallus gallus*. A correction to some of the units reported in the original paper was made in Briels *et al.* (2019b). F-53B or PFOS both with a purity of ≥98% were formed into an emulsion with peanut oil and water using lecithin as an emulsifier and injected either individually or in combination into eggs that were 2-3 days old. Two exposure concentrations of each substance (150 and 1500 ng/g egg) and all four possible combinations of F-53B and PFOS were tested, in addition to a control group that were exposed to the vehicle carrier only. The injection was made into the egg yolk before eggs were incubated at 37.5 to 38 °C and 60% humidity until the eggs began hatching after 20 days. Eggs were handled regularly and the heart rate of embryos (beats per minute, bpm) measured. Eggs that did not hatch were opened to determine the developmental stage that had been reached. Hatched chicks were euthanised before dissection. The concentration of the test substances in the liver of the hatched chicks was measured and it was found that 10 to 16% of the nominally injected F-53B and 15 to 19% of the nominally injected PFOS amount was found in the liver.

  No effects on embryo heart rate were found at 14 or 17 days after exposure. However, at 20 days post-exposure embryo heart rate was significantly lower than the controls in all exposures (range 248 to 279 bpm for F-53B, 247 to 267 bpm for PFOS and 298 bpm in controls). No statistically significant effects were observed for pipping (the point at which the chick breaks the outer egg shell membrane), hatching, survival or body mass. The highest exposure dose of F-53B resulted in a significant increase in the hepatosomatic index (HSI) of the chicks, indicating that the livers were enlarged relative to the body. Together with the concentrations detected in the liver, the authors state that this indicates that liver is a target organ for F-53B exposure. No significant change in HSI was observed for PFOS exposure.

  This non-guideline study investigates toxicity to birds via an exposure route that is considered of limited relevance to the standard dietary exposure route (ECHA, 2017c). Therefore, this study does not provide suitable information for use in a quantitative risk assessment. However, the data from this study indicate that acute apical effects were not observed at the doses tested, although F-53B may cause sub-lethal effects (e.g. on the heart) and can accumulate in the liver.

7.2.2 Data from structural analogues

Environment Agency (2004) includes a summary of the available acute and chronic terrestrial ecotoxicity data for PFOS. The lowest reported endpoints are:

- Earthworm 14-day LC_{50} 373 mg/kg ww
- Plant 21-day NOEC <3.91 mg/kg ww

There are no data for F-53B that can be directly compared with these values.

7.3 Microbiological activity in sewage treatment systems

7.3.1 Experimental data

No relevant information is available.

7.3.2 Data from structural analogues

Environment Agency (2004) reports a 3-h IC_{50} for activated sludge respiration inhibition of >905 mg/L for PFOS.

This suggests that sewage treatment micro-organisms are unlikely to be sensitive to F-53B.

7.4 Atmospheric effects

F-53B is not volatile, and so is not expected to partition significantly to the atmosphere (see Section 6.2.2).

7.5 Assessment of endocrine disrupting (ED) properties

The literature search identified 6 published studies on the potential endocrine disrupting properties of F-53B. Three papers investigated whether F-53B causes thyroid disruption and one paper investigated effects on estrogenic and androgenic pathways. The three papers of thyroid activity were published by three separate research groups. In addition, two studies were also identified that investigated whether F-53B could alter the activity of peroxisome proliferator activated receptors (PPAR) signalling pathways.

- Xin *et al*. (2018) used *in vitro* assays and an *in silico* simulation to study whether F-53B binds to and alters the activity of thyroid transport proteins and nuclear receptors. None of the assays followed a standard guideline, and none were conducted to GLP. The F-53B tested was composed of 77.6% 6:2 and 6% 8:2 Cl-PFESA, with the remaining 16.4% unidentified. Each individual component and PFOS (purity >95%) was also tested separately. For the purposes of this assessment, the Environment Agency has used the terms F-53B commercial to refer to the commercial product and F-53B to refer to the results for 6:2 chlorinated polyfluoroalkylether sulfonate.

  Stock solutions were prepared containing 1% DMSO and the maximum concentrations prepared from the stock solution were below that at which the substance would aggregate into micelles (the critical micelle concentration, CMC).

The authors report that in laboratory grade water and with DMSO at 1% the CMC were determined to be 2.9, 5.6, 0.82 and 2.7 mM for PFOS, F-53B, 8:2 Cl-PFESA and F-53 commercial respectively. For F-53B, this equates to a concentration of 3 036 mg/L.

In the first experiment, a competitive fluorescence binding assay was used to determine the relative binding affinities of the test substances to two thyroid transport proteins (transthyretin TTR and thyroxine-binding globulin TBG) and two thyroid receptors (TRα and TRβ). All 4 assays were conducted in 384 well plates, and each was conducted in triplicate. Results were expressed as the concentration of the substance required to displace half of the probe from the protein (IC_{50}). F-53B commercial, F-53B and PFOS were all found to have similar potency on a molar basis (Table 7.5). 8.2 Cl-PFESA was found to have a lower potency. None of the test compounds were found to bind to TBG.

Table 7.5 IC_{50} values for relative thyroid transport protein binding affinity

Substance	IC_{50}, μM		
	TTR	TRα	TRβ
F-53B commercial	7.3	8.4	12.9
F-53B	4.8	10.3	7.1
8:2 Cl-PFESA	>500	232.7	>500
PFOS	2.1	16.0	20.5

For F-53B the IC_{50} values have been converted by the Environment Agency to 2.6 mg/L for TTR, 5.6 mg/L for TRα and 3.8 mg/L for TRβ based on a molecular weight of 570.67 g/mol and a purity of 95%.

In the second experiment, human HEK293 cells with thyroid receptor mediated luciferase activity were exposed to the test compounds for 24 hours to determine whether agonistic activity was observed. Five nominal test concentrations were used (1.6, 3.1, 6.3, 12.5 and 25 μM). The authors state that the highest concentration is equivalent to 13.3 mg/L for F-53B. Cells exposed to 0.1% DMSO were used as the control, and triiodothyronine (T3) was used as a positive control. Each assay was conducted in triplicate. No effects on cell viability were observed for any of the test compounds. All test compounds were found to increase the luciferase transcriptional activity in a dose-dependent manner for both thyroid receptors, indicating agonistic activity (Table 7.6). F-53B was slightly more potent than PFOS in this assay.

Table 7.6 Fold increase in luciferase activity after exposure to 25 µM of test substance

Substance	TRα	TRβ
F-53B commercial	2.7	2.2
F-53B	2.8	1.8 (read from graph)
8:2 Cl-PFESA	1.4	1.5 (read from graph)
PFOS	1.6	1.7

In a third experiment, the effects of exposure to these compounds on rat pituitary GH3 cell proliferation was investigated, as this has previously been shown to be regulated by thyroid receptors. T3 was used as a positive control and was demonstrated to increase cell proliferation dose dependently. The nominal test concentration of 25 µM was stated to be equivalent to 13.3 mg/L for F-53B. Each assay was conducted in triplicate. All test compounds were found to increase cell proliferation in a dose-dependent manner, indicating agonistic activity (Table 7.7).

Table 7.7 Fold increase in cell proliferation after exposure to 25 µM of test substance

Substance	Increase in GH3 cell proliferation compared to T3
F-53B commercial	1.4
F-53B	1.7
8:2 Cl-PFESA	1.2
PFOS	1.5

Finally, Xin *et al*. (2018) conducted an *in silico* molecular docking analysis by comparing the structure of TTR, TRα and TRβ to the test compounds. The natural ligands T3 and thyroxine (T4) were also included for comparison. All test compounds were found to fit into the binding pockets of TTR and the two nuclear receptors.

The authors conclude that F-53B was found to have higher activity in thyroid related assays than PFOS, a commercial mixture of F-53B and 8:2 Cl-PFESA.

- Deng *et al*. (2018) used *in silico*, *in vitro* and *in vivo* techniques to study the effects of F-53B (purity of ≥99%) on thyroid disruption. None of the experiments followed a standard guideline, and none were conducted to GLP. A stock solution was prepared in 100% DMSO before this was diluted in culture media. The final DMSO concentration was 0.001% (v/v).

  The effect of F-53B exposure on rat pituitary GH3 cell proliferation was investigated, as this has previously been shown to be altered by thyroid hormone disrupting chemicals. The GH3 cell line was cultured in growth medium until 24 hours prior to exposure when they were placed in serum free medium. At the start of the test, cells were plated into 96 well plates and exposed to nominal F 53B concentrations of 0.01, 0.1, 1, 2, 5, or 10 mg/L. T3 was used as a positive control at

1.5 µg/L. Cells were exposed for 24 hours and all groups had six replicates. F-53B was found to increase cell proliferation in a dose-dependent manner, indicating agonistic activity. A statistically significant increase in cell proliferation was observed at the lowest exposure concentration (0.01 mg/L).

The in vivo study investigated the effect of F-53B exposure on thyroid related endpoints in Zebrafish. Beakers containing 200 2-hpf embryos were exposed to nominal F-53B concentrations of 0.0005, 0.02 or 0.2 mg/L in triplicate, with exposure solutions renewed every 24 hours. Triplicate blank and solvent controls were also included. Embryos were exposed for 5 days, before being transferred to clean water for a further 5 days. Temperature was maintained at 28 ± 1 °C and under a 14:10 hour light:dark cycle. At the end of the exposure, 20 larvae were sampled from each beaker for RNA analysis, 30 for western blot analysis and 100 for a thyroid hormone assay, with the remainder stored as back-up samples. Exposure concentrations were confirmed by LC-MS/MS analysis on days 0 and 1 (before renewal) of the uptake phase and days 1 and 5 of the depuration phase. Exposure concentrations were lower than nominal but maintained over 24 hours. The authors suggest that this is due to rapid initial adsorption to the glass surfaces. As the exposure concentrations were not found to decrease over the renewal period the arithmetic mean of the initial and day 1 concentrations were used to report the results. These were 0.00023, 0.0125, 0.135 mg/L.

At the end of the 5-day exposure period and after a further 5 days' depuration no statistically significant effects were observed on body length. Body weight was significantly reduced at the highest test concentration at both time points, and the authors comment that reduced body weight could be a result of disruption to the thyroid pathway, especially during early development. The Environment Agency notes that a reduction in body weight may be due to a number of toxicity pathways, and is not specific to this mode of action. Other observations made by Deng *et al*. (2018) were:

- After 5 days' exposure, concentrations of T4 were found to be significantly increased at all exposure concentrations. At the end of the 5-day depuration phase, T4 concentrations remained significantly increased compared to the control in the 0.135 mg/L group.

- No significant differences were observed in concentrations of T3 at either time point.

- TTR concentrations were found to be significantly increased after 5 days' exposure at all concentrations. After 5 days' depuration concentrations were not different to that of the control.

- Thyroglobulin (TG) concentrations were found to be significantly decreased after 5 days' exposure to 0.0125 mg/L and above. However, after 5 days' depuration this effect was reversed, with significantly increased concentrations of TG at concentrations of 0.0125 mg/L and above.

- Expression of some genes involved in the regulation, transport, synthesis and metabolism of thyroid hormones was also found to be significantly altered after exposure and depuration.

Finally, Deng *et al*. (2018) conducted an *in silico* molecular docking analysis by comparing the structure of Zebrafish TTR to F-53B. F-53B was found to fit into the binding pocket of Zebrafish TTR in the correct orientation and to form three hydrogen bonds. The authors consider that F-53B competes with thyroid hormones to bind to TTR, and thus can interfere with the thyroid system.

- Li *et al*. (2018) used *in vitro* assays and an *in silico* simulation to study whether F-53B binds to and alters the activity of peroxisome proliferator activated receptors (PPAR) signalling pathways. The F-53B (>95% purity) and 8:2 Cl-PFESA (>95% purity) were purified from a commercial F-53B product by preparative liquid chromatography. Each individual component and PFOS (purity >95%) was tested separately. Stock solutions were prepared using DMSO and the final DMSO concentration was <1%.

  In the first experiment, a competitive fluorescence binding assay was used to determine the relative binding affinities of the test substances to PPAR ligand binding domains (human PPARα, human PPARβ and human PPARɣ). The assays were conducted in 384 well plates, and each was conducted in triplicate. Results were expressed as the concentration of the substance required to displace half of the probe from the protein (IC_{50}). F-53B, 8:2 Cl-PFESA and PFOS were all found to bind to all three PPAR. F-53B was found to have a higher binding potency than PFOS for all three PPAR ligands (Table 7.8).

Table 7.8 IC_{50} values for PPAR ligand binding affinity

Substance	IC_{50}, µM		
	PPARα	PPARβ	PPARɣ
F-53B	105.3	114.0	98.5
8:2 Cl-PFESA	84.7	232.6	109.4
PFOS	247.7	456.5	189.5

In the second experiment, human HEK293 cells with PPAR mediated luciferase activity were exposed to the test compounds for 24 hours to determine whether agonistic activity was observed. Five nominal test concentrations were used (10, 25, 50, 75 and 100 µM). Cells exposed to 0.1% DMSO were used as the control, and WY14643, GW501516 and rosiglitazone were used as known PPAR agonists as positive controls. Each assay was conducted in triplicate. No effects on cell viability were observed for any of the test compounds. All test compounds were found to increase the luciferase transcriptional activity in a dose-dependent manner for all three PPAR receptors, indicating agonistic activity. F-53B and PFOS were found to have very similar activity levels, but 8:2 Cl-PFESA was more potent than both F-53B and PFOS in this assay.

In a third experiment, the effects of exposure to these compounds on mouse 3T3-L1 adipogenesis was investigated. WY14643, GW501516 and rosiglitazone were used as positive controls and were demonstrated to increase adipogenesis dose dependently. Exposure concentrations of 10, 50 and 100 µM were tested in triplicate. No effects on cell viability were observed for any of the test compounds. All test compounds were found promote adipogenesis in a dose-dependent manner, indicating agonistic activity (Table 7.9).

Table 7.9 Fold increase in adipogenesis after exposure to 100 µM of test substance

Substance	Increase in adipogenesis compared to solvent control
F-53B	2.3
8:2 Cl-PFESA	2.6
PFOS	2.1

Li *et al*. (2018) also measured the gene expression of four genes related to adipogenesis (C/EBPα, aP2, Adip and Lep) (Table 7.10).

Table 7.10 Fold increase in gene expression after exposure to 100 µM of test substance

Substance	C/EBPα	aP2	Adip	Lep
F-53B	5.6	610	3.0	3.7
8:2 Cl-PFESA	6.2	645	2.7	3.5
PFOS	6.8	966.3	2.6	2.6

Finally, Li *et al*. (2018) conducted an *in silico* molecular docking analysis by comparing the structure of PPARα, PPARβ and PPARɣ to the test compounds. The natural ligand decanoic acid was also included for comparison. All test compounds were found to fit into the binding pockets of the three PPAR.

The authors conclude that F-53B was found to activate PPAR signalling, primarily through the PPARɣ pathway, with a similar activity level to PFOS.

Shi *et al*. (2018), Shi *et al*. (2019a) and Shi *et al*. (2019b) report on additional *in vivo* endocrine-related endpoints as part of a chronic fish toxicity experiment as described in Shi *et al*. (2018) and Shi *et al*. (2019a) (both discussed in Section 7.1.1):

- Shi *et al*. (2018) report some statistically significant changes in the serum testosterone and oestradiol levels in F0 males and females, but these did not follow a dose response. Similarly, serum vitellogenin levels were found to be significantly increased in F0 males at 0.005 mg/L exposure, but not at 0.05 mg/L. Expression of some genes involved in the hypothalamic-pituitary-gonadal axis was found to be significantly altered at 0.005 mg/L exposure in males and females in the F0 and F1 generations.
- Shi *et al*. (2019b) report that after 180 days' exposure, T4 concentration in both male and female F0 fish was statistically significantly increased at 0.05 mg/L exposure. T3 concentration was statistically significantly decreased in F0 females at 0.005 and 0.05 mg/L, with the pattern in males unclear. F1 embryos from parents exposed to 0.05 mg/L had significantly higher concentrations of T4, which was assumed by the authors to be of maternal origin. After 5 days in clean water, F1 larvae still had significantly raised T4 at 0.05 mg/L and also had significantly reduced T3 at both 0.005 and 0.05 mg/L. F1 larvae from parents exposed to 0.05 mg/L also had significantly reduced

body length compared to the controls, and at both 0.005 and 0.05 mg/L increased mortality and uninflated swim bladders were observed. No significant differences in T4 or T3 were found in F2 fish which were raised in clean water. Expression of some genes involved in thyroid pathways was found to be significantly altered in F0, F1 and F2 fish.

- Shi *et al*. (2019a) measured the transcriptional levels of PPAR related genes in the livers of adult zebrafish exposed to 0.005 mg/L for 180 days using RNA-seq. Male and female fish appeared to have different responses, with pparαb up-regulated in males and pparda down-regulated in females. Shi *et al*. (2019a) also measured the transcriptional levels of PPARα (pparαa, pparαb), PPARβ (pparda, ppardb) and PPARɣ (pparg) and target genes in the livers of adult fish exposed to 0.005 and 0.05 mg/L using qRT-PCR. Again, differences between the sexes were observed with statistically significant increases in pparαa, pparαb, pparda and pparag in males at both exposure concentrations, but only for pparαa at the higher dose in females. A significant decrease in ppardb was observed in female fish at both doses. Finally, the livers of F1 larvae from parents exposed for F-53B at 0.005 and 0.05 mg/L were analysed by qRT-PCR. Transcription of pparαa was statistically higher in the 0.05 mg/L group and pparαb was statistically higher in larvae from parents exposed to both exposure concentrations in a dose dependent manner.

Although the six studies summarised here are not conducted to standard guidelines, the Environment Agency considers that they are sufficiently well designed and reported to be used as supporting evidence in this assessment. Together, they indicate that F-53B has the potential to disrupt the thyroid and PPAR signalling pathways.

8 Mammalian toxicology

The main focus of this report is the environmental hazards and risks from F-53B and no human health assessment is undertaken. However, certain longer term mammalian toxicology endpoints are potentially relevant for the determination of a substance as Toxic ('T') according to the REACH Annex 13 criteria (see Section 9.3) and for a wildlife secondary poisoning assessment (see Section 9.5). A summary of available and relevant mammalian endpoints located from the public domain is therefore included here.

For F-53B, there are no standard regulatory studies available for any human health end point. There are no studies available at all to inform on the acute toxicity, skin and eye irritation skin sensitisation, germ cell mutagenicity or carcinogenic potential of F-53B. The only information on the toxicology of F-53B comes from a small number of published studies, which were not designed to meet current regulatory requirements, but address specific scientific questions. None of these studies was similar in design to the relevant OECD test guidelines or conducted in a GLP environment.

There are 4 non-standard oral repeated dosing studies. Very limited information on sexual function and fertility comes from a non-standard reproductive toxicity study employing F-53B-treated male mice and untreated female mice.

Some *in vitro* studies purporting to inform on the developmental toxicity potential of F-53B are available. These investigated the ability of F-53B to perturb hESC (human embryonic stem cell differentiation), mESC (mouse embryonic stem cell differentiation) and hBMSC (human mesenchymal stem cell) differentiation. For the studies conducted with hESC and hMBSC, comparison experiments were conducted with PFOS.

There is an extensive human health toxicology database for PFOS for all human health end points, comprising standard and non-standard studies in experimental animals. These studies have been extensively reviewed by EFSA (EFSA CONTAM Panel 2020; EFSA CONTAM Panel, 2018; EFSA, 2008).

8.1 Absorption, Distribution, Metabolism, Excretion

There are no standard studies available to inform on the toxicokinetics of F-53B. The only information available comes from a number of limited studies conducted to investigate how F-53B partitions in mammalian cells, proteins or lipids. None of the assays followed a standard guideline and none were conducted to GLP. In all these studies, PFOS has also been tested using the same experimental design, and so results for both compounds are reported here when available.

- Allendorf *et al*. (2019) investigated the partitioning of several PFAS, including F-53B (purity not stated) and PFOS (98% purity), between bovine serum albumin (BSA) and water. Dialysis cells were used which were separated by a membrane that kept BSA on one side of the chamber whilst permitting the free movement of the test compound. A known amount of the test compound was added to the BSA-free side

of the dialysis cell and left to equilibrate at 37 °C and under constant agitation. In order to prevent saturation of the protein binding sites, the molar ratio of the compound to BSA was kept below 0.1. The concentration of the compound in the BSA-free side was measured every day by UPLC-MS/MS and when there was no difference in the values, equilibrium was considered to have been reached and the experiment terminated. All experiments were run in triplicate.

For PFOS and F-53B equilibrium was reached after 96 hours. The partition coefficient $K_{albumin/water}$ was calculated by dividing the concentration bound to BSA by the concentration in the water. The bound concentration could not be measured directly. Instead a mass balance approach was used whereby the mass in the water and the mass extracted from the interior of the dialysis cells using methanol was subtracted from the initially dosed amount to calculate the mass bound to BSA. The log $K_{albumin/water}$ was 5.01 L/kg for F-53B and 4.67 L/kg for PFOS. The bound fraction was 56% for F-53B and 37% for PFOS. Allendorf *et al*. (2019) suggest that the higher coefficient for F-53B could be due to its larger atom radius than PFOS being sterically favoured at certain binding sites, or due to van der Waals interactions between the chlorine atom and the albumin.

- Ebert *et al*. (2020) performed a study using the same experimental design to determine the partitioning of several PFAS, including F-53B (purity not stated) and PFOS (98% purity), between liposomes and water. For PFOS and F-53B equilibrium was reached after 96 hours. The log $K_{liposome/water}$ was 5.14 L/kg for F-53B and 4.89 L/kg for PFOS. The bound fraction was 52% for F-53B and 55% for PFOS. The authors conclude that there was no significant difference between the partitioning of F-53B and PFOS in this experiment.

  In addition, Ebert *et al.* (2020) investigated the anionic permeability of several PFAS by measuring the electrical signal resulting from a charged compound crossing an experimental planar lipid bilayer membrane. Measured permeability was 7.6×10^{-5} cm/sec for F-53B and 5.5×10^{-5} cm/s for PFOS, which the authors note would lead to a high fraction being absorbed across human cell membranes by passive diffusion for both these compounds, without the need for active uptake processes.

- Sheng *et al*. (2018) studied the binding affinity of various PFAS, including F-53B and PFOS (purities not stated), to human liver fatty acid binding protein (hL-FABP). Each test compound was tested in a ligand displacement assay, with a fluorometric probe used to determine the binding affinity. The $Kd_{hL\text{-}FABP}$ was 4.05 µM for F-53B and 4.99 µM for PFOS, which the authors consider to be equivalent to each other. In addition, Sheng *et al*. (2018) conducted an *in silico* molecular docking analysis by comparing the structure of hL-FABP to the test compounds. F-53B and PFOS were found to fit into the binding pocket of hL-FABP (although F-53B had some structural distortion) and had equivalent average docking energies. The difference between the results was not tested statistically.

- Cheng and Ng (2018) also conducted an *in silico* molecular docking analysis by comparing the structure of hL-FABP and rat liver fatty acid binding protein (rL-

FABP) to a number of PFAS. The docking energy of F-53B for both hL-FABP and rL-FABP was found to be similar or stronger than that of PFOS, although the statistical significance is not reported, indicating that the binding affinity of F-53B to these proteins is at least that of PFOS. The authors conclude that F-53B could therefore be as bioaccumulative as PFOS.

UNEP (2006) reported that the bioaccumulation potential of PFOS is not based on classical lipophilic behaviour; instead PFOS binds to proteins such as albumin, β-lipoproteins and liver fatty acid binding proteins. The studies summarised here indicate that F-53B also has the potential to partition to albumin, liposome and human liver and rat liver fatty acid binding protein with binding affinities similar or greater than those measured for PFOS.

8.2 Mammalian toxicology studies

8.2.1 Acute toxicity, irritation and sensitisation

There are no studies available to inform on the acute toxicity, skin and eye irritation or skin sensitisation potential of F-53B.

8.2.2 Repeated dose toxicity

There are no standard studies available to inform on the repeated dose toxicity of F-53B. Limited information on the repeated dose toxicity of F-53B comes from 3 non-standard oral dosing studies: a 28-day study investigating thyroid toxicity in rats, a 56-day study investigating hepatotoxicity in male mice and a 70-day study investigating toxicity to the colon, also conducted in mice. None of these studies was similar in design to the relevant OECD test guideline or conducted in a GLP environment.

8.2.2.1 *Studies in rats*

Thyroid Toxicity

Hong *et al*. (2020) administered F-53B to groups of rats (Sprague-Dawley 7/sex/dose) at doses of 0, 5, 20, and 100 mg/kg bw/day via gavage, for 28-days. The study was designed to investigate the potential effects of F-53B on thyroid function in rats.

Body weights were measured prior to commencing the study, twice a week during the treatment period and at necropsy. The haematology and clinical chemistry investigations were consistent with a standard OECD TG compliant study, including triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH). The following organs were examined for gross and histopathological changes: liver, kidneys, adrenal glands, brain, heart, thyroid gland, pituitary gland, spleen, thymus, testes, epididymis, prostate, seminal vesicle, coagulating gland, ovaries, and uterus. Immunohistochemistry, employing diaminobenzidine (DAB) staining, was used to quantify changes in thyroid peroxidase (TPO) and thyroid stimulating hormone receptor (TSHR) protein.

Although the study appears to have been well conducted and reported, there are a number of deficiencies compared to OECD TG 407 (adopted October 2008). Notably, no clinical observations were included, the range of organs assessed for gross and histopathological examination was very limited and no urinalysis was conducted.

At necropsy, body weights in all three treatment groups were comparable to controls. Haematology parameters were comparable between treated and control groups. Following clinical chemical analysis, with the exception of statistically significantly decreased T3 and T4 levels, all other parameters were comparable with controls. It was not possible to establish precise quantitative values, as these data were only presented as bar charts. No changes in TSH levels were observed at doses of up to 100 mg/kg/day, the highest dose tested.

At necropsy, gross appearance and organ weights of all organs examined, including liver and thyroids, were comparable to controls. Histopathological examination of the same organs only found treatment-related changes in the thyroid. Thyroid follicular hyperplasia was observed in all male dose groups, although without a clear dose response, and in mid and high dose females. Information is available on thyroid follicular cell incidence only (see Table 8.1), no information on severity was provided.

Table 8.1 Incidence of Thyroid Follicular Cell Hyperplasia

	Groups (n=5)			
	control	**5**	**20**	**100**
Males	0	1	3	1
Females	0	0	4	5

Immunohistochemical investigations found increased TPO protein (area DAB positive cells: total area) at all dose levels, in males and females, but none of the changes achieved statistical significance. A statistically significant increase in TSHR protein (area of DAB positive cells: total area) was confined to high dose females. No quantitative information was provided for these changes.

Overall, decreased T3 and T4 levels were observed in rats after 28-days' exposure to oral doses of 5 mg/kg bw/day and above. Although TSH levels were not affected by treatment, there is some evidence of a proliferative response in the thyroid (thyroid follicular cell hyperplasia) in treated males and clear evidence of a proliferative response in females from 20 mg/kg bw/day and above. There is insufficient information available from this study to inform on potential mechanisms underpinning the observed decreases in thyroid hormone levels.

It was not possible to establish a reliable NOAEL since the investigations were limited, the groups sizes small and the reporting was insufficient.

8.2.2.2 *Studies in mice*

Liver toxicity

Groups of male mice (BALB/c strain 15/dose) were administered F-53B at doses of 0, 0.04, 0.2, or 1.0 mg/kg bw/day via gavage, for 56-days (Zhang *et al*., 2018). The study was designed to investigate the hepatotoxicity of F53-B mice, screen for liver protein changes and explore the mechanism of hepatotoxicity.

At study termination all animals were sacrificed, and the following investigations conducted: terminal body weight, liver weight (n=15), limited clinical chemistry, liver histopathology, F-53B levels in bile and liver (n=4), and liver proteomic analysis.

At necropsy, body weights were reported to be comparable to controls in all treatment groups. Compared to controls, absolute liver weights were statistically significantly increased in all treatment groups (estimated from bar charts in the publication to be by 14 %, 36 % and 180 % at 0.04, 0.2 and 1.0 mg/kg bw/day, respectively). Relative (relative to body weight) liver weights were also statistically significantly increased, compared to controls at doses of 0.2 mg/kg bw/day and above (estimated from bar charts to be by 5 % and 132 % at 0.2 and 1.0 mg/kg bw/day respectively). No quantitative data on body or organ weights were provided in the publication, hence the changes presented here are estimates from the published charts.

Histopathological examination found lipid droplet accumulation at 0.2 mg/kg bw/day and above. Hepatocellular necrosis, hepatocyte steatosis and ballooning, and inflammatory infiltrate was observed at the top dose only.

At study termination, there were indications of increased F-53B levels in liver and bile with increasing administered dose. It is unclear whether F-53B in the bile had been absorbed from the GI tract but was not systemically available, or if it had been resorbed from the systemic circulation. No information was presented on F-53B levels as a proportion of the administered dose.

Tabulated clinical chemistry findings from the report are presented in Table 8.2. Dosing of mice for 65-days with F-53B caused statistically significant increases in ALT and AP at the top dose of 1.0 mg/kg bw/day only. Lipid markers, HDL and LDL were statistically significantly decreased, and triglycerides statistically significantly increased, at the top dose only. Liver triglycerides and total cholesterol were statistically significantly increased at doses of 0.04 mg/kg bw/day and above, the lowest dose tested. These clinical chemistry changes suggest a perturbation in liver lipid metabolism and liver damage, as reported from the histopathology investigations.

Proteomic analysis of liver samples suggested that the PPARα/RXR pathway was activated. The authors predicted, using computational tools, that F-53B would elevate PPARα inducible proteins, including Cyp 4As, although there is no direct evidence from this study. Western-blot analysis found that PPARα, RXR and PXR proteins were all

statistically significantly increased compared to controls. No quantitative information on this change is available.

Table 8.2 Clinical chemistry findings

Serum	control	0.04 mg/kg bw/day (%/fold change)	0.2 mg/kg bw/day (%/fold change)	1.0 mg/kg bw/day (%/fold change)
ALT; (IU/l) alanine transaminase	52.38+/- 9.1	65.13+/-7.89	88+/-24.44	161.75+/-28.79* (3-fold)
AST; (IU/l) aspartate aminotransferase	104.5+/-10.29	171.12+/-19.06 (63 %)	152.88+/-11.13 (46 %)	143.62+/-11.02 (37 %)
ALP; (IU/l) alkaline phosphatase	104.62+/-	89.88+/-5.7	149.38+/-15.04 (1.4 -fold)	1123.4+/-43.69* (10.7-fold)
TBA (µmol/L); total bile acid	1.46+/-0.29	1.24+/-0.26	1.14+/-1.16	1.57+/-0.38
ALB (g/L); albumin	23.66+/-0.3	22.83+/-0.4	24.08+/-0.34* (1.7 %)	25.54+/-0.25* (7.9 %)
TCHO (mmol/L); Total cholesterol	3.15+/-0.08	3.1+/-0.09	2.85+/-0.08*	2.98+/-0.09
TG (mmol/L); triglyceride	1.36+/-0.11	1.6+/-0.11	1.14+/-0.08	2.19+/-0.22* (61.0 %)
HDL (mmol/L); high-density lipoprotein cholesterol	3.32+/-0.1	3.22+/-0.07 (-3.6 %)	3.06+/-0.07* (-7 %)	2.73+/-0.06* (-17 %)
LDL(mmol/L); low-density lipoprotein cholesterol	0.19+/-0.01	0.2+/-0.02	0.18+/-0.01	0.14+/-0.04* (-26 %)
GLU (mmol/L); glucose	2.4+/-0.68	2.23+/-0.57	2.39+/-0.52	3.6+/-0.5
Liver				
TCHO (µmol/g); Total cholesterol	13.72+/-0.88	18.16+/-0.55* (32.2 %)	18.26+/-0.62* (33.0 %)	24.53+/-1.77* (78.7 %)
TG (µmol/g); triglyceride	73.72+/-3.52	84.21+/-3.56* (14.2 %)	90.98+/-1.94* (23.4 %)	88.14+/-1.86* (19.5 %)

*denotes statistically significantly change, compared to controls

F-53B caused a clear increase in absolute liver weight in male mice at doses of 0.04 mg/kg bw/day and above, the lowest dose tested. The increases observed at doses of up to 0.2 mg/kg/day are likely to be adaptive in nature, but the 180 % increase at the top dose of 1.0 mg/kg bw/day is considered to be toxicologically adverse. The increases in liver weight were clearly associated with elevated liver TCHO and TG and PPARα, RXR at all doses tested and PXR protein levels at the top dose only.

Overall, this study provides very limited information on the potential of F-53B to cause toxicity following repeated dosing.

Gastrointestinal tract toxicity

Groups of mice (C57 black strain 4/sex/dose) were administered F-53B at doses of 0, 1, 3, or 10 µg/L via the drinking water, for 70 days (Pan *et al.,* 2019c). The study was conducted specifically to investigate the potential adverse effects of F-53B on the GI-tract of mice. The intakes of F-53B were 0, 0.6, 2 and 6 µg/kg bw/day, which were estimated by the Health and Safety Executive using the ECHA (2012) appendix R.8-2 p 64.

At study termination all animals were sacrificed, and faecal samples, colon, ileum, serum and colonic contents were collected and snap frozen in liquid nitrogen and then stored at minus 80 °C.

Very limited investigations were conducted, which were confined to determination of F-53B levels in the serum, faeces, ileum and colon (3m and 3f), histology of the ileum and colon. Specific investigations of gene expression of mucous and AMPs (antimicrobial peptides) and inflammation associated genes, immunohistochemistry for dendritic cells and changes in gut microflora were conducted on colon samples (4m and 4 f) only. No further investigations were conducted, including those usually conducted as part of an OECD TG compliant study.

It was not possible to establish precise quantitative values for the tissue, serum and faecal F-53 concentrations, as these data were only presented as bar charts. No statistical comparisons were performed by the authors. However, it is noted that for all matrices investigated, F-53B concentration increased with increasing dose.

No histopathological abnormalities in samples of gastrointestinal tract examined were observed at doses of up to 6 µg/kg bw/day, the highest dose tested.

Genes involved in ion transport were elevated at the top dose, in particular Nkcc1 (Na-K-Cl-cotransporter) in both sexes and Ctfr (cystic fibrosis transmembrane conductance regulator) in females at the top dose of 6 µg/kg bw/day. In addition, some AMP genes were also upregulated at the top dose, most notably Pla2g4A (cytosolic lipophosphatase A2 family). Genes for a number of inflammatory markers were also elevated at the top dose. Immunohistochemical staining showed that mucus secretion decreased in the colon of both female and male mice, and dendritic cells were increased in both males and females at the top dose only.

Investigations of gut microflora in this study found that F-53B exposure altered the composition of gut microbiota at the phylum and genus levels.

Overall, the authors inferred from the gene expression investigations that subchronic exposure to environmentally-relevant concentrations of F-53B interfered with gut barrier function and caused sub-clinical colonic inflammation in both female and male mice. However, given the limited range of investigations conducted, the small group sizes and

the lack of any supportive histopathology, it is not possible to form any reliable conclusions on the repeated dose toxicity of F-53B in mice from this study.

Hepatotoxicity *in vitro*

Sheng *et al.* (2018) investigated cytotoxicity, effects on cell viability and cell cycle kinetics of a number of perfluoroalkyl substances including F-53B in a human hepatocyte cell line (HL-7072). A cell viability assay was also conducted using PFOS.

The binding mode and affinity to human liver fatty acid binding protein (hL-FABP) was also determined for F-53B and compared with PFOA. This was achieved by employing circular dichroism spectroscopy, fluorescent displacement assays and *in silico* molecular docking investigations.

For the cell viability assay, cells were seeded in 96-well plates at initial densities of 1×10^4 cells/well. Cytotoxicity was measured by MTT (3-(4,5-dimethyl-2-thiazyl)-2, 5-diphenyl-2H-tetrazolium bromide) reduction. For cell cycle analysis, cells were seeded in 6-well plates at initial densities of 4×10^5 cells/well. The HL-7702 cells, cultured in 6-well plates, were allowed to attach for 24 h and then exposed to F53B at concentrations of 0, 25, 50 and 100 µm for a further 24 hours. Following exposure, half of the cells were collected, washed, filtered and stained for flow cytometry, and the other half were collected for gene expression analysis. Cells in different cell cycle phases were then analysed on a flow cytometer. For gene expression analysis, mRNA levels were reported relative to an internal control.

The IC_{50} to HL-7072 hepatocytes was found to be $3.02.10^{-4}$ M for F-53B, and $4.17.10^{-4}$ M for PFOS. Gene expression analysis found evidence of statistically significant, concentration-related increases in relative mRNA levels of genes related to cell cycle control, and Cd36 (cluster differentiation 36). No changes in PPARµ expression were noted.

After the addition of F-53B to the hL-FABP solution, the β-sheet content decreased from 54.4 to 48.98 % (9.97 %) and the α-helix content increased by 4.11 %, approximating the changes induced by PFOS. The binding affinities (Kd) of F-53B and PFOS to hL-FABP were 4.05 and 4.99 µM respectively.

The authors concluded that F-53B was more toxic in the *in vitro* investigations and with greater protein binding affinity than PFOS for hl-FABP, suggesting F-53B may be more hepatotoxic than PFOS.

F-53B appeared to be more cytotoxic than PFOS *in vitro* against the hepatocyte cell line HL-7072 in this study. However, it is unclear whether F-53B would be more cytotoxic to primary hepatocytes *in vitro*, or *in vivo*.

8.2.2.3 *Summary of repeated dose toxicity*

There are no standard studies available to inform on the repeated dose toxicity of F-53B. Limited information on repeated dose toxicity of F-53B comes from three non-standard

oral dosing studies: a 28-day study investigating thyroid toxicity in rats, a 56-day study investigating hepatotoxicity in male mice, and a 70-day study investigating toxicity to the colon, also conducted in mice. It is not possible to compare each mouse study, as different strains and investigations were employed in each case.

In the mouse study, absolute and relative liver weights were statistically significantly increased at doses of 0.04 mg/kg bw/day and above, the lowest dose tested. From the limited information available, it is not possible to identify a potential mechanism for the liver changes observed. However, there is some *in vitro* evidence that F53-B can bind to PPARα activate PPAR signalling (PPARα, βand γ) in cell-free systems, and to promote adipogenesis in adipocyte derived mouse 3T3-L1 cells (Li *et al*., 2018).

In rats, T4 levels were statistically significantly decreased, in both males and females, from 5 mg/kg bw/day, the lowest dose tested, with T3 levels statistically significantly decreased at 5 mg/kg bw/day and above in females, and from 20 mg/kg bw/day and above in males. No changes in TSH levels were observed.

There is some evidence of a proliferative response in the thyroid (thyroid follicular cell hyperplasia) in treated males from 5 mg/kg bw/day and above, and clear evidence of a proliferative response in females from 20 mg/kg bw/day and above.

There is insufficient information available from this study to inform on potential mechanisms underpinning the observed decreases in thyroid hormone levels. However, Xin *et al*., 2018 and Deng *et al*., 2018 found using *in silico* models and *in vitro* assays that F-53B binds to TTR, TRα and TRβ, that it increases activity of TRα and TRβ in a human cell line and that cell proliferation was increased in a rat pituitary established cell line (GH3 cells).

Overall, there is insufficient information available from the studies conducted with F-53B to conclude on repeated dose toxicity. However, the available data suggests that F-53B is hepatotoxic in mice and has anti-thyroid activity in rats.

8.2.2.4 *Data from structural analogues*

There is an extensive repeated dose toxicity database for the structural analogue PFOS with studies conducted in rats (28 to 90 days), mice (3 to 49 days) and monkeys (90 to 183 days). These studies have been extensively reviewed by UNEP (UNEP, 2006) and EFSA (EFSA CONTAM Panel 2020; EFSA CONTAM Panel, 2018; EFSA, 2008). The liver is the most consistent target organ in all three species, with concomitant perturbations of lipid metabolism. There is limited evidence of anti-thyroid activity in Sprague–Dawley rats and cynomolgus monkeys.

EFSA identified an overall repeated dose NOAEL for PFOS of 0.03 mg/kg bw/day, as decreased serum HDL and decreased TSH were observed in cynomolgus monkeys at doses of 0.15 mg/kg bw/day and above for 183 days.

PFOS has a harmonised classification in Annex VI of the GB Mandatory Classification and Labelling (MCL) for repeated dose toxicity (STOT RE1; H372). No target organs were specified.

Considering the structural similarities of F-53B and PFOS and the limited information available on F-53B, it is likely that F-53B would be hepatotoxic in mice, rats and primates and share the same anti-thyroid activity in rats and primates. These adverse effects of F-53B should be considered relevant for human health. However, there is insufficient information available on F-53B to make predictions on other potential toxic effects of F-53B following repeated dosing.

8.3 Germ cell mutagenicity

No studies have been identified to inform on the mutagenic potential of F-53B, either *in vitro* or *in vivo*. Therefore, it is currently not possible to conclude on germ cell mutagenicity.

8.4 Carcinogenicity

No studies have been identified to inform on the carcinogenic potential of F-53B. Therefore, it is currently not possible to conclude on carcinogenicity.

8.5 Toxicity to reproduction (effects on fertility and developmental toxicity)

8.5.1 Sexual function and fertility

No standard studies are available to inform on the potential of F-53B to adversely affect sexual function and fertility. Very limited information comes from a non-standard reproductive toxicity study employing F-53B-treated male mice and untreated female mice.

Groups of male mice (BALB/c strain, 15/dose) were administered F-53B at doses of 0, 0.04, 0.2, or 1.0 mg/kg bw/day via gavage, for 56-days (Zhou *et al.,* 2018). At study termination, 6 top-dose animals were retained for a limited fertility study.

The remaining animals were sacrificed, and the following investigations conducted.

- Bodyweight, testis weight and epididymides weights (n=9).
- F-53B levels were measured in serum, testis and epididymides from 4 animals/dose.
- The testis and epididymides (6/dose) were processed for histopathological examination.
- Serum oestradiol, luteinising hormone (LH) follicle stimulating hormone (FSH), and serum and testicular testosterone (T) levels were determined (6/dose).

- Epidydimal sperm analysis was conducted on 6 animals/dose. Gene expression and western blot analysis were also conducted.
- The 6 top-dose males set aside for a limited fertility investigation were each mated with 3 untreated females. The following parameters were determined: mating index (number of females mating/cohabited females), litter size, gender ratio per litter, birth weight of the pups and weaning weight on lactation day 21 (LD 21).

No adverse effects on any of the reproductive parameters investigated were observed up to 1.0 mg/kg bw/day, the highest dose tested. However, given the small group size, low doses, single-sex dosing, and very limited number of investigations conducted, this study does not provide any useful information on the reproductive toxicity of F-53B in mice.

F-53B levels were elevated in all samples investigated (see Table 8.3 below).

Table 8.3 F-53B Concentration in serum, testis and epididymides

Dose F-53B mg/kg bw/day	F-53B content		
	Serum (µg/ml)	Testis (µg/g)	Epididymides (µg/g)
0	0.07	0.00095	0.065
0.04	5.89	5.6	1.69
0.2	18.89	14.4	2.68
1	58.33	49.93	9.64

No change in absolute testis weight was observed in the three F-53B dose groups. However, the relative testis weights showed a slight but statistically significant decrease of 8% in the 1.0 mg/kg bw/day F-53B group compared with the control. In addition, the absolute and relative epididymis weights were also statistically significantly decreased by 14% and 16%, respectively, at 1.0 mg/kg bw/day F-53B. There were no further treatment-related changes observed in any of the investigations conducted in this study.

Considering there were no histopathological abnormalities observed in the testis or epididymides, no hormone changes or epidydimal sperm changes, the decreases in relative testis and absolute and relative epidydimal weights are not considered to be toxicologically significant.

Gavage administration of F-53B for 56 days to male mice had no adverse effect on the reproductive performance, reproductive hormones and reproductive organs and tissues investigated in this study, at doses of up to 1.0 mg/kg bw/day, the highest dose tested. However, it is currently not possible to conclude on the potential of F-53B to adversely affect sexual function and fertility, because of the very limited information available from this study.

8.5.2 Effects on or via lactation

8.5.2.*1* *Human information*

Jin *et al*. (2020b) investigated the concentrations of PFAS in human breast milk and compared this to paired infant postnatal growth data from Anji, Zhejiang, China. Breast milk was collected within one week of delivery. There were 174 participants in the longitudinal birth cohort. Samples were collected from 2018 to 2019. F-53B was detected in 100% of breast milk samples (median concentration 0.016 ng/ml) and PFOS in 50% of samples (median concentration 0.001 ng/ml). Only infant length gain was found to be significantly negatively correlated with F-53B concentration. However, it is noted that the possibility of *in utero* exposure has not been considered by the authors. Limited further information on this study is provided in Section 8.6, below. It is not possible from this study to conclude that F-53B was responsible for the reported effect on length gain.

8.5.2.2 *Animal studies*

There are no studies available to inform on the potential for F-53B to cause adverse effects on or via lactation in experimental animals.

8.5.2.3 *Data from structural analogues*

The EFSA reviews did identify human investigations around lactation but each report was confounded by the presence of other PFAS, notably PFOA. It was therefore not possible to ascribe any potential adverse effects to PFOS alone.

8.5.3 Developmental Toxicity

8.5.3.1 *Studies in vivo*

There are no studies available to inform on the potential for F-53B to perturb development in humans or in experimental animals. Therefore, it is currently not possible to conclude on the developmental toxicity of F-53B.

8.5.3.2 *Studies in vitro*

Two studies are available which were conducted to investigate whether F-53B can perturb human embryonic stem cell (hESC) and mouse embryonic stem cell (mESC) differentiation. Another study was conducted to investigate whether F-53B can perturb differentiation in human mesenchymal stem cells (hBMSC).

<u>Human Embryonic Stem Cell Differentiation</u>

Yang *et al*. (2020) investigated the potential of F-53B to induce developmental toxicity in an *in vitro* hESC cardiac differentiation model and compare to PFOS. The overall intention being to predict whether F-53B and PFOS might perturb cardiac differentiation in intact animals. hESCs were dissociated to single cells and cardiac differentiation induced by culturing the cells in CDM3 medium. F-53B and PFOS in DMSO was added to the medium

starting from day 0 until the end of the differentiation process, on day 12, at concentrations of 0, 0.1, 1, 10, 30, and 60 μM. The maximum concentration was identified in an AlmarBlue cell viability assay, in which no cytotoxicity was observed at concentrations of up to 60 μM, for both F-53B and PFOS.

Relative to the housekeeping gene after 12-days of dosing, two cardiac specific marker genes (NKX2.5 and MYH6) were up regulated at 10 μM and above and third (TNNT2) at 30 μM and above. The 4th cardiac specific marker gene (MYL7) was upregulated at 0.1 to 10 μM, and downregulated at 30 and 60 μM, the highest concentration tested. Further investigations of gene expression changes found that F-53B stimulated differentiation of hESC to epicardial cells rather than cardiomyocytes.

For PFOS, relative to the housekeeping gene after 12 days' dosing, only the cardiac specific marker gene (MYL7) was upregulated at 0.1 and 1 μM, and downregulated at 60 μM, the highest concentration tested. Further investigations of gene expression changes found that PFOS stimulated differentiation of hESC to epicardial cells rather than cardiomyocytes.

Treatment with 60 μM F-53B or PFOS upregulated RUNX2, WT1, while repressing NKX2.5, MYL4, and TNNT2, all regulated as part of the WNT (wingless-related integration site gene) signalling pathway. These changes suggest that F-53B and PFOS are activating the WNT pathway. However, for the same genes, incubation with the WNT inhibitor (Wi) in the presence of chemical treatments did not affect their expression compared to the DMSO control group. This suggests that there are additional mechanisms underpinning the actions of F-53B and PFOS on gene expression during cardiac differentiation. The WNTs comprise a large family of protein ligands that are critical to the correct functioning of diverse processes such as embryonic induction, generation of cell polarity, and the specification of cell fate.

The authors concluded, based on these *in vitro* data, that F-53B and PFOS may adversely affect cardiac development via inappropriate modulation of WNT signalling. These data indicate that F-53B and PFOS perturbed the *in vitro* differentiation of hESC to cardiomyocytes, mediated at least in part via perturbation of WNT signalling. However, there is insufficient information available to indicate whether this would translate to an adverse effect of human relevance in an intact system.

Abnormalities of cardiac development were reported (ventral septal defects and cardiac enlargement – species not reported) in at least one of the *in vivo* developmental toxicity studies conducted with PFOS (EFSA CONTAM Panel, 2018; EFSA, 2008). However, there is insufficient information to infer a direct link between these *in vitro* findings with PFOS and cardiac development *in vivo* or predict whether F-53B might perturb cardiac development *in vivo*.

Human Mesenchymal Stem Cell Differentiation (F-53B/6:2 PFESA)

Pan *et al*. (2019b) investigated the potential of F-53B to adversely affect differentiation *in vitro* in hBMSC obtained from healthy donors. No information is available in relation to the status of the donors, including underlying health conditions, age and sex.

hBMSC were seeded in 0.1% gelatine coated plates and one day later, the medium was changed to one containing F-53B, PFOS, PFHxS, PFOA or 0.1% DMSO vehicle. After 7 days, total cellular RNA was extracted. The mRNA expression levels of several key differentially expressed genes identified in microarray data were further measured by RT-qPCR. In addition, the molecular biomarkers of osteogenic differentiation were measured by RT-qPCR in differentiating hBMSCs. Perturbations in calcium signalling were monitored by real time calcium imaging in hBMSCs using a calcium sensitive fluorescent probe. The degree of differentiation was measured by following CD44, the stemness-related surface marker of mesenchymal stem cells (MSC). All differentiation experiments were (apparently) conducted with 100 nM F-53B, a non-cytotoxic concentration. Investigations of intracellular calcium signalling employed F-53B concentrations of 0, 1 mM, 10 mM and 100 mM, and CD44 employed concentrations of 0, 100 nM, 1 mM and 10 mM.

- F-53B and PFOS statistically significantly decreased RUNX2 protein by 28.5% and 41%, respectively, compared to vehicle control. RUNX2 is a key osteoblast differentiation transcription factor.
- F-53B statistically significantly increased both the ALP mRNA expression and ALP specific activity (from 5.5 to 7.5 nmol/min/mg protein in control and F-53B treated cells respectively). ALP is a phenotypic marker for early differentiation. Similar changes were not observed with PFOS.
- P-SMAD1/5/8 levels were significantly decreased by 57% in Cl-PFESA-treated hBMSCs, compared to solvent controls. SMAD proteins are key components of the TGFβ signal transduction cascade. Similar changes were not observed with PFOS.
- Qualitatively, the number of CD44 positive cells was decreased, compared to controls, at a concentration 10 mM Cl-PFESA and PFOS, but not at lower concentrations of 100nm and 1mM. CD44 is a differentiation marker in MSC.
- Intracellular Ca^{2+} spikes were stimulated in hBMSC by the addition of 10 mM Cl-PFESA, but not at lower concentrations. PFOS induced intracellular calcium spikes at 100mM, the highest concentration tested. Intracellular Ca^{2+} plays a key role in cell signalling pathways in various stem cell differentiation stages.

Overall, the authors concluded that F-53B can impair the multipotency of hBMSC *in vitro* and may perturb bone marrow homeostasis. Compared to PFOS, F-53B may be at least as potent, and may be more so. However, there is insufficient information available to extrapolate these *in vitro* findings to bone development in experimental animals or humans.

Abnormalities of bone development were reported in at least one of the *in vivo* developmental toxicity studies conducted with PFOS (EFSA CONTAM Panel, 2018; EFSA,

2008). However, there is insufficient information to inform on any direct link between these *in vitro* findings with PFOS and bone development *in vivo* or predict whether F-53B might perturb bone development *in vivo*.

Mouse Embryonic Stem Cell Differentiation (F-53B)

Yin *et al*. (2018) investigated the potential of F-53B to induce developmental neurotoxicity in an *in vitro* mESC differentiation model. In this study, embryonic stem cell differentiation was explored using stem cell embryoid bodies (EB) and in monolayer culture.

For cell viability assays, ESC were seeded in 96-well plates, in ESC or N2B27 differentiation medium, in the presence of 0, 0.001, 0.01, 0.1, 1 or 10 mM F-53B or PFOS; 0.1% DMSO was used as a solvent control. After seven days for cells in ESC medium, and nine days for cells in N2B27 medium, cell viability was measured with an Alamar Blue cell viability test.

For alkaline phosphatase (AP) staining, ESCs were seeded into 12-well plates, at 1000 cells per well, and cultured in ESC medium supplemented with 0.001, 0.01, 0.1, 1 or 10 mM F-53B or PFOS; 0.1% DMSO was used as a solvent control. Spent media were changed every other day. After seven days, AP staining was performed using a commercial AP staining kit.

For the EB differentiation experiment, ESCs were seeded onto non-coated petri dishes supplemented with 10 mL EB differentiation medium and incubated with 0.1% DMSO, or 0.1 mM F-53B or 0.1 mM PFOS from day 0. EB samples were collected at days 0, 4, 9 and 20 for RNA isolation. Changes in mRNA levels were reported relative to glyceraldehyde-3-phosphate dehydrogenase (Gapdh) as the reference housekeeping gene.

For the monolayer differentiation experiments the ESC were seeded onto 0.1% gelatine coated 6-well plates To assess toxicity, cells were incubated with medium containing 0.1% DMSO, or PFOS (0.1 and 10 mM), or F-53B (0.1 and 10 mM), for up to nine days starting from the beginning of differentiation. The spent medium was changed every other day, until cells were collected for RNA isolation. Changes in mRNA levels were reported relative to glyceraldehyde-3-phosphate dehydrogenase (Gapdh) as the reference housekeeping gene.

There were no treatment-related changes on cell viability or reactive oxygen species generation at concentrations of up to 10 mM, the highest concentration tested.

In the EB differentiation experiment, compared to the control group, F-53B (0.1mM) statistically significantly down-regulated the expression of the neural ectoderm markers Sox1 and Sox3 at Day 4 and Day 20, with Pax6, a neuronal cell progenitor cell marker repressed by F-53B treatment at Day 20. In contrast, similar changes were not observed with PFOS.

During monolayer neural differentiation, the expression level of the pluripotency marker Nanog decreased while the expression level of the neural markers Sox1, Sox3, Nestin,

Pax6 and Map2 increased compared to Day 0. Both F-53B and PFOS exhibited a general inhibitory trend for neural marker gene expression in the monolayer differentiation system.

Based on these data, the authors concluded that F-53B poses a risk to human development *in utero*, given F-53B has been found to cross the placenta. However, these *in vitro* findings do not reliably inform on the potential for F-53B to cause developmental toxicity in experimental animals or in humans.

8.5.4 Summary of reproductive toxicity

8.5.4.1 *Sexual function and fertility*

No standard studies are available to inform on the potential of F-53B to adversely affect sexual function and fertility. Very limited information comes from a non-standard reproductive toxicity study employing treated male mice (doses up to 1.0 mg/kg bw/d for 56 days) and untreated female mice. No adverse effects on reproductive performance were recorded. However, compared to a standard multi-generation protocol, this study is so limited in terms of design, conduct and reporting that it is currently not possible to conclude on the potential of F-53B to adversely affect sexual function and fertility.

The potential for PFOS to adversely affect sexual function and fertility has been considered by UNEP (UNEP 2006) and EFSA (EFSA CONTAM Panel, 2018 and EFSA 2008). Two studies were reviewed, a standard two generation rat study and a limited study investigating reproductive toxicity in male mice.

In the two-generation study (EFSA 2008; Christian, Hoberman and York, 1999), gestation duration was significantly reduced and there also was a significant reduction in the number of implantation sites with a concomitant reduction in litter size at the high dose of 3.2 mg/kg bw/day, in the F1 generation. No further adverse effects on sexual function and fertility were reported in the F1 generation.

In the F2 generation, birth weight was reduced in the group treated with 0.4 mg/kg bw/day and above. No other toxicological changes were reported in the F2 generation, including gestation length and implantation sites. A NOAEL of 0.1 mg/kg/bw/day was identified, as birth weight of F2 pups was decreased at 0.4 mg/kg bw/day and above.

The changes reported in the F1 generation were not repeated in the F2, suggesting they may be chance findings, and not related to PFOS treatment.

In a very limited study, in male mice were treated with 0, 1, 5 and 10 mg/kg bw/day of PFOS for 7, 14 or 21 days. Serum testosterone levels and epididymal sperm counts were decreased at 10 mg/kg bw/day. No further information was included on these changes.

Overall, there is no consistent evidence for adverse effects on reproductive performance in rats at doses of up to 3.2 mg/kg bw/day, the highest dose tested. The information from the mouse study is insufficient to form any reliable conclusions.

There is insufficient information available from the two studies to conclude on any similarities which may exist between F-53B and PFOS in terms of adverse effects on sexual function and fertility. Therefore, it is currently not possible to use the sexual function and fertility data for PFOS to inform on the potential adverse effects of F-53B.

PFOS is not classified for adverse effects on sexual function or fertility.

8.5.4.2. *Adverse effects on or via lactation*

There are no studies available to inform on the potential for F-53B to cause adverse effects on or via lactation in experimental animals.

There is limited information from a rat 2-generation study (EFSA 2008; Christian *et al*., 1999) which found elevated perinatal mortality and reduced pup weight at maternal PFOS doses of 1.6 mg/kg bw/day and above in the F1 generation only. However, there is insufficient information to determine whether the increased perinatal mortality and pup weight represent an effect on or via lactation or is a developmental effect.

It is noted that F-53B was detected in human breast milk samples at levels well below 1 ng/mg. The study did not inform on the potential of F-53B to cause adverse effects on or via lactation in humans.

PFOS has a harmonised classification in Annex VI of the GB MCL for effects on or via lactation (H362).

8.5.4.3 *Developmental toxicity*

There are no *in vivo* studies available to inform on the potential of F-53B to adversely affect development.

Some *in vitro* studies purporting to inform on the developmental toxicity potential of F-53B are available. These investigated the ability of F-53B to perturb hESC (human embryonic stem cell differentiation), mESC (mouse embryonic stem cell differentiation) and hBMSC (human mesenchymal stem cell) differentiation. For the studies conducted with hESC and hMBSC, comparison experiments were conducted with PFOS.

These studies indicate that:

- F-53B and PFOS perturbed *in vitro* differentiation of hESC to cardiomyocytes, mediated at least in part via perturbation of WNT signalling.
- F-53B can impair the multipotency of hBMSC *in vitro* and may perturb bone marrow homeostasis. Compared to PFOS, F-53B may be at least as potent, and maybe more so.
- F-53B can modulate mESC differentiation.

The *in vitro* differentiation studies conducted with hESC and hBMSC provide some extremely limited information that F-53B and PFOS may share some common features.

However, there is insufficient information available to conclude on the human health relevance of these *in vitro* findings.

For PFOS, developmental toxicity has been well investigated in standard and non-standard studies conducted in rats, rabbits and mice. These studies have been thoroughly reviewed by EFSA (EFSA, 2008; EFSA CONTAM Panel, 2018, EFSA CONTAM Panel 2020).

Developmental studies on PFOS show effects in offspring at doses similar to, or below, those showing maternal toxicity (EFSA, 2008; EFSA CONTAM Panel, 2018). Among effects observed in rats and/or mice are high mortality early after birth, reduced fetal weight, reduced postnatal growth, increased liver weight, anasarca, impaired immune effects, cardiac abnormalities, cleft palate, delayed ossification of bones and a decrease in placental weight and capacity, with some evidence of developmental neurotoxicity in rodents. Increase in liver weight (NOAEL 0.3 mg/kg bw/ day) effects on placental physiology (LOAEL 0.5 mg/kg bw/day, NOAEL not determined) and aspects of glucose homoeostasis (LOAEL 0.3 mg/kg bw/day, NOAEL not determined) were the most sensitive endpoints (EFSA CONTAM Panel, 2018).

PFOS has a harmonised classification in Annex VI of the GB MCL for developmental toxicity (Repr 1B; H360D).

There are no *in vivo* developmental toxicity studies available conducted with F-53B. It is therefore not currently possible to directly compare the *in vivo* developmental toxicity of F-53B with PFOS.

The *in vitro* studies conducted with hESC and hBMSC provide some extremely limited information that F-53B and PFOS may share some common features in relation to perturbation of differentiation in non-standard *in vitro* systems, specifically cardiac and bone differentiation.

Abnormalities of bone and cardiac development were reported in at least one of the *in vivo* developmental toxicity studies conducted with PFOS. However, there is insufficient information to inform on any direct link between these *in vitro* findings with PFOS and cardiac and bone development *in vivo*.

It is, therefore, currently not possible to use these limited *in vitro* data to make any reliable predictions about the developmental toxicity of F-53B in experimental animals or humans.

8.6 Human biomonitoring

Seventeen studies were identified that reported measured concentrations of F-53B in human samples collected in China (fifteen studies) or North America. A variety of sample types were included in the studies (blood serum, umbilical cord serum, urine, breast milk, semen, hair and nails). Nearly all of these studies also reported concentrations of a variety of other PFAS. For the purposes of this assessment, reported concentrations of F-53B

have been collated together with those of 8:2 Cl-PFESA and PFOS when they have been measured in the same samples.

Table 8.4 Measured concentrations in human samples

Sample type	Concentration, ng/mL (unless stated otherwise)			Reference
	F-53B	8:2 Cl-PFESA	PFOS	
Serum				
Median concentration in 8 serum samples, control group	4.78	0.083	not stated	Shi *et al.* (2016)
Median concentration in 45 serum samples, high fish consumers group	93.7	1.64	not stated	Shi *et al.* (2016)
Median concentration in 19 serum samples, metal plating workers group	51.5	1.62	40	Shi *et al.* (2016)
Mean (minimum to maximum) serum concentration in 15 samples, ordinary people	0.22 (<LOD-0.73)	<LOD (<LOD-<LOD)	13.9 (0.68-45.97)	Gao *et al.* (2018)
Mean (minimum to maximum) serum concentration in 15 samples, occupational workers	0.42 (<LOD-1.39)	not calculated (<LOD-0.31)	1 064 (13.72-10 449)	Gao *et al.* (2018)
Median concentration in 8 serum samples, high exposure group	102.3	1.38	6 860	Wang *et al.* (2018)
Mean serum concentration without HBsAb data in 671 samples	2.21	0.04	13.7	Zeng *et al.* (2020)
Mean serum concentration with HBsAb data in 605 samples	2.05	0.03	12.8	Zeng *et al.* (2020)
Mean serum concentration HBsAb positive in 509 samples	1.75	0.01	10.1	Zeng *et al.* (2020)
Mean serum concentration HBsAb negative in 96 samples	2.38	0.02	14.1	Zeng *et al.* (2020)
Median measured serum concentrations in 252 samples	8.64	0.06	14.24	Duan *et al.* (2020)
Mean measured serum concentrations in 85 samples	6.0	0.17	5.9	Jin *et al.* (2020a)
Median cord serum concentration in 98 samples	0.731	0.021	4.07	Xu *et al.* (2019)
Median maternal serum concentration in 372 samples	2.405	0.001	7.153	Chu *et al.* (2020)
Median concentration in 50 serum samples	<LOD	not analysed for	1.7	Kato *et al.* (2018)
Median concentration in 664 serum samples	6.088	0.081	8.378	Pan *et al.* (2019a)

Sample type	Concentration, ng/mL (unless stated otherwise)			Reference
	F-53B	8:2 Cl-PFESA	PFOS	
Paired maternal and umbilical cord serum				
Median maternal serum concentration trimester 1 in 100 samples	2.22	0.05	14.23	Pan *et al.* (2017)
Median maternal serum concentration trimester 2 in 100 samples	1.93	0.05	13.20	Pan *et al.* (2017)
Median maternal serum concentration trimester 3 in 100 samples	1.89	0.05	12.32	Pan *et al.* (2017)
Median cord serum concentration in 100 samples	0.8	0.03	4.38	Pan *et al.* (2017)
Median maternal serum concentration in 32 samples	1.54	0.01	7.01	Chen *et al.* (2017a)
Median cord serum concentration in 32 samples	0.6	0.01	3.64	Chen *et al.* (2017a)
Median maternal serum concentration in 132 samples	0.094	<LOD	4.07	Gao *et al.* (2019b)
Median cord serum concentration in 132 samples	0.091	<LOD	1.8	Gao *et al.* (2019b)
Median maternal serum concentration in 424 samples	0.63	0.009	4.32	Cai *et al.* (2020)
Median cord serum concentration in 424 samples	0.32	0.008	1.93	Cai *et al.* (2020)
Semen				
Median concentration in 664 semen samples	0.064	<LOD	0.097	Pan *et al.* (2019a)
Urine				
Median concentration in 70 urine samples, all groups combined	0.005	<LOD	1.56	Shi *et al.* (2016)
Median concentration in 8 urine samples, high exposure group	0.01	<LOD	4.64	Wang *et al.* (2018)
Median concentration in 41 urine samples, low exposure group	1.98	<LOD	3.47	Wang *et al.* (2018)
Median concentration in 50 urine samples	<LOD	not analysed for	<LOD	Kato *et al.* (2018)
2682 urine samples	<LOD	not analysed for	0.1-0.6	Calafat *et al.* (2019)
Nails				

Sample type	Concentration, ng/mL (unless stated otherwise) F-53B	8:2 Cl-PFESA	PFOS	Reference
Median concentration in 8 nail samples, high exposure group	2.82 ng/g	<LOD	188.04 ng/g	Wang *et al*. (2018)
Median concentration in 41 nail samples, low exposure group	1.02 ng/g	0.46 ng/g	0.86 ng/g	Wang *et al*. (2018)
Hair				
Median concentration in 41 hair samples, low exposure group	0.53 ng/g	0.35 ng/g	0.33 ng/g	Wang *et al*. (2018)
Placenta				
Median placenta concentration in 32 samples	0.34 ng/g ww	<LOD	0.35 ng/g ww	Chen *et al*. (2017a)
Breast milk				
Median concentration in breast milk in 174 samples	0.016	<LOD	0.001	Jin *et al*. (2020b)

- Shi *et al*. (2016) analysed samples taken from three groups of participants. The first two groups were expected to have exposure (19 metal plating workers and 45 consumers of large amounts of freshwater fish who worked at a fishery) whilst the third was a control group with lower exposure (8 participants). Blood and urine samples were analysed by HPLC-ESI-MS/MS. In serum, F-53B was detected in 100% of samples and 8:2 Cl-PFESA in 98% of samples. In urine, F-53B was detected in 74% of samples and 8:2 Cl-PFESA in 3% of samples. PFOS was analysed for in the metal plating worker samples, but details on detection rates are not given. The PFOS measurements in the fishery workers and control groups had been previously determined in a larger number of samples (Zhou, Shi, Vestergren, Wang, Liang and Cai, 2014) and a subset was used here, again details were not given. Measured concentrations are shown in Table 8.4. The concentration in blood was significantly correlated to the concentration in urine for F-53B and PFOS. Workers at the metal plating site with over 1 year of employment were found to have significantly higher serum concentrations than those workers of less than 1 year's employment which the authors suggest could indicate that F-53B accumulates over time.

  Shi *et al*. (2016) used the measured concentrations in serum and urine to calculate the renal clearance rate of F-53B, 8:2 Cl-PFESA and PFOS for all sample groups combined. The median renal clearance rates are 0.0016 mL/kg/day F-53B, 0.0006 mL/kg/day 8:2 Cl-PFESA and 0.0074 mL/kg/day PFOS. The authors note that the clearance rate for 8:2 Cl-PFESA is only based on two samples so is uncertain. F-53B had a statistically significant slower renal elimination rate than PFOS. The authors also converted these renal clearance rates to median half-lives of 280 years for F-53B and 81.9 years for PFOS. In order to calculate these half-lives Shi *et al*. (2016) assumed that the partitioning behaviour of F-53B between blood and tissue in humans was the same as that of PFOS.

Shi *et al*. (2016) also estimated the total elimination half-lives for all routes of excretion of F-53B and PFOS, as intake could be estimated based on the rate of fish consumption and measured concentrations in fish muscle samples and the fact that serum concentrations appeared to be at steady state for fishery employees with over 6 years' employment. The median total elimination half-lives were calculated as 15.3 years for F-53B and 6.7 years for PFOS. The authors note that as these half-lives are considerably lower than those they calculated based on renal elimination this suggests that other clearance routes are important for F-53B.

- Gao *et al*. (2018) published a novel integrated analytical method using SPE-UHPLC-MS/MS that could determine the concentration of a wide number of PFAS in human serum, including F-53B, 8:2 Cl-PFESA and PFOS. After method development, they tested the analytical technique on 15 human serum samples from 'ordinary' people and 15 samples from occupational workers at a fluorochemical manufacturing site in Wuhan, China collected in 2017 (Table 8.4). Higher concentrations of all three compounds were detected in the serum from occupationally exposed workers, but F-53B and PFOS were also detected in the general population samples.

- Wang *et al*. (2018) investigated whether non-invasive sampling methods could be used to estimate the internal exposure of humans by comparing hair, nail, urine and blood samples. Two groups of participants were involved in the study; a group of 8 individuals from Wuhan, China who had high exposure to PFAS and 41 individuals from Shijiazhuang, China who had relatively little PFAS exposure. Samples were analysed by HPLC-MS/MS.

  In the highly exposed group, F-53B was detected in 100, 100 and 62.5 % of nail, serum and urine samples. PFOS was detected in 100% of all samples, and 8:2 Cl-PFESA was found in 100% serum samples but not in nail or urine. Paired serum-nail concentrations were found to be significantly correlated for both F-53B and PFOS. When a larger dataset of 55 samples collected concurrently by Zhou *et al*. (2014) was analysed the paired serum-urine concentrations were also significantly correlated for both F-53B and PFOS. In the low exposure group, F 53B was detected in 95, 88 and 95 % of nail, hair and urine samples. PFOS was detected in 98, 83 and 93% of nail, hair and urine samples, and 8:2 Cl-PFESA was found in 56, 61 and 2% of nail, hair and urine samples. Paired urine-hair and urine-nail concentrations were found to be significantly correlated for both F-53B and PFOS. Median measured concentrations are shown in Table 8.4.

- Gao *et al*. (2019a) published a novel analytical method using SPE-HPLC-MS/MS that could determine the free and bound concentration of a number of compounds in human serum. Blood samples were collected from 30 'ordinary' people from Wuhan, China in 2017. 8:2 Cl-PFESA was not analysed for in this study. Free F-53B was detected in 60% of samples and free PFOS in 67%. When the total and free concentrations were compared, Gao *et al*. (2019a) found that 85% of F-53B was bound to protein and 93% of PFOS was bound. The authors also calculated

partition coefficients to represent the binding affinity of each compound to human serum albumin (HSA) protein. The Kd_{HSA} for F-53B was 94 µmol/L and was 35 µmol/L for PFOS. The authors state that higher Kd values indicate lower binding affinities to proteins in serum, which may result in a higher concentration of free PFAS. These results would therefore suggest that F-53B may be more likely to be in the free form than PFOS in human blood.

- Zeng *et al*. (2020) measured concentrations of F-53B in human serum using UPLC-MS, together with other legacy and novel PFAS including 8:2 Cl-PFESA and PFOS. The measured concentrations were also compared to whether the volunteer had had their hepatitis B surface antibody (HBsAb) measured in the study, and if so whether they were positive or negative for this. The study participants were all members of the C8 Health Project in China, had a mean age of 58.9 years and 82.5% participants had occupations unrelated to PFAS exposure. Samples were taken from 2015 to 2016.

  Detection rates for the PFAS were high, with 100% of samples containing F-53B and PFOS and 67.1% of samples containing 8:2 Cl-PFESA. Of the samples, 671 did not have HBsAb data and 605 did. The mean measured serum concentrations are shown in Table 8.4 and were not found to be statistically different between the two groups.

  Of the samples with HBsAb data, 509 were seropositive and 96 were seronegative. When the data were grouped by this variable, significant differences in mean measured concentrations were found for all three compounds (Table 8.4). In addition, linear regression analysis was used to demonstrate that increasing concentrations of all three compounds were associated with decreasing HBsAb, with a stronger relationship for F-53B than PFOS**.** Zeng *et al*. (2020) conclude that their study demonstrates that higher serum concentrations of PFAS, including F-53B, are associated with lower hepatitis B virus antibodies and that this fits with previous studies showing that PFAS exposure can reduce the immune response.

- Duan *et al*. (2020) measured concentrations of F-53B in 252 serum samples using SPE-HPLC-MS, together with other legacy and novel PFAS including 8:2 Cl-PFESA and PFOS. The measured concentrations were also compared to two glycemic biomarkers (fasting glucose concentrations and glycated haemoglobinHbA1c). The age range of the participants was 19 to 87 years old, and all were staff or support workers at Nankai University, Tianjin, China who did not have diabetes or prediabetes. Samples were collected in June 2017.

  F-53B and PFOS were detected in 100% and 99.6% of samples respectively, whilst 8:2 Cl-PFESA was detected in 69.8%. The median measured serum concentrations are shown in Table 8.4. Duan *et al*. (2020) report that the concentrations of F-53B and PFOS were significantly positively correlated with age, and that the concentrations of all three compounds were significantly positively correlated with body mass index (BMI). Neither F-53B nor PFOS were found to be significantly related to the two glycemic biomarkers.

- Jin *et al*. (2020a) recruited 85 healthy people from Anji, Zhejiang, China to measure the concentrations of several PFAS in their blood. Participants ranged from 18 to 70 years' old and were generally healthy with no known occupational exposure to PFAS. The concentrations of PFAS were measured using UPLC-MS. F-53B was detected in 88% of samples, PFOS in 100% and 8:2 Cl-PFESA in 73%. The mean measured concentrations are shown in Table 8.4, with F-53B having a mean concentration above that of PFOS. No significant difference was found between F-53B concentrations in males and females, but in males the concentrations of F-53B were found to significantly increase with age. The authors speculate that this could be due to bioaccumulation of F-53B over time, with the potential for greater loss from females due to menstruation, pregnancy and lactation.
- Xu *et al*. (2019) measured the concentrations of PFAS in umbilical cord serum and compared this to birth outcomes. Participants were recruited from Hangzhou, China and 98 paired samples were collected from 2016 to 2017. All mothers were generally healthy, and all gave birth to single children. Birth weight, length, head circumference and gestational age were used as the indicators of birth outcomes. Cord samples were analysed by UPLC-MS.

  F-53B was found to be statistically significantly associated with older mothers, primiparous mothers (first time mothers) and overweight mothers. However, no significant relationships were identified between F-53B concentration and birth outcomes. PFOS was also statistically significantly related to primiparous and overweight mothers, and was negatively associated with birth weight and ponderal index (the ratio of birth weight to length). The authors suggest that the higher concentrations measured in older mothers could be due to higher accumulation over time, and that the lower concentrations in mothers who have older children could be due to elimination via placental transfer or breastfeeding.

- Chu *et al*. (2020) measured concentrations of F-53B in 372 maternal serum samples using UPLC-MS, together with other legacy and novel PFAS including 8:2 Cl-PFESA and PFOS. The measured concentrations were also compared to the birth outcomes, birth weight and gestational age. The mothers had a mean age of 27, were predominantly blue-collar workers and the study participants were all members of the Guangzhou Birth Cohort study in China. Samples were collected in 2013.

  Detection rates for PFAS were high, with 100% of samples containing F-53B and PFOS and 40.9% of samples containing 8:2 Cl-PFESA. The median measured serum concentrations are shown in Table 8.4. Statistically significant decreases in birth weight were associated with the concentrations of the individual PFAS concentrations. F-53B and PFOS also had statistically significant decreases in gestational age with maternal serum concentration.

- Pan *et al*. (2017) investigated the transfer of PFAS from mother to child via placental transfer by using matched maternal and umbilical cord serum samples. One hundred paired samples were collected in 2014 and 2015 from participants

recruited in Wuhan, China. All mothers were aged over 18 and were having their first child. Three maternal blood samples were taken, during the first, second and third trimester of the pregnancy. Cord blood samples were collected after delivery. All samples were analysed by UPLC-MS. F-53B and PFOS were detected in 100% of samples, while 8:2 Cl-PFESA was detected in 100% of maternal samples and 99% of cord samples. The median measured concentrations are shown in Table 8.4.

Concentrations of all PFAS were found to significantly decrease between the maternal trimester 1 and 2 samples. A further decline was observed by the third trimester, but this was only significant for 8:2 Cl-PFESA. The Spearman correlation coefficients between the maternal samples and the cord samples ranged from 0.83 to 0.93 for all time points, with higher correlation coefficients seen for the third trimester. It is not reported whether these coefficients were statistically significant. The authors suggest that this could have been due to blood volume expansion or increased renal clearance that occurs during pregnancy. The authors also calculated trans-placental transfer (TPT) as the ratio of the concentration in the cord samples to the concentration in the third trimester maternal samples. The geometric mean TPT for F-53B, 8:2 Cl-PFESA and PFOS were 0.41, 0.61 and 0.34 respectively.

The transfer efficiencies of PFAS were negatively associated with the level of maternal serum albumin and positively associated with cord serum albumin levels. This led Pan *et al*. (2017) to hypothesise that the maternal and child albumin were 'competing', with the maternal albumin preventing PFAS from crossing the placenta whilst child albumin facilitated transfer.

- Chen *et al*. (2017a) investigated the transfer of PFAS from mother to child via placental transfer by using matched maternal and umbilical cord serum samples and placenta samples. Thirty two participants were recruited from Wuhan, China and samples were collected from 2015 to 2016 and analysed by UPLC-MS. F-53B and PFOS were detected in 100% of samples, whilst 8:2 Cl-PFESA was detected in 84.4% maternal serum, 81.3% cord serum and 28.1% placenta samples. The median measured concentrations are shown in Table 8.4. The authors calculated the ratio of the concentration in the cord samples to the concentration in the maternal samples. Although the authors term this the R_{CM}, it is the same as the TPT defined above, so the Environment Agency will use TPT for consistency. The median TPT for F-53B, 8:2 Cl-PFESA and PFOS were 0.403, 0.557 and 0.399 respectively. The authors also calculated the ratio of the concentration in the placenta samples to the concentration in the maternal samples (adjusted for density to make the ratio dimensionless) and defined this as R_{PM}. The median R_{PM} for F-53B and PFOS were 0.201 and 0.045 respectively. An R_{PM} was not calculated for 8:2 Cl-PFESA because it was not frequently detected in placenta.
- Gao *et al*. (2019b) investigated the transfer of PFAS from mother to child via placental transfer by using matched maternal and umbilical cord serum samples. In total, 132 matched samples were collected in 2015 and 2016 and analysed by

HPLC-MS/MS. All participants were recruited from the Affiliated Hospital of Capital Medical University, Beijing, China. F-53B and PFOS were detected in 80 and 100% of maternal serum samples and 68 and 100% of cord samples respectively. 8:2 Cl-PFESA was not detected in any sample. F-53B was found to be significantly positively related to birth length, but no other significant relationships between F-53B or PFOS and birth outcomes were found. The mean TPT for PFOS was 0.44. The mean TPT for F-53B is not stated, but is approximately 0.75 based on Figure 3 in Gao *et al*. (2019b).

Human serum albumin (HSA), serum protein (SP) and liver-fatty acid binding protein (L-FABP) partition coefficients were also derived for each PFAS using an equilibrium dialysis method. The authors state that higher Kd values indicate lower binding affinities to proteins in serum, which may result in a higher concentration of free PFAS that can pass across the placenta more easily. The partition coefficients for F-53B were KdHSA 67 μM, KdSP 82 μM and KdL-FABP 102 μM. The partition coefficients for PFOS were KdHSA 38 μM, KdSP 49 μM and KdL-FABP 81 μM. These results would therefore suggest that F-53B may be more likely to transfer across the placenta than PFOS.

- Cai *et al*. (2020) investigated the transfer of PFAS from mother to child via placental transfer by using matched maternal and umbilical cord serum samples. In total, 424 matched samples were collected in 2015 to 2018 and analysed by UPLC-MS/MS. All the participants were part of the Maoming Birth Cohort, China, were aged 20 to 45 and in good health, and all gave birth to single children at full term. F-53B was detected in 99.8% of maternal serum samples and 99.5% of cord samples. 8:2 Cl-PFESA was detected in 85.1% of maternal samples and 70.8% of cord samples and PFOS was detected in 99.8% of maternal serum samples and 100% of cord samples. The median measured concentrations are shown in Table 8.4 and the authors note that the concentrations in maternal serum were higher than those detected in cord serum and that for all PFAS except 8:2 Cl-PFESA the cord and maternal concentrations were significantly positively correlated. The authors also calculated TPT as the ratio of the concentration in the cord samples to the concentration in the maternal samples. The median TPT for F-53B, 8:2 Cl-PFESA and PFOS were 0.46, 0.98 and 0.42 respectively.

- Jin *et al.* (2020b) investigated the concentrations of PFAS in breast milk and compared this to paired infant postnatal growth data. All infants in the study were exclusively breast fed. Breast milk was collected within one week of delivery and the children were approximately 5 months old when their growth was assessed. The 174 participants were from a longitudinal birth cohort in Hangzhou, China and samples were collected from 2018 to 2019 and analysed by HPLC-MS/MS. F-53B was detected in 100% of breast milk samples, PFOS in 50% of samples and 8:2 Cl-PFESA in 20% samples. Of the growth measurements, only infant length gain was found to be significantly negatively correlated with F-53B concentration. The authors also estimated the daily intake of each of the PFAS detected. F-53B was estimated to have a daily intake of 8.1 ng/kg/day and PFOS 9.9 ng/kg/day.

- Pan *et al*. (2019a) analysed 664 paired serum-semen samples collected in 2015 to 2016 from adult men in Nanjing, China. Participants were generally healthy, and were excluded from the study if they had occupational exposure to PFAS. Blood and semen samples were collected on the same day and analysed by UPLC-MS. Semen samples were also analysed for sperm count, morphology and motility. F-53B was detected in 100% of blood and semen samples, 8:2 Cl-PFESA in 96.5% of blood samples and 30.6% of semen samples and PFOS in 100% of blood and 96.1% semen samples. Median measured concentrations are shown in Table 8.4. Concentrations in serum were significantly correlated with semen concentrations for all three compounds. F-53B and PFOS were also found to be significantly associated with reduced progressive sperm (a measure of sperm motility) and an increase in sperm DNA fragmentation.

- Kato *et al*. (2018) analysed 50 paired serum-urine samples collected in 2016 and supplied commercially. No information was available on the demographic of the samples, but they are stated to be from American volunteers. Samples were analysed by SPE-HPLC-MS/MS. F-53B was not detected in any of the serum or urine samples (LOD 0.1 ng/mL). PFOS was detected in 98% of the serum samples, but none of the urine samples. 8:2 Cl-PFESA was not analysed in this study.

- Calafat *et al*. (2019) analysed 2 682 spot urine samples collected in 2013-2014 from participants in the NHANES study in America who were 6 years of age or older. The samples were analysed using HPLC-MS. F-53B was not detected in any of the samples (LOD 0.1 ng/mL) and PFOS was detected in <0.1% of samples. 8:2 Cl-PFESA was not analysed for in this study.

None of the studies summarised here have attempted to estimate the bioaccumulation rate of F-53B in humans. However, these studies do demonstrate that:

- F-53B is widely detected in human biomonitoring samples from both high exposure groups (e.g. occupational workers) and expected low exposure groups in China.

- The two studies that took samples from American participants (Kato *et al*., 2018; Calafat *et al*., 2019) between 2013 and 2016 did not detect F-53B in any samples. This may reflect lower usage and therefore exposure compared to China.

- F-53B has been detected in human blood serum, umbilical cord serum, urine, breast milk, semen, hair and nails.

- Three studies (Duan *et al*., 2020; Jin *et al*., 2020a; Xu *et al*., 2019) noted a significant positive relationship between F-53B concentrations and the age of the volunteer. The authors suggest that the higher concentrations measured in older participants could be due to higher accumulation over time.

- F-53B was always detected at higher concentrations than 8:2 Cl-PFESA in the same samples, with many of the authors noting that this is to be expected as 8:2 Cl-PFESA is mainly present as an impurity in F-53B.

- F-53B was generally present at lower concentrations than PFOS in the same samples, although some studies did find similar or higher concentrations of F-53B.

- Two studies (Gao *et al*., 2019a and 2019b) calculated partition coefficients to represent the binding affinity of each compound to proteins in the blood. The authors state that higher Kd values indicate lower binding affinities to proteins in serum, which may result in a higher concentration of free PFAS. The Kd values are summarised in Table 8.5. The results from both studies are consistent and suggest that F-53B may be more likely to be in the free form than PFOS in human blood. However, this is the opposite result reported to that in Allendorf *et al*. (2019) which found that F-53B was more likely than PFOS to be bound to bovine serum albumin (Section 8.1).

Table 8.5 Kd values in human blood (Gao *et al*., 2019a)

Partition coefficient	F-53B	PFOS
Kd_{HSA}	94 µM	35 µM
Kd_{HSA}	67 µM	38 µM
Kd_{SP}	82 µM	49 µM
$Kd_{L\text{-}FABP}$	102 µM	81 µM

Several studies investigated paired maternal blood serum and umbilical cord serum concentrations to determine whether PFAS were transferred from mother to child during pregnancy. Four studies reported TPT values and are summarised in Table 8.6. The TPT values are consistent between the studies and indicate that 8:2 Cl-PFESA is transferred to the cord blood to the greatest extent, followed by F-53B then PFOS.

Table 8.6 Trans-placental transfer values

F-53B	8:2 Cl-PFESA	PFOS	Reference
0.41	0.61	0.34	Pan *et al*. (2017)
0.403	0.557	0.399	Chen *et al*. (2017a)
0.75	Not calculated	0.44	Gao *et al*. (2019b)
0.46	0.98	0.42	Cai *et al*. 2020

- Shi *et al*. (2016) was the only study that attempted to use measured concentrations in serum and urine and link these to estimated exposure to generate elimination half-lives. F-53B was found to have a statistically significant slower renal elimination rate than PFOS. The authors also converted these renal clearance rates to median half-lives of 280 years for F-53B and 81.9 years for PFOS based on the assumption that F-53B partitioned between blood and tissue in the same way as PFOS, which the Environment Agency considers to be an acceptable assumption based on the experimental and observational studies summarised in Section 8.1 and 8.6. Based on the estimated dietary intake of F-35B and PFOS from fish, the median total elimination half-lives were calculated as 15.3 years for F-53B and 6.7 years for

PFOS. The author's note that as these half-lives are considerably lower than those they calculated based on renal elimination this suggests that other clearance routes are important for F-53B. The Environment Agency also notes that these total elimination half-lives are based on a number of input variables that are uncertain: dietary intake is estimated from fish sampled at a single time point and estimates of fish consumption based on participant questionnaires, steady state is assumed to have been reached and other potential exposure routes are not considered.

The observational data suggest that F-53B can be taken up and distributed within humans to different parts of the body and can be present in a wide range of tissues. It can also be transferred across the placenta (to a greater extent than PFOS) and has been detected in breast milk, indicating that infants can be exposed before and immediately after birth. A single study on human clearance time (Shi *et al*. 2016) reports half-lives longer than those for PFOS, which suggests that F-53B is potentially more bioaccumulative than PFOS.

9 Environmental hazard assessment

9.1 Classification and labelling

9.1.1 Harmonised classification

There is no current harmonised entry in Annex VI of the Classification, Labelling and Packaging (CLP) Regulation (EC) No 1272/2008, nor a mandatory classification under UK CLP.

9.1.2 Self-classification

No self-classifications have been made to the ECHA Classification and Labelling (C&L) Inventory (https://echa.europa.eu/information-on-chemicals/cl-inventory-database [accessed 29/9/20]).

9.1.3 Conclusions for classification and labelling

F-53B is not readily biodegradable and there is no evidence that it degrades significantly via abiotic or biotic mechanisms (see Section 6.1). It is therefore considered to be “not rapidly degradable” for the purposes of hazard classification.

The substance has a reported fish BCF above 500 L/kg from non-standard studies using fish larvae (see Section 6.3.1). There is evidence that the substance is rapidly taken up by adult fish, with slower depuration. It is therefore considered potentially bioaccumulative for classification purposes.

Acute ecotoxicity endpoints are available for fish and algae (see Section 7.1). The key study for the purposes of classification is an acute fish study by Wang *et al*. (2013), which reported a 96-hour LC_{50} of 15.5 mg/L. Since this is higher than 1 mg/L, **Aquatic Acute classification is not required**. However, it should be noted that there are no aquatic invertebrate data, so this classification should be considered provisional.

Chronic aquatic toxicity data are available for fish and algae. No data are available for aquatic invertebrates. The key study for the purposes of classification is the chronic fish study by Shi *et al*. (2018), which reported a 177-d NOEC of <0.005 mg/L for effects on fecundity, embryo malformation and survival. Since this is below 0.1 mg/L, this results in a classification of **Aquatic Chronic 1**. As the NOEC is unbounded it is not possible to determine the Chronic M-factor. However, based on the NOEC of <0.005 mg/L, the lack of rapid degradation and bioaccumulation potential, the Chronic M-factor would be a minimum of 10.

There is currently insufficient information on mammalian endpoints to conclude on any human health classification of F-53B.

9.2 Assessment of endocrine disrupting (ED) properties

The studies reporting endpoints relevant to the assessment of ED are summarised in Section 7.5. Together, these studies indicate that F-53B is a potential thyroid disruptor. Two studies (Xin *et al*., 2018; Deng *et al*., 2018) used *in silico* models to demonstrate that F-53B is able fit into the binding pockets of TTR and TRα and TRβ. These same two papers also used a variety of non-standard *in vitro* assays to investigate whether F-53B is able to bind to and interact with thyroid receptors and thyroid transport proteins. Xin *et al*. (2018) used three different assays to demonstrate that F-53B binds to TTR, TRα and TRβ, that it increases activity of TRα and TRβ in a human cell line and that cell proliferation was increased in a rat cell line, an endpoint which has previously been shown to be regulated by thyroid receptors. Deng *et al*. (2018) used the same rat cell line and also found cell proliferation increased in a dose-dependent manner, indicating agonistic activity.

Two *in vivo* studies using Zebrafish embryos have also reported effects on thyroid-related endpoints, with T4 and TTR concentrations significantly increased, and body weight and TG significantly decreased after 5 days' exposure (Deng *et al*., 2018) and T4 concentrations significantly increased and T3 significantly decreased (females only) after 180 days' exposure (Shi *et al*. 2019b). Trans-generational effects on thyroid have also been reported by Shi *et al*. (2019b), who link the changes in thyroid related endpoints to the increased mortality and malformation rate (uninflated swim bladders) observed in the F1 generation. The lowest concentration of F-53B at which possible endocrine related effects have been reported is 0.00023 mg/L (Deng *et al*., 2018), but this was also the lowest concentration that they tested.

A single 28-day repeated dose study in rats reported significant reductions in T3 and T4, although effects on apical endpoints were not observed.

Table 9.1 F-53B thyroid related effects

OECD GD 150 conceptual framework level	Available data
1	Two *in silico* studies (Xin *et al*. 2018, Deng *et al*. 2018) F-53B fits into the binding pockets of transthyretin and the two nuclear receptors TRα and TRβ
2	Two *in vitro* studies (Xin *et al*. 2018, Deng *et al*. 2018) F-53B competitively binds to transthyretin, TRα and TRβ. F-53B increased activity in human HEK293 cells with thyroid receptor mediated luciferase activity. F-53B increased cell proliferation in rat pituitary GH3 cell line.
3	One *in vivo* study in fish (Deng *et al*. 2018)

OECD GD 150 conceptual framework level	Available data
	F-53B exposure for 5 days resulted in changes in transthyretin, T4 and thyroid related gene expression.
4	One *in vivo* study in fish (Shi *et al*. 2019b) F-53B exposure for 180 days resulted in changes in T4 and T3. Trans-generational effects on thyroid have also been reported and authors link the changes in thyroid related endpoints to the increased mortality and malformation rate observed in the F1 generation. One *in vivo* study in rats (Hong *et al*., 2020) Repeated F-53B exposure for 28-days resulted in changes to T3 and T4 concentrations, and thyroid follicular cell hyperplasia.
5	No data

Although these studies are not conducted to standard guidelines, and in some cases only minimal descriptions are given for each assay, the Environment Agency considers that they are sufficiently well designed and described to conclude that F-53B has the potential to be a thyroid disrupting compound. The available data are presented against the OECD Guidance Document 150 'conceptual framework' (OECD, 2018b) in Table 9.1. Data indicating the potential to disrupt the thyroid pathway are available for studies that would sit within OECD GD 150 Levels 1, 2, 3 and 4. It must be emphasised that, at the time of writing, there are no validated OECD test guidelines to investigate thyroid disruption in fish and to allow mechanistic endpoints to be linked to apical effects in order to conclude that a compound is an endocrine disruptor with a thyroid disrupting mode of action. In order to reach a definite conclusion on whether F-53B can be considered to be an endocrine disruptor it would be necessary to conduct a study that could link endpoints indicating a thyroid disrupting mode of action to adverse effects, for example an OECD TG 241 Larval Amphibian Growth and Development Assay (LAGDA).

There is also evidence to suggest that F-53B can activate the PPAR signalling pathway (Shi *et al*., 2019a; Li *et al*.,2018). The single study investigating some endpoints related to estrogenic and androgenic activity in fish (Shi *et al*., 2018) did not report any conclusive findings. Gavage administration of F-53B for 56 days to male mice had no adverse on the reproductive performance, reproductive hormones and reproductive organs and tissues investigated, at doses of up to 1.0 mg/kg bw/day, the highest dose tested (Zhou *et al.,* 2018).

9.3 PBT and vPvB assessment

Persistence: No environmental half-life data are available for comparison with the definitive criteria in REACH Annex 13. F-53B is not readily biodegradable and there is no evidence that it degrades significantly via abiotic mechanisms (see Section 6.1). It therefore meets the screening criterion in REACH Annex 13 for being potentially persistent

(P) or very persistent (vP). Despite the level of biodegradation in the available screening test for F-53B, the strength of the carbon-fluorine chemical bond, and laboratory and field data for similar PFAS such as PFOS suggests that the substance is likely to be persistent. Although there is an absence of measured data to establish a reliable half-life, it is precautionary to conclude that the substance likely meets the vP criterion in one or more environmental compartments, based on evidence from other PFAS.

Bioaccumulation: A standard test guideline bioconcentration study is not available, so there is no unequivocal evidence that F-53B meets the definitive criteria in REACH Annex 13.

However, a number of lines of evidence indicate that it has the potential for significant bioaccumulation in aquatic gill-breathing organisms such as fish (see Section 6.3). A BCF above 2 000 L/kg (non-lipid normalised BCF_k of 3 615) has been reported from a non-standard study using Zebrafish larvae (see Section 6.3.1). A number of studies have reported rapid uptake and slower depuration in adult Zebrafish, with significant accumulation in specific tissues, including liver and ovaries. There is also maternal transfer to eggs, which spans at least 2 generations. There is some evidence that protein binding may be relevant for F-53B.

Several observational studies have reported that F-53B is detected in a wide range of aquatic organisms and water samples collected from different areas of China, with TMF's above 1 reported in 2 studies (range: 3.43 to 4.32). Monitoring studies also indicate accumulation in organisms at the top of the food chain, including at locations where use is likely to be limited (such as otters in the UK). Where studies have included both F-53B and PFOS, the available information suggests that F-53B is at least as bioaccumulative as PFOS.

Based on this evidence, the Environment Agency considers that the substance likely meets at least the bioaccumulative (B) criterion for aquatic organisms in REACH Annex 13.

Evidence from other highly fluorinated substances, such as PFOS (UNEP, 2006), suggests that terrestrial bioaccumulation may be relevant for this type of substance. There are experimental and observational data to suggest that F-53B can be taken up by plants, monkeys and locusts. Bioaccumulation in air-breathing organisms is also suggested by screening data (see Section 6.3.2). The available human biomonitoring data indicate that F-53B is widely detected in both high exposure and low exposure groups in China, with positive detections in human blood, umbilical cord serum, urine, breast milk, semen, hair and nails. F-53B can be translocated within the human body and transferred to the next generation across the placenta or in breast milk. A single study Shi *et al*. (2016) calculates a renal elimination rate for F-53B that was found to be statistically significantly slower than the rate for PFOS calculated in the same study and a total elimination half-life that is higher than that of PFOS. A definitive conclusion on the bioaccumulation potential of F-53B in air-breathing organisms would require further data on the human clearance time via all clearance routes or better predictive methods, but the available information suggests that F-53B may be at least as bioaccumulative as PFOS.

Toxicity: Chronic ecotoxicity data are available for fish and algae (see Section 7.1). A 177-d NOEC of <0.005 mg/L for effects on fecundity, embryo malformation and survival has been reported from a non-standard (but sufficiently reliable) multi-generational study with Zebrafish. Since this is below 0.01 mg/L, F-53B meets the definitive toxic (T) criterion in REACH Annex 13.

There are also indications that the substance can affect thyroid function in aquatic organisms. However, there are no definitive data for mammals or birds to draw a conclusion for non-aquatic wildlife.

Overall conclusion: F-53B is likely to be vP and likely meets the B criterion (and possibly vB criterion) in aquatic organisms. It also meets the definitive criterion to be considered T based on evidence from aquatic organisms.

Based on comparative information, F-53B appears to be at least as bioaccumulative as PFOS and potentially more toxic.

9.4 Groundwater hazard

Draft persistence, mobility and toxicity (PMT) criteria have been developed by the German Federal Environment Agency as intrinsic hazard criteria to identify substances that are difficult to remove during normal wastewater treatment practices and may be a threat to remote aquatic environments and drinking water sources, including groundwater (Arp and Hale, 2019). The criteria for P and vP are consistent with those in REACH Annex 13, whereas the mobile criterion is unique to PMT assessments. The T criteria include those in REACH Annex 13, in addition to considerations for carcinogenicity, effects via lactation, long-term toxicity to the general human population and endocrine disruption potential

There is no legal basis for these criteria under the REACH Regulation, but for completeness, a brief evaluation is included here.

Persistence: F-53B is likely to be P or vP (see Section 9.3).

Mobility: Experimental log Kd values for six soils are reported by Wei *et al.* (2019) and these have been converted to log K_{OC} values of 3.65-3.98 by the Environment Agency. F-53B would therefore meet the draft criterion as being mobile (M) (log K_{OC} <4) but not the criterion for very mobile (vM) (log K_{OC} <3).

Toxicity: F-53B meets the definitive T criterion based on chronic fish ecotoxicity (see Section 9.3). The available data also indicate that F-53B may be an endocrine disruptor in wildlife. Human health effects have not been fully considered.

Overall conclusion: F-53B is likely to be vP and meets the proposed M and T criteria.

9.5 Limit values

9.5.1 Predicted No Effect Concentration (PNEC) derivation

A PNEC is an indication of an acceptable environmental concentration based on evidence from (eco) toxicity studies. Available hazard data are discussed in Section 7. PNECs have been derived by the Environment Agency following EU REACH guidance (ECHA, 2008) as applied in the EUSES model (ECHA, 2020).

For the derivation of the aquatic PNEC, F-53B has acute and chronic data available for fish and algae, but no data are available for invertebrates. Based on read across from the structural analogue PFOS, freshwater fish are expected to be the most sensitive taxa, whilst marine water invertebrates appear to be more sensitive. Therefore, the freshwater PNEC for F-53B has been derived using an assessment factor of 10 on the basis that fish are likely to be most sensitive and the missing invertebrate data would not alter the PNEC. The marine PNEC for F-53B has been derived using an assessment factor of 500 on the basis that the available data may not cover the most sensitive taxa.

Table 9.2 PNECs derived by the Environment Agency

Protection goal	Most sensitive toxicity descriptor	Assess-ment factor	PNEC	Justification/ remarks
Fresh surface water	Chronic fish toxicity NOEC <0.005 mg/L (fecundity, embryo malformation and survival)	10	<0.0005 mg/L	Chronic data available for fish and algae. No acute or chronic data available for invertebrates. Based on read across from PFOS, freshwater invertebrates are expected to be less sensitive than fish.
Freshwater sediment	-	-	<0.0775 mg/kg ww	No sediment data available. Calculated using Equilibrium Partitioning in EUSES (parameters in Table 10.2).
Sewage treatment micro-organisms	-	-	-	No data available.
Marine surface water	Chronic fish toxicity NOEC <0.005 mg/L (fecundity, embryo malformation and survival)	500	<0.00001 mg/L	Chronic data available for fish and algae. No acute or chronic data available for invertebrates. Based on read across from PFOS, marine invertebrates may be more sensitive than fish.

Protection goal	Most sensitive toxicity descriptor	Assess-ment factor	PNEC	Justification/ remarks
Marine sediment	-	-	<0.00155 mg/kg ww	No sediment data available. Calculated using Equilibrium Partitioning in EUSES (parameters in Table 10.2).
Soil	-	-	<0.0626 mg/kg ww	No soil data available. Calculated using Equilibrium Partitioning in EUSES (parameters in Table 10.2).
Secondary poisoning				Unable to calculate as no reliable mammalian or avian data available.

F-53B is potentially an endocrine disrupting substance. If these properties are confirmed, the PNECs above may need to be adjusted with an additional assessment factor to take account of the uncertainties that are introduced (e.g. heightened sensitivity of critical life stages for untested species).

These PNECs are for the substance alone, and do not take account of potential interactions with other substances that may be found in the environment, especially those with similar modes of action (e.g. other PFAS).

9.5.2 Qualitative/semi-quantitative descriptors for other critical hazards

F-53B is likely to be a PBT (and possibly a vPvB) and PMT substance. Risk management for PBT substances focusses on minimizing environmental releases, and the same approach may be adopted for PMT substances in future. For practical purposes, this means that there is an assumption of no safe level of exposure. If these properties are confirmed, risk management should be based on this assumption rather than the PNECs derived in Section 9.5.1. However, in these circumstances it may still be appropriate to set a recommended maximum acceptable concentration or *de minimis* level for monitoring purposes, under legislation such as the Water Framework Directive. This would require further policy discussion.

10 Exposure assessment

10.1 Environment

10.1.1 Release assumptions made by the Environment Agency

As F-53B is not registered for use under REACH, an assessment of exposure is based on hypothetical use as a PFOS replacement in mist suppressants for chrome plating, using information from the PFOS evaluation report (Environment Agency, 2004). For the purposes of this evaluation, the Environment Agency has assumed that F-53B is used as a mist suppressant in chrome plating with a UK market volume of 0.5 tonnes/year (Section 3). This is an over-estimate as other substances are known to be used for this application, but it demonstrates what might happen if the market moves away from those substances in future.

It is assumed that a large scale processor treats 40 m^2 of metal per hour, over a 12 hour day for 240 days per year. Losses can occur from the treatment tank through solution remaining on the metal articles as they are removed from the tank - this is called drag-out and is a potential emission to wastewater. For a rack deposition system, the typical drag-out rate is 5 litres per 100 m^2 of surface treated. Assuming a level of 10 ppm, then the removal rate for F-53B would be 20 mg per hour.

Chrome plating takes place at around 40 °C, and consequently there is some evaporation of water from the tank. The drag out is removed from the metal object by rinsing in water, which is returned to the treatment tank. Approximately, 25% of the drag-out can be returned in this way, and so the amount lost is reduced by 25%, to 15 mg per hour. The drag out is removed from the metal by rinsing, so this substance is diluted in the rinse water, but the rate of loss is not affected by this. For a 12-hour day, the daily local loss to wastewater is 180 mg/day, and over 240 release days the annual loss is 43 g/year.

There may also be the possibility of emissions to air from this process. This should be low, as the function of the substance is to prevent mist formation during the plating process, and the substance has a low vapour pressure. An approach to estimating such emissions is to consider the maximum limit for chromium (VI) in air of 0.05 mg/m^3, and to assume that all components of the treatment bath are present in any mist at their ‘working’ concentrations. The concentration of chromium (VI) in a hard hexavalent chromium bath is around 130 g/L. The volume containing 0.05 mg is therefore 3.8×10^{-7} litres. At a concentration of 10 ppm, this contains 3.8×10^{-6} mg of F-53B, hence the air concentration of F-53B is 3.8×10^{-6} mg/m^3. A rate of 7 200 m^3/hour has been assumed for air flow rates in chromium plating works. For a 12-hour day, this gives a daily removal of air of 86 400 m^3, and hence a release of F-53B of 0.33 mg/day and over 240 release days the annual loss is 79 mg/year.

A regional scale scenario is used to provide a background concentration arising from multiple sources, for addition to concentrations arising from a single site. Assuming a

steady state would mean that the liquid in the treatment tanks needs to be topped up periodically, and entirely replaced over the course of the year. It is therefore assumed that all of the F-53B would be released to waste water during the course of a year, while emissions to air are considered to be less than <0.1 %. Releases are assumed to be 10% (0.05 tonnes/year) to wastewater at the regional level and 90% (0.45 tonnes/year) at the continental scale.

Therefore, environmental releases can be expected to waste water, surface water and air compartments at the local scale and wastewater and surface water at the regional scale.

Table 10.1 Summary of assumed emissions from chrome plating

Local (mg/day)	Regional (kg/year)	Continental (kg/year)
180 (waste water) 0.33 (air) 0 (soil)	50 (waste water) 0 (air) 0 (soil)	450 (waste water) 0 (air) 0 (soil)

10.1.2 Predicted Environmental Concentrations (PECs)

Chemical concentrations can be predicted for various environmental compartments by inputting the environmental releases to the European Union System for the Evaluation of Substances (EUSES) computer program (v2.0.3). This is the best model currently available for assessing environmental exposure of novel chemicals in a standardised way. The EUSES output file for this assessment is confidential because of the information it contains on tonnage and use pattern.

In the following discussion, the 'local' environment is considered to be an area close to a site of release (e.g. an industrial facility). The 'regional' PEC is a background concentration arising from direct emissions of the substance from industrial processes and diffuse emissions as a consequence of the use of end products within a highly developed region, 200 km × 200 km in area, with 20 million inhabitants. The 'regional' scenario is equivalent to around 31% of the land area (130,279 km²) and 36% of the population (approximately 56 million people) of England (https://www.ons.gov.uk/peoplepopulationandcommunity/populationandmigration/populationestimates/bulletins/annualmidyearpopulationestimates/mid2019). The equivalent figures for the UK are around 16% for land area and 30% for population. The continental environment is the size of the EU and is generally used for mass balance purposes. The assessment is generic, representing a realistic worst case approach for a hypothetical environment that broadly reflects average European conditions. It is not intended to represent any specific part of the UK, with the exception of the local environment.

The key properties of F-53B used in the EUSES calculations are summarised in Table 10.2. Unless stated otherwise, all other partitioning coefficients are derived using the log K_{OW} using the hydrophobic QSAR contained within the model. The local and regional PECs are summarised in Table 10.3 and Table 10.4.

Table 10.2 Substance-specific input parameters for the EUSES model

Parameter	Values used in this evaluation
Physical state	Solid
Molecular weight, g/mol	560.67
Vapour pressure at 25 °C, kPa	3×10^{-7}
Water solubility at 25 °□C, mg/L	500
Octanol-water partition coefficient (log K_{ow})	4.63 (range of 4.38 to 4.79)
Organic carbon-water partition coefficient (log K_{OC})	3.85 (range of 3.65 to 3.98)
Suspended matter–water partitioning coefficient (log $K_{SUSP-WATER}$)	2.85
BCF_{fish} (L/kg ww)	3 615
$BCF_{earthworm}$ (L/kg ww)	513
Half-life for degradation in air, hours	2.4×10^{40} k_{OH} = 0 cm^3/molecule/s
Biodegradability	Not readily biodegradable
Sewage treatment works removal rate: Air Sludge	 <0.1% 45.3 %

10.1.2.1 *Fresh surface water*

Concentrations are estimated in sewage effluent from a 'standard' wastewater treatment works (WWTPs) for each life cycle step, based on the influent concentration and the partitioning properties described in Section 6. The concentration in the receiving water is calculated by assuming a default dilution factor of 10. The PEC_{local} is made up of a local water concentration (C_{local}) resulting from the relevant process emission, added to the $PEC_{regional}$.

Regional PECs and local PECs for surface water have been estimated and are presented in Table 10.3 and Table 10.4.

10.1.2.2 *Freshwater sediment*

The PEC for sediment can be derived from the PEC_{local} for surface water using the suspended matter–water partitioning coefficient (see Section 6 and Table 10.2), assuming equilibrium partitioning.

Regional PECs and local PECs for freshwater sediment have been estimated and are presented in Table 10.3 and Table 10.4.

10.1.2.3 *WWTP micro-organisms*

PECs for WWTPs are based on effluent concentrations arising from direct releases. A PEC_{WWTP} has been estimated for the assumed use pattern (see Table 10.3).

10.1.2.4 *Marine surface water*

Regional PECs and local PECs for marine surface water have been estimated and are presented in Table 10.3 and Table 10.4.

10.1.2.5 *Marine sediment*

As for freshwater sediment, the $PEC_{marine\ sediment}$ is calculated from the marine surface water concentration assuming equilibrium partitioning. Regional PECs and local PECs for marine sediment have been estimated and are presented in Table 10.3 and Table 10.4.

10.1.2.6 *Groundwater*

Local PECs for groundwater have been estimated for each use pattern (see Table 10.3) and the porewater concentration for agricultural soil is used to represent groundwater.

10.1.2.7 *Air*

The local air compartment is assumed to receive emissions from the process and via volatilisation from the WWTP. Local PECs for air represent the concentration at the site boundary, and have been estimated for each use pattern (see Table 10.3).

10.1.2.8 *Soil (including sewage sludge)*

At both local and regional scales, EUSES takes into account the application of sewage sludge containing the substance, atmospheric deposition and direct releases to soil at regional scale. Industrial soil is not considered a protection target for direct releases at local scale and no direct releases to soil are permitted at local scale.

Three different soil PECs are calculated in EUSES: soil (PEC_{soil}), agricultural soil ($PEC_{agr,soil}$) and grassland ($PEC_{grassland}$). These vary in terms of the depth of soil considered and the duration and/or route of exposure which include the repeated application of sludge from a WWTPs that occurs over a 10 year period and aerial deposition from atmosphere. The 30-day average for soil represents the PEC for soil organisms, while the 180-day averages for agricultural and grassland soils are used to estimate exposure of farmed animals and people through the food chain. The various soil PECs are summarised in Table 10.3.

At the regional level the soil concentration in unpolluted or 'natural' soil is used as the background concentration, to avoid double counting of application through sludge. The estimated regional concentrations for the soil compartment are summarised in Table 10.4.

10.1.2.9 *Secondary poisoning in the freshwater food chain*

If a substance accumulates in the food chain, it might reach a concentration in food that could cause toxic effects in a predator that eats that food. This is referred to as secondary poisoning.

PECs for fish have been calculated and are presented in Table 10.3 below. These are estimated from the surface water concentration (annual average PEC) using the fish BCF and biomagnification factors (BMFs) and assumes that the predator gets half of its food from a local site and half from regional background.

10.1.2.10 *Secondary poisoning in the marine aquatic food chain*

PECs for marine fish-eating predators have been calculated and are presented in Table 10.3 below.

10.1.2.11 *Secondary poisoning in the terrestrial food chain*

The EUSES model estimates concentrations in earthworms using an estimated earthworm bioconcentration factor and the pore water concentration of the substance in agricultural soil.

Table 10.3 Local PECs

Life cycle stage	Compartment	PEC_{local}	Unit
Mist suppressant at metal plating works	Fresh surface water (annual average)	4.83×10^{-6}	mg/L
	Freshwater sediment	4.35×10^{-4}	mg/kg ww
	Marine surface water (annual average)	4.83×10^{-7}	mg/L
	Marine sediment	4.35×10^{-5}	mg/kg ww
	Air	6.05×10^{-11}	mg/m^3
	Agricultural soil - 30 day average	6.66×10^{-4}	mg/kg ww
	Agricultural soil - 180 day average	6.64×10^{-4}	mg/kg ww
	Grassland - 180 days	2.5×10^{-4}	mg/kg ww
	Groundwater*	1.19×10^{-5}	mg/L
	WWTP	4.93×10^{-5}	mg/L
	Concentration in fish (fresh water)	0.0233	mg/kg ww
	Concentration in fish (marine water)	2.34×10^{-3}	mg/kg ww
	Concentration in marine top predators	2.82×10^{-3}	mg/kg ww
	Concentration in earthworms	3.29×10^{-3}	mg/kg ww

Note: ww - wet weight

* The pore water concentration for agricultural soil is used to represent groundwater.

Table 10.4 Regional PECs

Compartment	$PEC_{regional}$	Unit
Fresh surface water	1.62×10^{-6}	mg/L
Freshwater sediment	4.62×10^{-4}	mg/kg ww
Marine surface water	1.63×10^{-7}	mg/L
Marine sediment	4.15×10^{-5}	mg/kg ww
Air	2.44×10^{-13}	mg/m^3
Agricultural soil	2.57×10^{-4}	mg/kg ww
Natural soil	9.37×10^{-7}	mg/kg ww
Industrial soil	9.37×10^{-7}	mg/kg ww
Groundwater*	2.05×10^{-6}	mg/L

Note: ww - wet weight

* The pore water concentration for agricultural soil is used to represent groundwater

10.1.3 Sensitivity analysis

10.1.3.1 *Variation in log Kow*

There is uncertainty regarding the reliability of several of the input parameters for EUSES, in particular key physico-chemical and fate properties of F-53B. To determine the effect on local and regional PEC generation, the log K_{OW} of F-53B has been varied within the range 4.38 to 4.79. Log K_{OW} and the Henry's law constant strongly influence the distribution of a substance in the environment. As F-53B has a very low vapour pressure, log K_{OW} is considered the key sensitive input parameter for EUSES to vary.

Changing the K_{OW} had no effect on the air concentration, as would be expected for this property. It could affect the surface water, sediment, soil and groundwater PECs because partitioning is dependent on the K_{OC} which is derived from the K_{OW}. However, although an increase / decrease in PECs was observed from the modification to K_{OW} in the range described in Section 5.4 , the surface water PECs are generally of the same order of magnitude although the freshwater sediment PEC is very sensitive to K_{OW} / K_{OC} (see Table 10.5).

Table 10.5 Sensitivity analysis of PEC_{local} by varying K_{OW}

Life cycle stage	Compartment	PEC_{local} log K_{OW}			Unit
		4.38	4.63	4.79	
Mist suppressant at metal plating works	Fresh surface water (annual average)	5.59×10^{-6}	4.83×10^{-6}	4.32×10^{-6}	mg/L
	Freshwater sediment	7.4×10^{-4}	4.35×10^{-4}	1.2×10^{-3}	mg/kg ww
	Marine surface water (annual average)	7.6×10^{-7}	4.83×10^{-7}	5.78×10^{-7}	mg/L
	Marine sediment	7.4×10^{-5}	4.35×10^{-5}	1.21×10^{-4}	mg/kg ww
	Air	9.17×10^{-11}	6.05×10^{-11}	6.05×10^{-11}	mg/m^3
	Agricultural soil - 30 day average	1.13×10^{-3}	6.66×10^{-4}	1.72×10^{-3}	mg/kg ww
	Agricultural soil - 180 day average	1.13×10^{-3}	6.64×10^{-4}	1.72×10^{-3}	mg/kg ww
	Grassland - 180 days	4.37×10^{-4}	2.5×10^{-4}	6.79×10^{-4}	mg/kg ww
	Groundwater*	1.43×10^{-5}	1.19×10^{-5}	1.02×10^{-5}	mg/L
	WWTP	5.87×10^{-5}	4.93×10^{-5}	4.31×10^{-5}	mg/L
	Concentration in fish (fresh water)	0.0266	0.0233	0.021	mg/kg ww
	Concentration in fish (marine water)	2.66×10^{-3}	2.34×10^{-3}	2.12×10^{-3}	mg/kg ww
	Concentration in marine top predators	3.1×10^{-3}	2.82×10^{-3}	2.62×10^{-3}	mg/kg ww
	Concentration in earthworms	2.13×10^{-3}	3.29×10^{-3}	4.27×10^{-3}	mg/kg ww

Note: ww - wet weight
* The pore water concentration for agricultural soil is used to represent groundwater.

10.1.3.2 *Future climate scenarios*

The sensitivity of the modelled PECs to potential changes under future climate change scenarios has been considered to highlight whether pre-emptive controls may be necessary.

The default temperature of most environmental compartments within the EUSES model is 12 °C and a default value of 10 has been used as a dilution factor for WWTP effluent. Experimentally derived chemical properties are usually measured at room temperature (20 to 25 °C). For most chemicals and most properties, a temperature correction is not applied to take account of differences at lower temperatures (an exception may be degradation rate).

Research into UK climate change projections has been published by the Met Office (2020) for different warming levels (+1.5, 2 and 4 °C). Increasing the environmental compartment temperature to 16 °C has no effect on the generated PEC values. This is because the

substance is assumed to be extremely persistent over a range of ambient temperatures and also has a very low vapour pressure, so its general environmental behaviour is likely to be unaffected by a change in temperature of 4 °C.

A default dilution factor of 10 is also assumed for STP effluent. The Environment Agency (2013) reported that this default is insufficiently protective for a great deal of surface watercourses in England. In addition, dry weather is also likely to become more frequent in summer months, which will reduce river flows. A dilution factor of 2 was recommended by Environment Agency (2013) to be protective of surface watercourses. Reducing the dilution factor from 10 to 5 and also 2 increased the surface water and freshwater sediment PEC values as reported in Table 10.6.

Table 10.6 Sensitivity analysis of PEC_{local} by varying dilution factor

Life cycle stage	Compartment	PEC_{local} Dilution factor 2	5	10	Unit
Mist suppressant at metal plating works	Fresh surface water (annual average)	1.77×10^{-5}	8.03×10^{-6}	4.83×10^{-6}	mg/L
	Freshwater sediment	4.03×10^{-3}	1.76×10^{-3}	4.35×10^{-4}	mg/kg ww
	Marine surface water (annual average)	4.83×10^{-7}	4.83×10^{-7}	4.83×10^{-7}	mg/L
	Marine sediment	1.01×10^{-4}	1.01×10^{-4}	4.35×10^{-5}	mg/kg ww
	Air	6.05×10^{-11}	6.05×10^{-11}	6.05×10^{-11}	mg/m^3
	Agricultural soil - 30 day average	1.49×10^{-3}	1.49×10^{-3}	6.66×10^{-4}	mg/kg ww
	Agricultural soil - 180 day average	1.49×10^{-3}	1.49×10^{-3}	6.64×10^{-4}	mg/kg ww
	Grassland - 180 days	5.83×10^{-4}	5.83×10^{-4}	2.5×10^{-4}	mg/kg ww
	Groundwater*	1.19×10^{-5}	1.19×10^{-5}	1.19×10^{-5}	mg/L
	WWTP	4.93×10^{-5}	4.93×10^{-5}	4.93×10^{-5}	mg/L
	Concentration in fish (fresh water)	0.0697	0.0349	0.0233	mg/kg ww
	Concentration in fish (marine water)	2.34×10^{-3}	2.34×10^{-3}	2.34×10^{-3}	mg/kg ww
	Concentration in marine top predators	2.82×10^{-3}	2.82×10^{-3}	2.82×10^{-3}	mg/kg ww
	Concentration in earthworms	3.29×10^{-3}	3.29×10^{-3}	3.29×10^{-3}	mg/kg ww

Note: ww - wet weight

* The porewater concentration for agricultural soil is used to represent groundwater.

Although an increase in PECs is observed as the dilution factor reduces, they are generally in the same order of magnitude.

10.1.3.3 *Discussion*

An increase/decrease in PECs was observed from the modification of K_{OW} in the range described in Section 5.4. Although the surface water PECs are generally in the same order of magnitude, the freshwater sediment PEC is very sensitive to K_{OW} / K_{OC} changes.

Information from the Met Office as summarised above indicates the future climate will experience periods of drought. In this situation, where there is less dilution in surface water courses, this could lead to potentially higher concentrations in environmental media. In the exposure assessment, a decrease in the default dilution factor from 10 to 5 and 2 has the effect of increasing the surface water and sediment PECs accordingly. This parameter change only affects surface water media after mixing with effluent from the wastewater treatment plant and partitioning with the surface water sediment.

10.1.4 Monitoring data

A concise overview of monitoring data is presented below for the UK, Europe and global environmental media. Appendix D contains summary tables of monitoring data collated for F-53B in all available environmental media. Also see Sections 6.2, 6.3 and 8.6 for further discussion.

10.1.4.1 *UK*

F-53B has been measured in six samples of surface water (River Thames) with concentrations ranging from 0.01 to 0.08 ng/L (Pan *et al*. 2018), one sample of estuarine sediment at a concentration of 0.054 µg/kg dw (Barber *et al*. 2021) and in fifty samples of Eurasian Otter liver with a maximum concentration of 2.1 µg/kg ww (O'Rourke *et al*., 2022).

The surface water data are two orders of magnitude lower than the predicted regional background concentration and the sediment data point is of the same order of magnitude as the predicted marine background concentration. However, the sample numbers are insufficient to establish a reliable local or regional concentration.

The calculated PEC for fish eaten by predators is 23.3 µg/kg ww, although this is highly uncertain. Levels in air-breathing organisms are expected to be higher than those in aquatic gill-breathing organisms in general. The maximum measured concentration in otter liver is an order of magnitude lower than the fish PEC, and this suggests that the fish PEC may be not be reliable. Further data for fish (and their predators) might be useful. For example if sources of exposure of F-53B through interpretation of surface water and groundwater monitoring data, further targeted monitoring of biota can be undertaken in the localities of concern.

10.1.4.2 *Europe*

F-53B has been measured in various environmental media in Europe with the following maximum reported concentrations in fresh surface water (0.38 ng/L), marine surface water (<LOQ), marine sediment (<LOQ), drinking water (<LOQ). No other environmental media with reported concentrations of F-53B in Europe were identified.

The data suggest that higher surface water concentrations may occur in the UK than currently reported.

10.1.4.3 *Countries outside of Europe*

F-53B has been measured in a range of environmental media outside of Europe, principally in China where it is widely detected. Maximum reported concentrations are as follows, although it is not known whether any of these are equivalent to a local or regional scenario as would be modelled in Europe: fresh surface water 8.37 mg/L; freshwater sediment 71.6 µg/kg dw; marine surface water 7.85 ng/L; marine sediment 37.3 µg/kg dw; sewage effluent 78 µg/L; drinking water 4.4 µg/L; groundwater 1.83 ng/L; soil 9.4 µg/kg dw; and air 0.722 ng/m^3. F-53B has been detected in many species of biota with some of the highest concentrations being reported for Black-spotted Frogs (maximum 119 µg/kg).

Risk management measures in China are likely to be different to those in the UK. However, the Chinese data give an indication of levels that might occur here if the substance increases in volume in future. In particular, the substance appears to be capable of wide dispersal in the environment.

10.1.5 Discussion

The derivation of the modelled PECs for F-53B is influenced by a range of uncertainties including:

- Emission uncertainty (use pattern, emission scenarios and volumes);
- Parameter uncertainty (predicted physico-chemical and fate inputs, dilution factor);
- Modelling uncertainty (modelled WWTPs removal); and
- Monitoring data uncertainty (limited sampling and measurements available).

In the absence of more detailed information regarding emissions, use pattern and measured environmental concentrations, there remains significant uncertainty in the exposure assessment. Therefore, the PECs derived in this evaluation are highly tentative. However, because use appears to be currently relatively low, environmental concentrations in the UK are also likely to be low. This may change in future if the substance becomes as commercially important as it is in China (see Section 3).

Reliability could be improved with more detailed information on the amounts in commercial supply, use pattern and emissions to each environmental compartment along with more reliable information on environmental partitioning. This could be supplemented with

monitoring data to help reduce the uncertainty. For the time being, further refinement is a low priority, but this should be kept under review.

11 Risk characterisation

11.1 Introduction

The following sections characterise risks for the aquatic, terrestrial and atmospheric compartments, secondary poisoning of predators in aquatic and terrestrial food chains. The risk characterisation is performed by comparing the PECs with the PNECs, to derive a risk characterisation ratio (RCR). An RCR of less than 1 implies that any risk resulting from that level of exposure is acceptable. An RCR above one implies a potential risk, and all such values are highlighted in bold in Table 11.1.

A critical release level to surface water ($E_{localwater}$ or local emission rate to wastewater during an episode (kg/d)) of <0.015 kg/d (15 g/day) would equal the PNEC of <0.0005 mg/L i.e. an RCR of 1.

It should be noted that if PBT or 'equivalent level of concern' properties are confirmed, the assumption would be that there is no acceptable level of environmental exposure, and so the RCRs would not be used.

11.2 Environment

11.2.1 Aquatic compartment

F-53B is highly toxic to aquatic organisms. The freshwater $PNEC_{water}$ is <0.0005 mg/L and the marine water PNEC is <0.00001 mg/L. The PECs are very low, but given the low PNEC values, RCRs are greater than 0.01 for both surface fresh and marine waters (and since the PNEC is a 'less than' value, the RCRs could in theory exceed 1, indicative of a potential risk). The measured concentrations from the River Thames are below the $PNEC_{water}$. The deterministic risk therefore appears to be currently low, but a risk cannot be ruled out. Further refinement of the effect endpoints and PNECs may be possible if additional public data become available.

Reducing the dilution factor from 10 to 5 and also 2, increased the surface water and freshwater sediment PECs. As well as increased the RCRs above 0.01, but below 0.1.

As noted in Section 9.2, F-53B is a potential endocrine disruptor. This could be elucidated further if, for example, a relevant Level 4 or Level 5 study from the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters (OECD, 2018b) became available. Such information would only be worth pursuing if the substance is confirmed not to be persistent within the meaning of the REACH Annex 13 criteria (since it would otherwise be dealt with as a non-threshold concern as a PBT substance).

11.2.2 WWTP micro-organisms

In the absence of a suitable PNEC for micro-organisms in a WWTPs, it's not possible to derive an RCR. However, based on an analogy with PFOS, a risk is unlikely.

11.2.3 Sediment compartment

PNECs have been generated using equilibrium partitioning within EUSES with a value of <0.0775 mg/kg ww for freshwater sediment and <1.55 x 10^{-3} mg/kg ww for marine sediment. Given the very low PECs, RCRs are greater than 0.01 for both freshwater sediment and marine water sediment but less than 1. As for surface waters, since the PNEC is a 'less than' value, the RCRs could in theory exceed 1.

11.2.4 Terrestrial compartment

A PNEC for terrestrial organisms has been generated using equilibrium partitioning within EUSES with a value of $PNEC_{soil}$ of <0.0626 mg/kg ww. Despite the very low PECs, RCRs are greater than 0.01 for soil. As for surface waters, since the PNEC is a 'less than' value, the RCRs could in theory exceed 1. So, whilst the deterministic risk therefore appears to be currently low, a risk cannot be ruled out. Further refinement of the effect endpoints and PNECs may be possible if additional public data become available.

11.2.5 Groundwater

No quantitative risk characterisation has been performed for the groundwater compartment. As noted in Section 9.5.2, F-53B is likely to be a PMT substance. If these properties are confirmed, a policy decision would be needed to decide whether this makes the substance a priority for more detailed risk assessment or monitoring, or a candidate for risk management based on the assumption that there is no safe level of exposure.

11.2.6 Atmospheric compartment

No quantitative risk characterisation is possible for the atmospheric compartment in the absence of any effects data. Direct risks are likely to be low given the low predicted concentrations. However, there is a potential for long-range transport (see Section 6.2.4).

11.2.7 Non-compartment-specific effects relevant to the food chain (secondary poisoning)

RCRs were not calculated for the risk to predators via secondary poisoning as it was not possible to derive a PNEC based on the available data. However, as noted in Section 9.5.2, F-53B is likely to be a PBT (and possibly vPvB) substance. **If these properties are confirmed, risk management should be based on the assumption that there is no safe level of exposure.**

Table 11.1 Risk characterisation ratios derived by the Environment Agency

Life cycle stage	Fresh water	Fresh water sediment	Marine water	Marine sediment	Soil
Mist suppressant at metal plating works	>0.013	>0.013	>0.065	>0.065	>0.024
Regional	>0.0032	>0.016	>0.006	>0.027	>0.004

11.3 Human health

11.3.1 Risks to human health following environmental exposure

PECs for human foodstuffs and drinking water have not been estimated in the absence of reliable information on use pattern or hazards to human health. Risks to human health via environmental exposure have therefore not been evaluated.

12 Conclusion and recommendations

12.1 Conclusion

F-53B is a PFAS that belongs to the group of perfluoroether sulfonic acids. It is not registered under EU or UK REACH. There is currently no information about the actual quantities on the UK market (including in imported goods). However, F-53B may be a PFOS replacement given their structural similarity.

Based on the available hazard data the following conclusions can be reached:

- F-53B is likely to meet the criteria to be classified as Aquatic Chronic 1 for environmental hazard under the CLP Regulation.
- F-53B is not readily biodegradable and there is no evidence that it degrades significantly via abiotic mechanisms. In addition, there is no information on environmental degradation rates or half-lives available from simulation studies. F-53B therefore screens as potentially persistent or very persistent, although based on evidence from related substances (like PFOS) it is likely to be extremely persistent.
- Non-standard data on bioconcentration in fish and data from monitoring studies suggest that F-53B is likely to be bioaccumulative or very bioaccumulative (B/vB). It is not possible to draw definitive conclusions on the bioaccumulation potential of F-53B in air-breathing organisms, but the available data suggests that F-53B may be at least as bioaccumulative as PFOS.
- F-53B is therefore likely to be PBT and vPvB.
- F-53B is also likely to meet the draft PMT/vPvM criteria, suggesting that it might be hazardous for groundwaters and has the potential for transport over long distances via water. A UK policy position on the actual criteria and their risk management implications is not yet available.
- Based on the available *in vitro* and *in vivo* data, F-53B has the potential to be a thyroid disrupting compound. None of the studies were conducted to standard guidelines, so further testing would be needed to reach a definitive conclusion.

An indicative exposure assessment has been carried out by the Environment Agency based on an assumed use as a mist suppressant in chrome plating. No risks have been identified for any environmental compartments or life cycle stages based on risk characterisation ratios. However, if the PBT properties of F-53B are confirmed, emission minimisation would need to be considered.

12.2 Recommendations

There are several ways to improve the dataset to allow a more robust assessment of the hazards and risks posed by F-53B. Any future UK importer or REACH Registrant of F-53B may wish to consider these recommendations as well as ensuring that the tonnage-based information requirements are met:

- Vapour pressure should be measured using an appropriate standard method.
- Surface tension should be measured in aqueous solution.
- Water solubility should be measured using an appropriate standard method, taking care to minimise colloid formation.
- A log K_{OW} value should be estimated using measured data (either the ratio of solubility in water and in n-octanol, or back-calculated from either a reliable organic carbon-water partition coefficient or fish bioconcentration factor). This is a key input parameter for estimating long-range transport.
- Ideally further substance properties such as K_{OA}, K_{AW}, pKa, K_d and K_{OC} should be measured using appropriate standard methods.
- Further studies should be performed to confirm the environmental half-life of F-53B, along with transformation products and pathways.
- A definitive conclusion on the bioaccumulation potential of F-53B in air-breathing organisms would require further data on the human clearance time via all clearance routes or better predictive methods, but the available information suggests that F-53B may be at least as bioaccumulative as PFOS.
- Until data are available to demonstrate that the PBT/vPvB criteria are *not* met, F-53B should be treated as if it is PBT.
- The endocrine disrupting potential of F-53B could be further investigated by following an appropriate testing strategy, subject to the outcome of a refined PBT assessment.
- Further evidence should be provided on the level of supply and use pattern of F-53B in the UK.

The Environment Agency along with HSE have been undertaking a Regulatory Management Options Analysis (RMOA) for PFAS, and the information summarised in this evaluation has fed into that analysis to identify the most appropriate risk management measures for PFAS in a UK context.

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14 List of abbreviations

6:2 Cl-PFESA	6:2 chlorinated polyfluorinated ether sulfonate
%	Percentage
B	Bioaccumulative
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BMF	Biomagnification factor
CAS	Chemical Abstracts Service
CLP	Classification, labelling and packaging (of substances and mixtures)
cm	Centimetre
CoRAP	Community Rolling Action Plan
CSR	Chemical Safety Report
d	Day
$DegT_{50}$	Degradation half-life or transformation half-life (days)
DMEL	Derived Minimal Effect Level
DNEL	Derived No Effect Level
DSD	Dangerous Substances Directive
DT_{50}	Dissipation half-life (days)
dw	Dry weight
EC_{10}	10% effect concentration
EC_{50}	50% effect concentration
ECETOC TRA	European Centre for Ecotoxicology and Toxicology of Chemicals Targeted Risk Assessment
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency
EPM	Equilibrium Partitioning Method

EQS	Environmental Quality Standard
ERC	Environmental release category
ES	Exposure Scenario
EU	European Union
EUSES	European Union System for the Evaluation of Substances
FSDT	Fish Sexual Development Test
g	Gramme
GC	Gas chromatography
GC/FID	Gas chromatography – Flame Ionisation Detection
GC/MS	Gas chromatography – mass spectrometry
GLP	Good laboratory practice
H	Hours
HLC	Henry's Law Constant
hPa	Hectopascal
HPLC	High performance liquid chromatography
HSE	Health and Safety Executive
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database
IUPAC	International Union of Pure and Applied Chemistry
kg	Kilogram
kJ	Kilojoule
km	Kilometre
K_{AW}	Air-water partition coefficient
K_{OA}	Octanol-air partition coefficient
K_{OC}	Organic carbon-water partition coefficient
K_{OW}	Octanol-water partition coefficient

kPa	Kilopascal
$K_{SUSP–WATER}$	Suspended matter–water partitioning coefficient
k_X	Rate constants (days-1)
L	Litre
LC_{50}	50% lethal effect concentration
LEV	Local Exhaust Ventilation
LOD	Limit of detection
Log	Logarithmic value
LOQ	Limit of quantitation
M	Molar
m/z	Mass to charge ratio
mg	Milligram
min	Minute
mL	Millilitre
mol	Mole
MS	Mass spectrometry
nm	Nanometre
NOAEL	No observed adverse effect level
NOEC	No-observed effect concentration
NOEL	No observed effect level
NONS	Notification of New Substances Regulations 1993
OC	Operational condition
OECD	Organisation for Economic Co-operation and Development
OSPAR	Oslo and Paris Convention for the Protection of the Marine Environment of the North-East Atlantic
NICNAS	National Industrial Chemicals Notification and Assessment Scheme

p	Statistical probability
Pa	Pascal
PACT	Public Activities Co-ordination Tool
PBT	Persistent, Bioaccumulative and Toxic
PC	Product category
PEC	Predicted environmental concentration
pg	Picogramme
PFAS	Per- and polyfluorinated alkyl substances
PFCA	Perfluoroalkyl carboxylic acids
PFOS	Perfluorooctanesulfonate
PFOA	Perfluorooctanoic acid
pKa	Acid dissociation constant
PNEC	Predicted no effect concentration
POP	Persistent organic pollutant
ppb	Parts per billion
PPE	Personal Protective Equipment
ppm	Parts per million
PROC	Process Category
QSAR	Quantitative structure-activity relationship
OPERA	OPEn structure–activity/property Relationship App
r^2	Correlation coefficient
RCR	Risk characterisation ratio
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals (EU Regulation No. 1907/2006)
RMM	Risk Management Measures
RPE	Respiratory protective equipment
rpm	Revolutions per minute

SMILES	Simplified Molecular Input Line Entry System
SVHC	Substance of Very High Concern
t	Tonne
T.E.S.T.	Toxicity Estimation Software Tool
TG	Test Guideline
TMF	Trophic Magnification Factor
TSCA	Toxic Substances Control Act
UNEP	United Nations Environment Programme
UK	United Kingdom
US EPA	United Stated Environmental Protection Agency
UV	Ultraviolet
vB	Very bioaccumulative
vP	Very persistent
VP	Vapour pressure
vPvB	Very persistent and very bioaccumulative
WAF	Water Accommodated Fraction
WSF	Water Soluble Fraction
wt	Weight
ww	Wet weight
WWTP	Wastewater Treatment Plant
µg	Microgram

Appendix A: Literature search

A literature search was undertaken by the Environment Agency on the 31 March 2020 to identify published information relevant to the assessment of F-53B. The keywords listed in Table A.1 were searched for in PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Science Direct (https://www.sciencedirect.com/). In order to maximise the number of records identified. Keywords were based on the substance name only, and not on the endpoints of interest or year of publication.

For human health only the literature search was updated April 2021, by HSE, using the same key words.

Table A.1 Literature search terms and number of hits

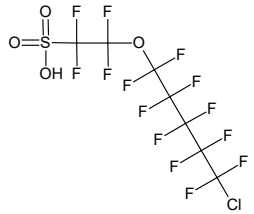
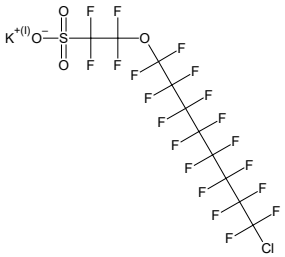
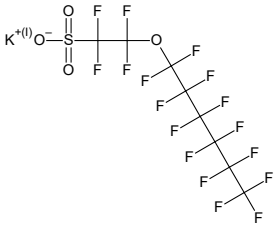
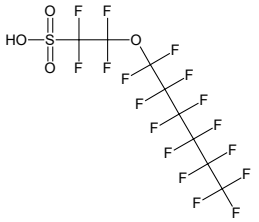
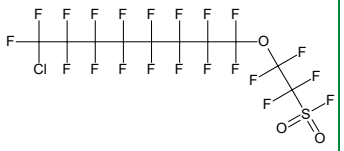
Search terms	PubMed	Science Direct
73606-19-6	0	33
6:2 chlorinated polyfluorinated ether sulfonate	30	34
6:2 Cl-PFESA	2	37
F-53B	39	117
F53-B	1	5
F53B	0	17
9Cl-PF3ONS	3	6
Total unique records	57	147

The identified records were screened manually for relevance to this assessment based on the title and abstract. Articles identified as of potential interest were obtained and reviewed for relevance. Those that were found to be relevant are discussed in the appropriate sections of this report.

Appendix B: Additional analogues of F-53B

The US EPA CompTox Chemicals database (US EPA, 2020a; accessed July 2020) was used to identify relevant structures.

Table B.1 Structural identifiers for additional analogues of F-53B

Public name	2-[(5-Chloro-1,1,2,2,3,3,4,4,5,5-decafluoropentyl)oxy]-1,1,2,2-tetrafluoroethane-1-sulfonic acid	2-[(8-Chloro-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-hexadecafluorooctyl)oxy]-1,1,2,2-tetrafluoroethane-1-sulfonic acid	Potassium 1,1,2,2-tetrafluoro-2-[(tridecafluorohexyl)oxy]-ethane-1-sulfonate [F-53]	1,1,2,2-Tetrafluoro-2-[(tridecafluorohexyl)oxy]-ethane-1-sulfonic acid	11-Chloroperfluoro-3-oxaundecanesulfonyl fluoride
CAS number	2196242-85-8	763051-92-9	68136-88-9	754925-54-7	73606-15-2
EC number	-	-	-	-	-
Structural formula					
Molecular formula	$C_7HClF_{14}O_4S$	$C_{10}HClF_{20}O_4S$	$C_8F_{17}KO_4S$	$C_8HF_{17}O_4S$	$C_{10}ClF_{21}O_3S$
Molecular weight	482.57 g/mol	632.59 g/mol	554.22 g/mol	516.12 g/mol	634.58 g/mol

SMILES code	OS(=O)(=O)C(F)(F)C(F)(F)OC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)Cl	OS(=O)(=O)C(F)(F)C(F)(F)OC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C	[K+].[O-]S(=O)(=O)C(F)(F)C(F)(F)OC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F	OS(=O)(=O)C(F)(F)C(F)(F)OC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F	FC(F)(Cl)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)OC(F)(F)C(F)(F)S(F)(=O)=O
Source	US EPA (2020f)	US EPA (2020g)	US EPA (2020h)	US EPA (2020i)	US EPA (2020j)
Comment	5:2 Cl-PFESA	Parent acid of 8:2 Cl-PFESA	As 6:2 Cl-PFESA but Cl atom replaced by F	F-53 parent acid	Sulfonyl fluoride derivative of 8:2 Cl-PFESA

Appendix C: QSAR models

Two main databases were used to source *in silico* data for this evaluation when required. These were the United States Environmental Protection Agency (US EPA) CompTox Dashboard (US EPA, 2020a) and the Royal Society of Chemistry (RSC) ChemSpider portal (RSC, 2020a). Both integrate diverse types of relevant domain data through a cheminformatics platform, and are built upon a database of curated substance properties linked to chemical structures (Williams *et al*., 2017).

The QSAR models available from these two platforms are presented in Table C.1 (data from other open access models are available in the CompTox dashboard, but for the sake of brevity, these have not been used for the purposes of this evaluation).

Table C.1 QSAR model outline

Name	Brief description
ACD/Labs	Predicts physicochemical properties via the Percepta Platform[2].
EPISuite™ **Estimation Programs Interface Suite™ for Microsoft® Windows**	A Windows®-based suite of physical/chemical, environmental fate and ecotoxicity property estimation programs developed by the US EPA and Syracuse Research Corp. It uses a single input (typically a SMILES string) to run the following estimation programs: AOPWIN™, AEROWIN™, BCFBAF™, BioHCwin, BIOWIN™, ECOSAR™, HENRYWIN™, HYDROWIN™, KOAWIN™, KOCWIN™, KOWWIN™, LEV3EPI™, MPBPWIN™, STPWIN™, WATERNT™, WSKOWWIN™ and WVOLWIN™.
OPEn structure–activity/property Relationship App (OPERA)	Open source suite of QSAR models providing predictions and additional information including applicability domain and accuracy assessment, as described in Williams *et al*. (2017). All models were built on curated data and standardized chemical structures as described in Williams *et al*. (2016). All OPERA properties are predicted under ambient conditions of 760 mmHg (103 kPa) at 25 °C.

[2] http://www.acdlabs.com/products/percepta/

TEST Toxicity Estimation Software Tool	US EPA software application for estimating the toxicity of chemicals using QSAR methods. EPISuite™ is the model used to generate some physico-chemical data, although TEST does not report K_{OW} values and uses a different database for surface tension. (US EPA, 2016).

EPISuite™

Table C.2 summarises the PFCs identified in the training/validation sets for EPISuite™. Applicability domain (US EPA, 2020c).

Table C.2 EPISuite™ PFCs included in training and validation sets

EPISuite model	Training set	Validation set
MPBPVP v 1.42	tetrafluoromethane hexafluoroethane tetrafluoroethylene octafluoropropane hexafluoropropene decafluorobutane perfluorocyclobutane perfluoro-n-hexane perfluorocyclohexane perfluoroheptane perfluoromethylcyclohexane	not available
WSKOWWIN v 1.41	none identified	octafluoropropane octafluorocyclobutane
Water solubility estimate from fragments (v 1.01 est)	trifluoromethane	tetrafluoromethane hexafluoroethane octafluoropropane perfluorocyclobutane tetrafluoroethylene
KOAWIN v 1.1	Uses KOWWIN and HENRYWIN databases	
KOCWIN v 1.66	none identified	none identified
KOWWIN v 1.67	tetrafluoromethane hexafluoroethane	perfluorocyclohexane
HENRYWIN v 3.1	tetrafluoromethane hexafluoroethane tetrafluoroethene	octafluoropropane perfluorocyclobutane

Open Structure-activity/property Relationship App (OPERA)

OPERA is a free and open-source/open-data suite of QSAR models providing predictions for physicochemical properties, environmental fate parameters, and toxicity endpoints.

Applicability domain (AD) (Williams *et al.,* 2017):

- If a chemical is considered outside the global AD and has a low local AD index (< 0.4), the prediction can be unreliable.
- If a chemical is considered outside the global AD but the local AD index is average (0.4–0.6), the query chemical is on the boundary of the training set but has quite similar neighbours (average reliability). If the local AD index is high (> 0.6), the prediction can be trusted.
- If a chemical is considered inside the global AD but the local AD index is average (0.4–0.6), the query chemical falls in a "gap" of the chemical space of the model but still falls within the boundaries of the training set and is surrounded with training chemicals. The prediction therefore should be considered with caution.
- If a chemical is considered inside the global AD and has a high local AD index (> 0.6), the prediction can be considered reliable.

T.E.S.T. (Toxicity Estimation Software Tool)

Data sets used in T.E.S.T. (US EPA, 2016) for parameters reported at 25°C:

- Surface tension: Dataset for 1 416 chemicals obtained from the data compilation of Jasper 1972;
- Water solubility: Dataset of 5 020 chemicals was compiled from the database in EPI Suite™. Chemicals with water solubilities exceeding 1,000,000 mg/L were omitted from the overall dataset;
- Vapour pressure: Dataset of 2 511 chemicals was compiled from the database in EPI Suite™.

T.E.S.T. displays structures for substances from the test and training sets that are closest to the substance where a predicted value is required. A comparison between the experimental and predicted value for the substances in the test and training sets provides a similarity coefficient. If the predicted values match the experimental values for similar chemicals in the test and training set (and the similar chemicals were predicted well), there is greater confidence in the predicted value for the substance under evaluation.

Appendix D: Monitoring data

Table D.1 Fresh surface water monitoring data

Samples	Location	Analytical method	Concentration range	Reference
7 sites, upstream and downstream of a municipal sewage treatment works	Wenzhou city, China	HPLC-MS/MS	10-50 ng/L	Wang *et al.*, 2013
41 surface water samples	Baiyangdian lake and surrounding rivers	UPLC-MS	0.05-23.54 ng/L	Cui *et al.*, 2018b
35 surface water samples	Rivers surrounding the Bohai sea, China	HPLC-MS/MS	<LOQ-36.9 ng/L	Zhao *et al.*, 2020
83 surface water samples	Fen River and Wei River, China	UPLC-MS/MS	2 x 10^4 to 8.37 x 10^6 ng/L	Zhou *et al.*, 2019
60 surface water samples, upstream and downstream of electroplating works	Fenghuajiang River and Pan River, China	UPLC-MS	0.59-77 ng/L	Lin *et al.*, 2017
53 surface water samples	19 rivers, China	UHPLC-MS	<LOQ-78.3 ng/L	Wang *et al.*, 2016
65 surface water samples	Rivers around the Bohai Sea, China	HPLC-MS/MS	<LOQ-50.6 ng/L	Chen *et al.*, 2017b
40 surface water samples	Hai River basin, China	UPLC-MS/MS	<LOQ-1.76 ng/L	Li *et al.*, 2020b
16 samples	Descheng and Yangtze river, China	HPLC-MS/MS	0.71-3.8 ng/L	Qu *et al.*, 2019
13 samples	Main rivers in Eastern cities, China	LC-Orbitrap Tribrid HRMS LC-MS/MS	2.0-44.2 ng/L 1.9-40.2 ng/L	Lin *et al.*, 2016
35 samples	Yangtzee river, China	UPLC-MS	0.12-12.94 ng/L	Pan *et al.*, 2018
15 samples	Yellow river, China		0.01-0.29 ng/L	
13 samples	Pearl river, China		0.13-11.06 ng/L	
6 samples	Liao river, China		0.24-2.29 ng/L	
9 samples	Huai river, China		0.36-21.38 ng/L	

Samples	Location	Analytical method	Concentration range	Reference
13 samples	Chao Lake, China		0.69-52.2 ng/L	
15 samples	Tai Lake, China		0.21-27.6 ng/L	
6 samples	River Thames, UK		0.01-0.08 ng/L	
20 samples	River Rhine, Germany and the Netherlands		0.02-0.38 ng/L	
12 samples	Delaware River, USA		<LOQ-0.08 ng/L	
10 samples	Malaren Lake, Sweden		<LOQ-0.05 ng/L	
6 samples	Han river, South Korea		0.02-0.06 ng/L	
5 samples	Xiaoqing river and Tangrun lake, China	HPLC-MS/MS	0.114-0.452 ng/L	Shi *et al*., 2015
18 samples	Upstream and downstream of a fluorochemical production plant	UPLC-ESI-MS/MA	<LOQ	Gebbink *et al*., 2017

Table D.2 Drinking water monitoring data

Samples	Location	Analytical method	Concentration range	Reference
2 tap water samples	Baiyangdian lake area	UPLC-MS	0.03-0.05 ng/L	Cui *et al*., 2018b
Finished water (post treatment) **3 water supplies**	Largest drinking water treatment plant in Changzhou, China	HPLC-MS/MS	0.74-3.9 ng/L 0.78-4.4 ng/L	Qu *et al*., 2019
6 drinking water supplies	City halls in the Netherlands	UPLC-ESI-MS/MA	<LOQ	Gebbink *et al*., 2017

Table D.3 Freshwater sediment monitoring data

Samples	Location	Analytical method	Concentration range	Reference
35 suspended particulate matter samples	Rivers surrounding the Bohai sea, China	HPLC-MS/MS	<LOQ-71.6 ng/g dw	Zhao *et al*., 2020
60 sediment samples, upstream and downstream of electroplating works	Fenghuajiang River and Pan River, China	UPLC-MS	46-7240 pg/g dw	Lin *et al*., 2017
40 suspended particulate matter samples **20 sediment samples**	Hai River basin, China	UPLC-MS/MS	<LOQ-49.44 ng/g 0.006-4.49 ng/g	Li *et al*., 2020b
Lake Hazen core, 0-17 cm **Lake B35 core, 0-5 cm**	Canada	UPLC-MS/MS	<LOQ <LOQ	MacInnis *et al*., 2019

Table D.4 Marine surface water monitoring data

Samples	Location	Analytical method	Concentration range	Reference
250 seawater samples	South China sea, coastal region	UPLC-MS	<LOQ-0.307 ng/L	Wang *et al*., 2019a
20 sea water samples	Bohai sea, China	HPLC-MS/MS	0.72-5.87 ng/L	Liu *et al*., 2019a
90 sea water samples	German Bight and German Baltic sea	HPLC-MS/MS	<LOQ	Joerss *et al*., 2019
52 sea water samples	Bohai sea, China	HPLC-MS/MS	<LOQ-0.32 ng/L	Zhao *et al*., 2020
27 samples	7 European countries	UPLC-HRMS	<LOQ	Aminot *et al*., 2019
250 sea water samples	Pearl River Delta, China	UPLC-MS/MS	<LOQ-0.307 ng/L	Wang *et al*., 2019a
120 sea water samples	Bohai Sea, China	HPLC-MS/MS	<LOQ-7.85 ng/L	Chen *et al*., 2017b
30 sea water samples	Yellow Sea, China	UPLC-MS	0.04-0.17 ng/L	Feng *et al*., 2020

Table D.5 Marine sediment monitoring data

Samples	Location	Analytical method	Concentration range	Reference
53 sediment samples	South China sea, coastal region	UPLC-MS	<LOQ -0.0158 ng/g dw	Wang *et al*., 2019a
18 sediment samples	Bohai sea, China	HPLC-MS/MS	<LOQ – 3.45 ng/g dw	Liu *et al*., 2019a
20 suspended particulate matter samples	Bohai sea, China	HPLC-MS/MS	0.082 – 37.3 ng/g dw	Liu *et al*., 2019a
48 sediment samples	German Bight, German Baltic sea, North Sea, the Skagerrak and Kattegat and Baltic Sea	HPLC-MS/MS	<LOQ	Joerss *et al*., 2019
30 sediment samples	Bohai sea, China	HPLC-MS/MS	<LOQ-0.04 ng/g dw	Zhao *et al*., 2020
52 suspended particulate matter samples	Bohai sea, China	HPLC-MS/MS	<LOQ-3.95 ng/g dw	Zhao *et al*., 2020
24 samples	7 European countries	UPLC-HRMS	<LOQ	Aminot *et al*., 2019
53 sediment samples	Pearl River Delta, China	UPLC-MS/MS	<LOQ-0.0158 ng/g dw	Wang *et al*., 2019a

Samples	Location	Analytical method	Concentration range	Reference
30 sediment samples **30 pore water samples**	Yellow Sea, China	UPLC-MS	0.0043-0.370 ng/g dw 0.021-9.84 ng/L	Feng *et al*., 2020
5 sediment samples	Fildes peninsula, King George Island and Ardley Island, Antartic	HPLC-ESI-MS/MS	0.1±0.09 ng/g dw	Gao *et al*., 2020
103 sediment samples	15 estuaries, England	UPLC-MS	<LOQ -0.054 ng/g dw	Barber *et al*. 2021

Table D.6 Groundwater monitoring data

Samples	Location	Analytical method	Concentration range	Reference
102 samples from non-industrial areas	13 cities in Jiangsu province, China	HPLC-MS	0.17-1.83 ng/L	Wei *et al*., 2018

Table D.7 Air monitoring data

Samples	Location	Analytical method	Concentration range	Reference
Particulate matter samples	Dalian University campus, China	HPLC-MS	85.9 – 722 pg/m^3	Liu *et al*., 2017b
Particulate phase	Bohai and Yellow sea, China	HPLC-MS/MS	<0.04 – 3.9 pg/m^3	Fang *et al*., 2018
6 air samples	Bohai sea, China	HPLC-MS/MS	<LOQ-0.42 pg/cm^3	Zhao *et al*., 2020
Rain water				
39 samples	28 cities, China	HPLC-MS/MS	<LOQ-6.5 ng/L	Chen *et al*., 2019

Table D.8 Soil monitoring data

Samples	Location	Analytical method	Concentration range	Reference
Samples from 89 cities	31 provincial level administrative regions, China	HPLC-MS/MS	<LOQ-1.117 ng/g dw	Li *et al*., 2020a
98 soil samples	Cornfields across 576 km^2, China	HPLC-MS/MS	<LOQ-1.20 ng/g dw	Lan *et al*., 2020
9 soil samples	Most heavily contaminated cornfields across 576 km^2, China	HPLC-MS/MS	0.27-0.69 ng/g dw	Lan *et al*., 2020
1 site, samples from 14 different depths (0.5-12.5m)	Chrome plating site, China	HPLC-MS/MS	<LOQ-9.4 µg/kg	Li *et al*., 2017

Table D.9 Sewage treatment works monitoring data

Samples	Location	Analytical method	Concentration range	Reference
A municipal works which also receives wastewater from electroplating industry	Wenzhou city, China	HPLC-MS/MS	65−112 µg/L influent 43−78 µg/L effluent	Wang *et al.*, 2013
19 works, with no known industrial sources	Australia	LC-MS/MS	<LOQ in aqueous and solid samples	Coggan *et al.*, 2019
Waste water treatment plant effluent	Changzhou, China	SPE-LC-MS/MS	Data available in supplementary file	Zhong *et al.*, 2019
56 sewage sludge samples	20 provinces and municipalities, China	UPLC-HRMS	0.02-209 ng/g dw	Ruan *et al.*, 2015
34 drain outlets	Emptying into the Bohai Sea, China	HPLC-MS/MS	<LOQ-7595 ng/L	Chen *et al.*, 2017b

Table D.10 Biota monitoring data

Samples	Location	Analytical method	Concentration range	Reference
11 fish species **crab** **shrimp** **duck (tissue specific)**	Baiyangdian lake and surrounding rivers	UPLC-MS	0.18-8.26 ng/g ww 0.98-1.89 ng/g ww 0.36 ng/g ww 3.92-53.75 ng/g ww	Cui *et al.*, 2018b
50 Eurasian otter liver samples	Sites near the Severn, Thames, Brue rivers and the Fylde Coast, UK	UPLC-MS	<LOQ – 2.1 ng/g ww	O'Rourke *et al.*, 2022
17 mussel samples	7 European countries	UPLC-HRMS	<LOQ	Aminot *et al.*, 2019
Poplar leaves **Maize leaves**	3 sites across 576 km^2, China	HPLC-MS/MS	<LOQ-0.87 ng/g dw <LOQ-1.9 ng/g dw	Lan *et al.*, 2020

Samples	Location	Analytical method	Concentration range	Reference
Locusts			<LOQ-2.77 ng/g dw	
43 crucian carp, tissue specific samples **10 crucian carp, tissue specific samples**	Xiaoqing river and Tangrun lake, China Beijing food market	HPLC-MS/MS	0.61-82.85 ng/g mean 1.06-27.99 ng/g	Shi *et al*., 2015
Ringed seal liver **Polar bear liver** **Killer whale liver**	East Greenland	UPLC-MS	mean 0.045 ± 0.004 ng/g mean 0.27 ± 0.04 ng/g mean 0.023 ± 0.009 ng/g	Gebbink *et al*., 2016
Skate	Bohai Sea, China	UPLC-MS	0.042-0.056 ng/g	Liu *et a*l., 2017a
Greenling			0.025-0.059 ng/g	
Branded goby			0.087-0.221 ng/g	
Korean rockfish			0.028-0.195 ng/g	
Red seabream			<LOQ-0.058 ng/g	
Javelin goby			0.034-0.087 ng/g	
Black-barred halfbeak			0.058-0.081 ng/g	
Spotted sea bass			0.030-0.125 ng/g	
Octopus			0.033-0.312 ng/g	
Mantis shrimp			0.213-0.575 ng/g	
Gazami crab			<LOQ-0.1 ng/g	
Asian paddle crab			<LOQ-0.176 ng/g	
Sand shrimp			<LOQ-0.06 ng/g	
Veined rapa whelk			<LOQ-0.034 ng/g	
Ark shell			<LOQ-0.056 ng/g	
Bladder moon snail			<LOQ-0.09 ng/g	
Oyster			<LOQ-0.026 ng/g	
Venus clam			<LOQ-0.325 ng/g	
Zhikong scallop			<LOQ-0.064 ng/g	
56 Black-spotted frog *(Pelophylax nigromaculatus)* liver	Changshu, Huantai, Quzhou and Zhoushan, China	UPLC-MS	0.13-119.05 ng/g	Cui *et al*., 2018a
Rapa whelk	Bohai Sea, China		0.351 ± 0.261 ng/g	

Samples	Location	Analytical method	Concentration range	Reference
Mactra quadrangularis		HPLC-MS/MS	0.117 ± 0.052 ng/g	Chen *et al*., 2018
Short necked clam			0.106 ± 0.056 ng/g	
Bay scallop			0.069 ± 0.057 ng/g	
Chinese shrimp			0.099 ± 0.063 ng/g	
Octopus			0.197 ± 0.17 ng/g	
Mullet			0.218 ± 0.310 ng/g	
Asian goby			0.142 ± 0.068 ng/g	
Bluefin gurnard			0.119 ± 0.074 ng/g	
Dotted gizzard shad			0.116 ± 0.053 ng/g	
White croaker			0.249 ± 0.273 ng/g	
Flathead fish			0.157 ± 0.049 ng/g	
Black porgy			0.075 ± 0.055 ng/g	
Schlegel's black rockfish			0.116 ± 0.065 ng/g	
Japanese flounder			0.188 ± 0.055 ng/g	
Yellowsnout sea bass			0.116 ± 0.034 ng/g	
Black tailed gull			0.725 ± 0.092 ng/g	
Finless porpoise			3.84 ± 0.271 ng/g	
Northern Goshawk (*Accipiter gentilis*)	Norway	UHPLC-MS/MS	<LOQ	Briels *et al*., 2019a
6 algae, 3 fish, 5 neogastropoda, 15 archaeogastropoda, 13 cape petrel feathers, 5 penguin feathers	Fildes peninsula, King George Island and Ardley Island, Antartic	HPLC-ESI-MS/MS	<LOQ	Gao *et al*., 2020
64 blood samples golden snub-nosed monkey (*Rhinopithecus roxellana*) and Francois' leaf monkey (*Trachypithecus francoisi*)	Wuzhou Breeding Center, Tongling zoo, Shanghai wild zoo and Shennongjia nature reserve, China	UPLC-MS/MS	<LOQ-0.13 ng/mL	Cui *et al*., 2019
26 bark samples from Chinese red pine (*Pinus tabulaeformis* Carr.), Chinese	Beijing, China	UPLC-MS/MS	<0.13–0.15 ng/g dw	Liu *et al*., 2019b

Samples	Location	Analytical method	Concentration range	Reference
scholartree (*Sophora japonica* Linn.), weeping willow (*Salix babylonica*) and Canadian poplar (*Populus* × *canadensis*)				
46 fish muscle samples (yellow catfish, common carp, white amur bream, grass carp, silver carp, bighead carp)	Tangxun Lake, China	HPLC-ESI-MS/MS	0.507-4.77 ng/g ww	Shi *et al*., 2016

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